A next-generation functional genomics strategy to deconvolute compound genetic drivers and genotype-to-phenotype relationships in bladder cancer

2019 Eula and Donald S. Coffey Innovative Research Award Finalist

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Background:
Next-generation sequencing has uncovered the sheer genomic heterogeneity of bladder cancer. Current approaches to model tumorigenesis are poorly suited for the systematic functional interrogation of the diverse, higher-order genetic interactions that drive bladder cancer due to the lack of scale, throughput, and economy.

Methods
We are developing a strategy to address this issue by leveraging organoid cultures, multiplex transduction with a barcoded lentiviral library of genetic perturbations, *in vivo* selection for tumorigenesis, and digital profiling of resultant tumors by single-cell sequencing. We initiated bladder urothelial organoids from basal cells (Lin^- $EpCAM^+CD49f^{high}$) isolated from C57BL/6J bladders. A lentiviral library was constructed that encodes gain- and loss-of-function genetic events recapitulating recurrent alterations in bladder cancer. Each vector contains matching 10-nucleotide barcodes at the 5' and 3' ends. Cells were transduced by mixing lentivirus into cell-Matrigel suspensions prior to seeding of organoid culture droplets. Organoids were transplanted subcutaneously into the flanks of NSG mice. Targeted single-cell DNA sequencing on the Mission Bio Tapestri platform was performed to recover compound lentiviral barcodes from tumor cells.

Results:
Bladder urothelial cells were transduced in organoid culture with lentivirus to achieve viral copy numbers (VCN) of up to 20 with a linear relationship between VCN and lentiviral dose. Barcoded lentiviruses encoding mutant *PIK3CA* E545K, mutant *FGFR3* S243C, *PVRL4*, *PPARG*, and *YWHAZ* generated a squamous cell carcinoma phenotype within our mouse bladder urothelial tumorigenesis assay. Single-cell characterization of ~3,000 tumor cells from this model showed the co-existence of multiple clonal populations driven by distinct combinations of oncogenic drivers.

Conclusions:
We achieved efficient lentiviral transduction of bladder urothelial cells to enable the interrogation of higher-order genetic interactions in cancer initiation. Genetic heterogeneity can be introduced via the Poisson distribution of lentiviral transduction and sensitively recovered by massively parallel targeted scDNA-seq of lentiviral barcodes. With this approach, we have generated genetically-defined models of bladder cancer and provide preliminary proof-of-principle for a strategy to rapidly deconvolute complex genotype-to-phenotype relationships in bladder cancer initiation.

Myeloid-Derived Suppressor Cells Inhibit T Cell Activation in Prostate Cancer through Nitrating LCK *2019 Eula and Donald S. Coffey Innovative Research Award Finalist*

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Shan Feng, Ph.D., Postdoctoral Fellow, Liang Cheng, M.D., Virgil Moon Professor of Pathology

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Background: Patients with advanced prostate cancer (PCa) benefit from androgen deprivation therapy initially, but often later develop lethal metastatic castration-resistant prostate cancer (mCRPC). Immune checkpoint blockade (ICB) using antibodies against CTLA4 or PD1/PD-L1 generates durable therapeutic responses in a variety of cancer types. However, mCRPC showed overwhelming de novo resistance to ICB. Our previous publications established that myeloid-derived suppressor cells (MDSCs) are the key player in PCa immune evasion, and targeting MDSCs with multikinase inhibitors or CXCR2 inhibitors could synergize with ICB to elicit potent anti-mCRPC effect (*Cancer Discovery, 2016; Nature, 2017*). More effective and specific MDSC inhibition relies on deeper mechanistic understanding of MDSCs. One main mechanism for MDSCs to induce T cell tolerance is through secretion of reactive nitrogen species (RNS, e.g. peroxynitrite). However, this mechanism remains poorly understood and very few nitrated proteins are known.

Methods: We developed a new nitroproteomic approach for the identification of nitropeptides from cells and tissues (*JoVE.* 2019. PMID: 31259896). The biological models we used for target identification and functional validation are *Pten/p53/Smad4* PCa transgenic model and syngeneic Lewis Lung Carcinoma (LLC) model.

Results: We identified that lymphocyte-specific protein tyrosine kinase (LCK), an initiating tyrosine kinase in the T cell receptor signaling cascade, is nitrated at Tyr394 by MDSCs. LCK nitration inhibited T cell activation, leading to reduced interleukin-2 production and proliferation. In human T cells with defective endogenous LCK, wild type, but not nitrated LCK, rescued IL2 production. We documented elevated 3-nitrotyrosine signals in clinical samples of CRPC. In both the Pten/p53/Smad4 mCRPC model and LLC model, we showed that ICB therapy (PD1 and CTLA4 antibody cocktail) elicited strong anti-tumor efficacy when combined with a RNS neutralizing agent.

Conclusions: We have identified a previously unknown mechanism of T cell inactivation by MDSC-induced protein nitration and illuminated a clinical path hypothesis for combining ICB with RNS-reducing agents in the treatment of mCRPC (Figure 1).

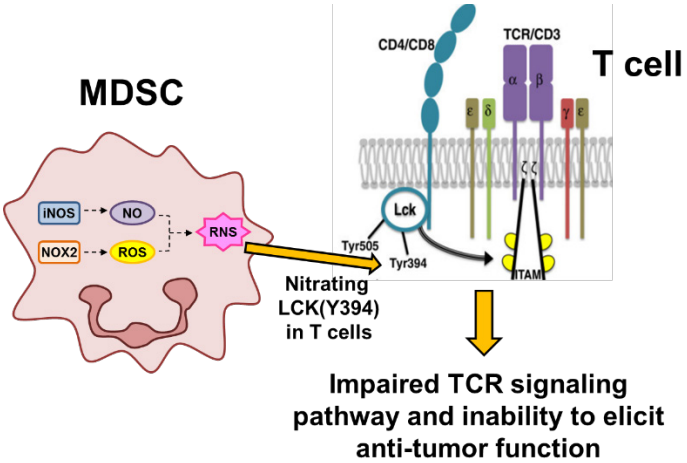

Figure 1. Schematic showing the mechanism of LCK-Y394 nitration by MDSC-derived RNS.

First Genetically Engineered Mouse Model of Penile Cancer and Its Application in Preclinical Immunotherapy

Xin Lu, Ph.D., Boler Assistant Professor, University of Notre Dame; Indiana University

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Background: Penile cancer is a rare but highly morbid disease, with penile squamous cell carcinoma (PSCC) accounting for over 95% of all cases. PSCC often results in devastating disfigurement and only half of the patients survive beyond 5 years. The molecular etiology of PSCC remains poorly understood, which causes limited treatment options for advanced tumors incurable with surgery or radiotherapy alone.

Methods: Herein, we describe the development of the first genetically engineered mouse (GEM) model of PSCC. Transcriptomics of the penile tumor and normal penile epithelium was profiled by RNA-seq for mechanistic investigation and comparison to human penile cancer transcriptomics. Immunophenotyping of the model was performed by CyTOF. The model was subjected to unbiased molecular annotation with RPPA and inhibitor screening for potential novel drugs against PSCC. Pharmacological targeting of the tumor microenvironment was combined with immune checkpoint blockade antibodies (PD1, CTLA4) for the discovery of new treatment strategy of lethal PSCC.

Results: The GEM model is highly relevant to the human disease by virtue of sharing up- and down-regulated genes and pathways with human PSCC based on comparative transcriptomic and biomarker analyses. In silico and animal experiments elucidated both cancer cell-intrinsic (β-catenin and SOX2 transcription activation) and extrinsic (COX2-dependent inflammatory microenvironment) mechanisms that were operative to drive the progression of PSCC. Mouse PSCC fosters an immunosuppressive microenvironment with myeloid-derived suppressor cells (MDSCs) as a dominant population. Preclinical trials in the model demonstrate synergistic efficacy of immune checkpoint blockade with the MDSC-diminishing drugs cabozantinib or celecoxib. Drug screen studies informed by targeted proteomics identified a few potential therapeutic strategies for PSCC.

Conclusion: Our studies have established what we believe to be essential resources for studying PSCC biology and developing new therapies (Figure 1).

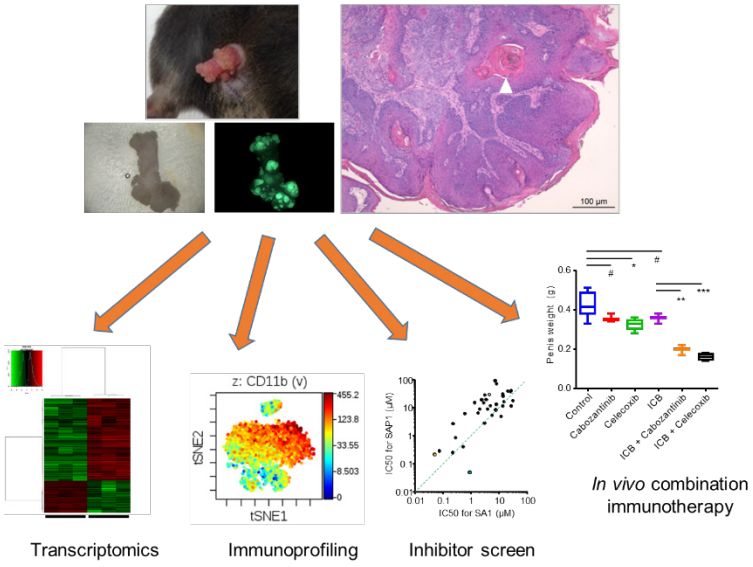

Figure 1. First Genetically Engineered Mouse Model of Penile Cancer and Its Application in Preclinical Studies

A Novel Therapeutic Target For the Treatment Lethal Prostate Cancer

2019 Eula and Donald S. Coffey Innovative Research Award finalist

Salma Kaochar, PhD, Baylor College of Medicine *Additional Authors - Darlene Skapura, Matthew Robertson, Christel Davis, Erik Ehli, Kimal Rajapakshe, Cristian Coarfa, Bert O'Malley, Nicholas Mitsiades*

A Novel Therapeutic Target for the Treatment Lethal Prostate Cancer

Salma Kaochar, Darlene Skapura, Matthew Robertson, Kimal Rajapakshe, Christel Davis, Erik Ehli, Cristian Coarfa, Bert W. O'Malley, Nicholas Mitsiades

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Background: The p160 Steroid Receptor Coactivators (SRCs): SRC-1, SRC-2 (NCOA2), and SRC-3 (AIB1 or NCOA3) are critical components of many transcription factor (TF) complexes and master regulators of TF activity necessary for cancer cell proliferation, survival, metabolism, motility, invasion, metastasis, progression, and resistance to therapy. The p160 SRCs are overexpressed in many cancers, including prostate cancer (PC), where they are mediating the function of androgen receptor (AR), a major TF for PC. However, while numerous studies have demonstrated their important roles as oncogenes in many cancers, they were historically considered "undruggable", because they lack a natural ligand-binding site or enzymatic activity that can be targeted by small molecule inhibitors (SMIs).

Methods: We recently identified a novel family of SRC SMIs with improved drug-like properties and strong potential for clinical development (Song X, et al. PNAS 2016;113(18):4970-5). We examined the effects of SI-2, the parent compound of this new class, and SI-12, a derivative with better PK properties (longer half-life in the circulation), against prostate cancer cell lines in vitro and in vivo.

Results: Our novel p160 SRC SMIs exert potent anti-proliferative activity against PC cell lines (IC50s in the low nM range), including against cell lines resistant to conventional therapies. Gene expression profiling revealed that they strongly suppress genes associated with cellular proliferation, DNA repair, WNT signaling and overall transcriptional programs associated with stemness or stem-like cell state. They also suppress the transcriptomic footprint of the p160 SRCs, block the ability of cancer cells to migrate in an in vitro assay and have potent in vivo anticancer activity against xenograft models.

Conclusions: We propose a 'first-in-field' approach to target the previously undruggable family of the p160 SRC oncogenes. Our preclinical studies demonstrate that our novel p160 SRC SMIs exert potent activity against PC cell lines in vitro and in vivo, including against cells that are resistant to conventional therapies, at low nM concentrations that are easily achievable in vivo in mouse models. Based on these strong preliminary studies, we are confident that a class of SI-2 derivative compounds are highly promising drug candidates as p160 SRC SMIs for cancer treatment.

PD-L1 is associated with the clinical features of human primary prostate tumors

2019 Travel Award Winner

Dongxia Ge, Tulane University, New Orleans, LA

PD-L1 is associated with the clinical features of human primary prostate tumors

Dongxia Ge[1], Peng Xian[1,3], Victor J Wu[1], Avi Patel[4], Wendell W Tang[5], Xiaocheng Wu[6], Kun Zhang[7], Li Li[4], and Zongbing You[1,2]

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Background: Immunotherapy targeting programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) has been approved for the treatment of a variety of solid tumors. However, prostate cancer does not respond to anti-PD-1/PD-L1 therapies. The objective of this study was to determine PD-1 and PD-L1 expression status and their correlation with clinical features of human primary prostate tumors.

Methods: A total of 279 prostate cancer patients who underwent radical prostatectomy included in this study. PD-1 and PD-L1 expression was detected using immunohistochemical staining. Analyses were made between PD-1/PD-L1 status and patients' age, ethnicity, body mass index (BMI), diabetes mellitus, tumor stage, lymph node metastasis, prostate-specific antigen (PSA), Gleason score, grade group, and survival.

Results: We found that 6.5 tumor-infiltrating lymphocytes (standard deviation 14.3; range 0-161.6) per high power field were positive for PD-1 staining and 50/279 (17.9%) tumors were positive for PD-L1 staining (Figure 1 and Table 1). PD-L1-positive tumors had significantly more PD-1-positive lymphocytes than PD-L1-negative ones. The number of PD-1-positive lymphocytes was not correlated with any clinical features. In contrast, more PD-L1-positive tumors were found in older patients (≥ 65 years), obese patients (BMI ≥ 30), and patients with advanced tumor stage, lymph node metastasis, and high Gleason score. Neither PD-1 nor PD-L1 status was correlated with ethnicity, PSA, or survival.

Conclusions: Our findings suggest that PD-L1 rather than PD-1 status is associated with the clinical features of human primary prostate tumors.

Figure 1

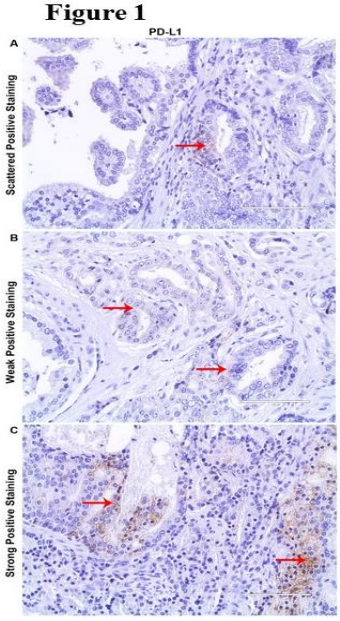

Table 1. Clinical features and their associations with PD-L1 status.

	Total Cases (N=279)	PD-L1 Status		P-value
		PD-L1 (-)	PD-L1 (+)	
Age (%)				
<65	186 (66.7%)	159 (85.5%)	27 (14.5%)	0.0360
≥65	93 (33.3%)	70 (75.3%)	23 (24.7%)	
BMI (%)				
<25	54 (19.4%)	43 (79.6%)	11 (20.4%)	0.0026
25-29.99	137 (49.1%)	123 (89.8%)	14 (10.2%)	
≥30	81 (29.0%)	58 (71.6%)	23 (28.4%)	
Unknown	7 (2.5%)	5 (71.4%)	2 (28.6%)	
Tumor stage (%)				
T1 (%)	16 (5.7%)	14 (87.5%)	2 (12.5%)	0.0196
T2 (%)	152 (54.5%)	133 (87.5)	19 (12.5%)	
T3 (%)	88 (31.5%)	67 (76.1%)	21 (23.9%)	
T4 (%)	23 (8.2%)	15 (65.2%)	8 (34.8%)	
T1+T2 (%)	168 (60.2%)	147 (87.5%)	21 (12.5%)	0.0037
T3+T4 (%)	111 (39.8%)	82 (73.9%)	29 (26.1%)	
N Stage (%)				
N0 (%)	255 (91.4%)	213 (83.5%)	42 (16.5%)	0.0294
N1 (%)	21 (7.5%)	13 (61.9%)	8 (38.1%)	
Unknown (%)	3 (1.0%)			
Gleason score (%)				
≤6 (%)	109 (39.1%)	102 (93.5%)	7 (6.5%)	0.0001
=7 (%)	97 (34.8%)	76 (78.4%)	21 (21.6%)	
≥8 (%)	73 (26.2%)	51 (69.9%)	22 (30.1%)	

LSD1 activates PI3K/AKT signaling through regulating p85 expression in prostate cancer cells

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Prof. Shuai Gao, Dr. Dong Han, Wanting Han, Muqing Li, Prof. Changmeng Cai

LSD1 activates PI3K/AKT signaling through regulating p85 expression in prostate cancer cells

Introduction & Objective

Lysine specific demethylase 1 (LSD1/KDM1A), a specific demethylase of mono- or di-methylated histone lysine 4 (H3K4me1,2, enhancer-associated histone marks), was first identified as a component of REST repressor complex through interaction with CoREST and histone deacetylases 1, 2 (HDAC1,2). LSD1 is also found in Mi-2/nucleosome remodeling and deacetylase (NuRD) repressor complex with interaction with MTA proteins. While LSD1 is well known for its transcription repressor activity, it also activates gene transcription through demethylating repressive histone marks, such as methylated histone 3 lysine 9 (H3K9me1,2), and possibly other non-histone proteins. In particular, LSD1 functions as a major androgen receptor (AR) coactivator in prostate cancer (PCa) cells. This coactivator function was thought to be attributed to androgen-induced phosphorylation of histone 3 threonine 6 and 11 (H3T6/T11ph), which lead to the switch of LSD1 substrate from H3K4me1,2 to H3K9me1,2. However, our recent study indicates that the H3K4 demethylase activity of LSD1 persists at AR-mediated enhancers marked with H3T6ph, suggesting additional mechanism(s) mediating its coactivator activity of AR. Indeed, we reported that LSD1 interacts and colocalizes with FOXA1, a pioneer factor of AR, at AR-mediated enhancers, and that this interaction may facilitate AR transcription activity. Nonetheless, since AR signaling is critical to PCa development and progression to the lethal stage of castration-resistant PCa (CRPC), studies from us and others highly suggest that targeting LSD1 may be a potential treatment strategy for PCa and particularly CRPC, where AR signaling is commonly restored. However, whether LSD1 regulates other major tumor-promoting pathways in PCa cells remains to be determined.

Methods

To identify the additional pathways that may be regulated by LSD1, we conducted RNA-seq analyses in LSD1 inhibitor treated PCa cell lines and subsequently validated the results in multiple PCa cells lines. In addition, we also generated LSD1 knockout lines using CRISPR/CAS9 approach to validate the results from the transcriptome studies. Furthermore, we also validated the results *in vivo* using LSD1 inhibitor-treated LuCaP35CR CRPC xenograft model.

Results

From the RNA-seq analyses of LSD1 inhibitor treated PCa cells and the subsequent functional annotation analyses, we found that LSD1-activated genes were enriched for PI3K/AKT pathway in absence of androgen stimulation. We further confirmed *in vitro* and *in vivo* that LSD1 inhibition and gene knock-out can significantly decrease AKT phosphorylation. Mechanistically, we identified the regulatory subunit of PI3K, p85α (and possibly its isoform p85β), as a critical transcriptional target of LSD1 that mediates its effect on PI3K/AKT pathway activation in PCa cells. We also show that the combination treatment of a PI3K inhibitor with a LSD1 inhibitor can suppressed PCa cell proliferation more effectively.

Conclusions

In this study, we show that LSD1 can activate PI3K/AKT pathways in absence of androgen stimulation, and we further demonstrate that this effect is mediated by transcriptional regulation of PI3K regulatory subunit, p85, possibly through epigenetic reprogramming of enhancer landscape in PCa cells. Our study suggests that the LSD1 has dual functions in promoting PCa development, that it enhances AR signaling through its coactivator function, and that it activates PI3K/AKT signaling through increasing p85 gene expression. Based on these findings, it is plausible that the effectiveness of LSD1 inhibitor treatment in CRPC may be due to inhibition of both AR signaling and PI3K/AKT signaling pathways.

Interleukin-17 Upregulates MTA1 Expression to Promote Cancer Cell Migration and Invasion *2019 Travel Award Winner*

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Interleukin-17 Upregulates MTA1 Expression to Promote Cancer Cell Migration and Invasion

Ahmed A. Moustafa[1], Na Guo [1,7], Ge Shen [1], Ying Zhang[8], Dongxia Ge[1], and Zongbing You [1,2,3,4,5,6]

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Background: Interleukin-17 (IL-17) has been shown to promote development of primary prostate cancer and lymph node metastasis in mouse models. However, the mechanisms of how IL-17 promotes metastasis are not clear. The purpose of this study was to determine if IL-17 regulates metastasis associated 1 (MTA1) expression and its biological consequences.

Methods: Human prostate cancer DU-145 and human cervical cancer HeLa cell lines were used to test if IL-17 regulates MTA1 mRNA and protein expression using qRT-PCR and Western blot analysis, respectively. Cell migration and invasion were studied using wound healing assays and invasion chamber assays. Thirty-four human cervical tissues were stained for IL-17 and MTA1 using immunohistochemical staining.

Results: We found that IL-17 increased MTA1 mRNA and protein expression in both cell lines. Cell migration was accelerated by IL-17, which was abolished by knockdown of MTA1 expression with small interference RNA (siRNA). Further, cell invasion was enhanced by IL-17, which was eliminated by MTA1 knockdown (Fig.1). Human cervical intra-epithelial neoplasia (CIN) and cervical cancer tissues had increased number of IL-17-positive cells and MTA1 expression compared to normal cervical tissues. The number of IL-17-positive cells was positively correlated with MTA1 expression.

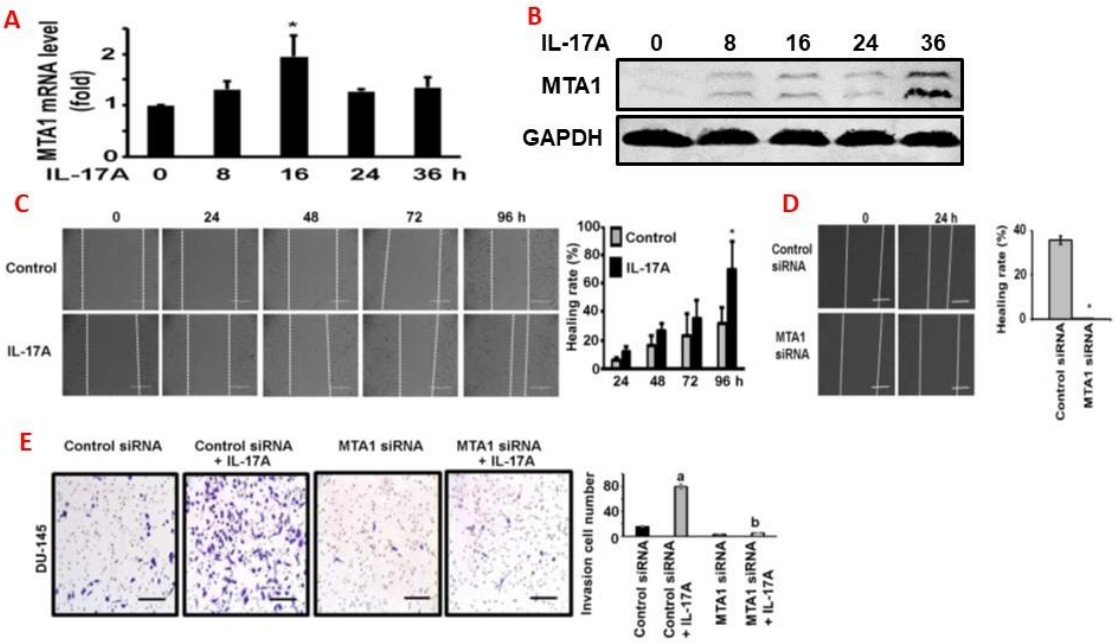

Fig.1. A. IL-17A (also named IL-17) induced MTA1 mRNA expression. B. IL-17A induced MTA1 protein expression. C. IL-17A induced DU-145 cell migration. D. MTA1 siRNA blocked IL-17A-induced DU-145 cell migration. E. MTA1 siRNA blocked IL-17A-induced DU-145 cell invasion.

Conclusions: These findings demonstrate that IL-17 upregulates MTA1 mRNA and protein expression to promote DU-145 and HeLa cell migration and invasion.

A novel model of prostate cancer suggests enzalutamide functions through the immune system to diminish castration resistant and metastatic growth

Steven Kregel, PhD, University of Michigan

Jae Eun Choi, Kristen Juckette, Brooke Mccollum, Stephanie Simko, Parth Desai, Yuanyuan Qiao PhD, Paul A. Volden PhD, and Arul M. Chinnaiyan MD, PhD

Background: There is tremendous need for improved prostate cancer (PCa) models. The murine prostate is anatomically and developmentally different from the human prostate, and does not form sporadic tumors. Furthermore, engineered mouse models lack the heterogeneity of human disease, are often driven in a contrived manner, and rarely (if ever) establish metastatic growth. Human xenografts represent an alternative, but they rely on tumor growth in an immunocompromised host, preventing the study of tumor-immune interactions and immunotherapies. Accordingly, we generated PCa xenograft models in a murine system with an intact human immune system to test the hypothesis that humanizing tumor-immune interactions would improve modeling of metastatic PCa, and further-enable improved modeling of hormonal and immunotherapies.

Methods: Male huNOG mice were produced at Taconic Biosciences by engrafted juvenile NOG mice with human CD34+ hematopoietic stem cells. These mice stably maintain multiple human cell linages, including functional human T-cells. We utilized two human PCa xenograft cell line models transduced with luciferase to assay organ-specific metastatic growth. First, castrated and intact control mice were injected subcutaneously with 22Rv1 cells. When tumors reached >100mm^3, half of the castrated mice were treated with enzalutamide (enza), then tumor growth was monitored to endpoint. Additionally, VCaP tumor–bearing mice were castrated when the tumors were ~200 mm^3 in size, and once the tumor grew back, they were randomized and treated with enza and/or the anti-PD-1 antibody pembrolizumab (pembro), with vehicle controls. At sacrifice, organs were *ex-vivo* analyzed for metastatic growth, tumor infiltrating lymphocytes, and splenic immune reconstitution.

Results: With 22Rv1, subcutaneous tumor size was not significantly altered across conditions; however, the extent and growth at the secondary sites differed markedly in castrate huNOG vs conventional NOG mice treated with enza. VCaP xenograft tumors showed marked decreases in growth with enza and pembro treatments in huNOG mice, and no effect was seen for either of these treatments in NOG mice. Furthermore, enza responses in huNOG and NOG mice were distinct, and associated with increased CD3+ T-cells within tumors of enza treated huNOG mice, and increased CD3+ T-cell activation, accessed by intracellular interferon-γ.

Conclusions: These results illustrate, to the best of our knowledge, the first model of human PCa that metastasizes to clinically relevant locations, has intact human immune system, and responds appropriately to standard-of-care hormonal therapies.

Androgen Receptor Degraders Overcome Common Resistance Mechanisms Developed During Prostate Cancer Treatment

Steven Kregel, PhD, University of Michigan

Chao Wang, PhD, Xin Han, PhD, Lanbo Xiao, PhD, Ester Fernandez-Salas, PhD, Pushpinder Bawa, PhD, Brooke L. McCollum, Kari Wilder-Romans, BS, Xuhong Cao, MS, Corey Speers, MD/PhD, Shaomeng Wang, PhD, and Arul M. Chinnaiyan, MD, PhD

Background: Androgen receptor (AR) signaling is critical for prostate development and homeostasis as well as the initiation and progression of prostate cancer, including in the castration and enzalutamide resistant state. Indeed, the clinical development of second-generation androgen receptor (AR) antagonists, including enzalutamide, has confirmed that the AR remains a key oncogene in castration-resistant prostate cancer (CRPC). Furthermore, response to enzalutamide is temporary and incremental, and prostate cancer (PCa) cells that develop resistance to AR-targeted therapy usually maintain AR expression. Therefore, with the hypothesis that AR protein is still active even during castration- and enzalutamide-resistant states, we employed the proteolysis targeting chimera (PROTAC) strategy to build compounds that targeted the AR through proteasomal degradation. We developed an optimized functional molecule, ARD-61b, which targets enzalutamide-resistant and AR splice variant (including AR-V7 that lacks the ligand binding domain) overexpressing cells *in vitro* and *in vivo*.

Methods: Western Blotting, qRT-PCR, Growth Assays, Xenograft studies, Proteomic and RNA-sequencing Genomic analyses, CRISPR-CAS9 mediated stable AR-V7 overexpression in LNCaP cells.

Results: ARD-61b functions to rapidly and specifically degrade AR-protein, and similarly decreases downstream AR-signaling in a wide variety of AR-positive cell lines and tumor xenografts, both enzalutamide sensitive, AR-mutated, and enzalutamide resistant. Enzalutamide sensitive and resistant cell lines, including those that overexpress high levels of AR-V7, are all similarly sensitive to ARD-61b, *in vivo* and *in vitro*. Endogenous overexpression of AR-V7 has no effect on the sensitivity of cells to ARD-61b.

Conclusions: Together, these data show the necessity for full-length AR in all stages of prostate cancer. Enzalutamide resistant cell lines require full length AR for sustained survival, despite continued AR-antagonization, and AR-variant overexpression, shown previously to promote resistance to AR-targeted therapies, still require AR protein with an intact ligand-binding domain to maintain growth in our models. These data presented illustrate the importance of continued targeting of AR in advanced prostate cancer, and the efficacy of the potent AR-PROTAC, ARD-61b. Ultimately, through its clinical translation, we anticipate the development of ARD-61b to be a therapeutic advance for patients with the metastatic CRPC.

Trop2 as a Driver and Therapeutic Target for Metastatic Castration-Resistant Prostate Cancer with Neuroendocrine Phenotype

Tanya Stoyanova, PhD, Stanford University

En-Chi Hsu, Meghan A. Rice, Abel Bermudez, Fernando Jose Garcia Marques, Merve Aslan, Ali Ghoochani, Chiyuan Amy Zhang, Yun-Sheng Chen, Aimen Zlitni, Frezghi Habte, Sahil Kumar, Shiqin Liu, Kashyap Koul, Michelle Shen, Rosalie Nolley, Donna M. Peehl, Amina Zoubeidi, Sanjiv Sam Gambhir, Christian Kunder, Sharon Pitteri, James D. Brooks and Tanya Stoyanova

Trop2 as a Novel Driver and Therapeutic Target for Metastatic Castration-Resistant Prostate Cancer with Neuroendocrine Phenotype

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Background:
Advanced prostate cancer, whether present at the time of diagnosis or arising after treatment of localized disease, responds to androgen deprivation, but invariably fails and recurs as castration resistant prostate cancer (CRPC). Over the last 15 years, new therapies for CRPC have prolonged the duration of survival. However, CRPC remains the cause of prostate cancer associated mortality. Heavily treated tumors, particularly those treated with secondary hormone therapies, frequently acquire a neuroendocrine phenotype (NEPC), which currently accounts for 10-20% of CRPC. NEPC is commonly characterized by downregulation or loss of androgen receptor (AR) and is thus not responsive to androgen deprivation therapies, expression of neuroendocrine markers, and an aggressive clinical course, making it the most lethal and currently untreatable subset of prostate cancer.

Methods:
We used tissue microarrays to assess the correlation of Trop2 levels with clinical outcomes. CRISPR/Cas9 technology and lentiviral infection were used to achieve Trop2 gene deletion and Trop2 overexpression in prostate cancer cells. To evaluate the role of Trop2 in prostate tumorigenesis, we utilized in vitro functional assays such as colony formation, tumorsphere formation, and cell migration assays including transwell and wound healing assays. In vivo tumor growth was assessed by subcutaneous xenograft tumor model. In vivo metastasis was addressed by intracardiac injection and subcutaneous xenograft tumor models. Whole proteomic profiling was utilized to identify Trop2 downstream targets and mediators. To target Trop2, we utilized antibody-based strategies.

Results:
Tumor-associated calcium signal transducer 2 (Trop2) is a cell surface glycoprotein overexpressed in multiple epithelial cancers. Here, we identify that Trop2 is significantly elevated in CRPC and NEPC and represents a novel driver of metastatic CRPC with neuroendocrine features. Trop2 overexpression increases tumor growth and drives prostate cancer metastasis to diverse organs

including bone and liver. Furthermore, inhibition of Trop2 or deletion of the *TROP-2* gene significantly slows prostate cancer growth and metastasis of prostate cancer cells.

Conclusions:
Our findings establish Trop2 as a novel driver and therapeutic target for metastatic CRPC with neuroendocrine phenotype and suggest that inhibition of Trop2 may represent a new therapeutic strategy for CRPC and NEPC.

3D Renal Organoids of Human Urinary Stem Cells for Nephrotoxicity Testing

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3D Renal Organoids of Human Urinary Stem Cells for Nephrotoxicity Testing

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Background: Despite recent achievements, development of in vitro renal tissue model has been limited due to the challenges in differentiating iPSC/ESC into functional renal cells. We demonstrated that human urine-derived stem cells (USC), originating from glomerular parietal stem cells, possess self-renewal and multipotent capacity. The goal of this study is to induce human USC to differentiate into renal tubule epithelial cells when grown on kidney extracellular matrix (k-ECM) to generate in vitro 3D renal tubular organoids for potential use as biological models to evaluate drug-induced nephrotoxicity.

Methods: USC obtained from 4 healthy individuals were isolated and expanded in vitro. Normal human renal cells were used as a control. Porcine-derived k-ECM hydrogel was produced when k-ECM was mixed with hydraulic acid gel (1:9). To generate the renal tubular organoids, USC were induced within 1% k-ECM gel for one week, compared to USC aggregates with k-ECM-free gel. The organoids of USC+k-ECM gel were cultured in Hanging Drop 96-well plates for 4 days and then transferred to ultra-low attachment plates for 7 days. To optimize the protocols and measure cell viability, different cell concentrations were assessed by ATPase and LIVE/DEAD Kit. The size of organoids and the renal tubule structures were assessed by histology, immunofluorescence staining and Western blot using renal tubular epithelial cell markers. Renal tubular cell function and injury were measured after Ethanol and Cisplatin as nephrotoxic agents were added into the culture medium.

Results: In vitro 3D organoids (about 350μm at diameter) were generated by USC ($4x10^3$) cultured for 7 days. ATPase and LIVE/DEAD assays showed that majority of USC (>95%) remained viable up to 4 weeks. Histologically, renal tubular-like structures were formed within the induced USC, but less or no within the USC aggregates without k-ECM gel. The induced-USC expressed proximal renal tubule epithelial cells marker (Aquaporin 1) within whole mount staining. In addition, induced USC expressing γ-Glutamyltransferase activity was similar to normal renal cells after ethanol treatment. In nephrotoxic testing, in vitro 3D organoids in which renal tubules expressed significantly higher levels of kidney injury molecular-1 and cytochrome P450 2E1 in response to cisplatin, compared to those in acetone or non-treated groups.

Conclusions: Our date demonstrated that in vitro 3D human renal tubular organoids were generated from kidney ECM-induced USC. Human USC-generated organoids responded well to nephrotoxic agent exposures, which could potentially be used as personalized testing platforms for nephrotoxicity.

Impaired Regeneration Potential of Urine-derived Stem Cells in Chronic Kidney Disease

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Background: Stem cells present in urine possess regenerative capacity to repair kidney injury. However, the unique characteristics of urinary stem cells (USC) from patients with chronic kidney disease (CKD) i.e. diabetic nephropathy (d-USC) are unknown. The goal of this study was to investigate stemness properties in cell phenotype and regenerative potential of d-USC, compared to USC from healthy individuals.

Methods: Thirty-six urine samples collected from patients (n=12, age range 60–75 years) with diabetic nephropathy (Stage 3-4 CKD) were compared with 30 urine samples from healthy age-matched donors (n=10).

Results: There were approximately six times as many cells in urine samples from patients with diabetic nephropathy, including twice as many USC clones as healthy donors. However, approximately 70% of d-USC were significantly weaker in regenerative capacity as assessed by cell proliferation, less secretion of paracrine factors, weaker telomerase activity, and lower renal tubular epithelial differentiation potential compared to healthy controls. In addition, the levels of inflammatory factors (IL-1β and Cx43) and apoptotic markers (Caspase-3, and TUNEL) were significantly increased in d-USC compared to USC ($p<0.01$). Protein levels of autophagy marker (LC3-II) and mTOR signaling molecules (p-mTOR/mTOR, p-Raptor/Raptor and p-S6K1) were significantly lower in patient with diabetic nephropathy ($p<0.01$). Nevertheless, up to 30% of d-USC possessed similar regenerative capacity as USC from healthy donors.

Conclusions: Regenerative performance of most d-USC was significantly lower than normal controls. Understanding the specific changes in d-USC regeneration capability will help elucidate the pathobiology of diabetic nephropathy and lead to prevent USC from diabetic insults, recover the stemness function and also identify novel biomarkers to predict progression of this chronic kidney disease.

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Key Words: chronic kidney disease, diabetic nephropathy; urine; stem cells; renal insufficiency

Prostatic fibrosis is coupled to a mesenchymal IL4/IL13 autocrine loop

2019 Travel Award Winner

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Prostatic fibrosis is coupled to a mesenchymal IL4/IL13 autocrine loop

Background

Prostate fibrosis is a major cause of lower urinary tract dysfunction (LUTD), a condition characterized by nocturia and incomplete bladder voiding. Prostate biopsies from men experiencing significant LUTD symptoms had increased levels of CD4+ T cells expressing interleukin (IL) 4 and IL13. IL4 and IL13 dysregulation has been involved in other fibrotic diseases, therefore, we hypothesized that the IL4/IL13 axis may be coupled to the development of prostate fibrosis and consequent LUTD.

Methods

Prostate fibroblasts were treated with 20 ng/mL IL4 or IL13 for up to 72 h, or with 4 ng/mL TGFβ1 as a positive control. Markers of myofibroblast phenoconversion and signaling pathways activated in response to IL4 and IL13 were investigated using Western Blot, qPCR and ELISA. IL4 and IL13 receptors levels in human prostate tissues were assessed by immunohistochemistry.

Results

Immunohistochemistry of the peri-urethral prostate tissue from patients with moderate/severe LUTD had significantly elevated IL4 and IL13 receptors levels. *In vitro* treatment of prostate fibroblasts with IL4 or IL13 for 72 h led to upregulation of collagen 1, but not α-smooth muscle actin, while TGFβ1 treatment led to upregulation of both. IL4 or IL13, but not TGF-β1, induced a strong activation of the Signal Transducer and Activation of Transcription 3 (STAT3) and STAT6 transcription factors, which have been implicated in fibrotic diseases. Because IL4 and IL13 promoters contain STAT binding domains, the IL4 or IL13 levels were measured by ELISA after 24 h of IL4 or IL13 treatment: IL4 selectively auto-upregulated, while IL13 upregulated IL4 and IL13, indicative of a positive autocrine loop. Western blot data also indicated that STAT6 remained activated for at least 72 h after initial exposure to IL4 or IL13.

Conclusions

IL4 and IL13 receptor levels are elevated in peri-urethral tissue from patients with moderate/severe LUTD concurrent with high levels of collagen expression. IL4/IL13 induces collagen expression in prostate fibroblasts consistent with tissue fibrosis, which has been associated with tissue stiffness and reduced urethral compliance contributing to LUTD. Moreover, intracellular signaling mediated by IL4 and IL13 was coupled with STAT3 and STAT6 activation, and appeared independent of TGFβ-mediated signaling pathways in prostate fibroblasts. In sum, these data suggest that exposure to CD4+ T-cells may “kick-start” an autocrine loop that promotes IL4/IL13 auto-secretion by prostate fibroblasts and initiates and maintains peri-urethral prostatic tissue fibrosis.

Targeting P2X4 Purinergic Receptors in Aggressive Prostate Cancer

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Targeting P2X4 Purinergic Receptors in Aggressive Prostate Cancer

Background: Chronic inflammation is associated with prostate cancer (PCa) aggressiveness. Cell injury or apoptosis result in ATP release, which activates P2 purinergic receptors driving inflammation, cell proliferation, and migration. Higher ATP concentrations are measured in mouse tumor microenvironments compared to healthy tissues, and P2 receptors are overexpressed in some cancer tissues. Multiple public datasets show that the P2 receptor P2X4 is upregulated in prostate cancer compared to benign tissues.

Methods: P2 receptor expression was assessed in PCa tissues by RNA-sequencing (RNA-seq) analysis. P2X4 receptor cellular localization was evaluated using RNA *in situ* hybridization (RISH) or immunohistochemistry (IHC) on formalin-fixed paraffin-embedded PCa tissues with a range of Gleason scores. RISH was also performed on tissue microarray (TMA) sets containing primary PCa tissues obtained by radical prostatectomy or distant metastatic tissues obtained by autopsy. Dual IHC analysis was used to assess P2X4 expression on immune cells. MTT assays, transwell migration/invasion assays, and western blotting were used to assess the effects of P2X4 receptor activation or blockade on PCa cells.

Results: We observed P2X4 protein expression primarily in epithelial cells of the prostate, and identified P2X4 positive immune cells. Corroborating our RNA-seq data, RISH analysis of our primary tumor TMA showed significantly elevated P2X4 expression in cancer compared to benign spots ($p < 0.0001$) and there was higher P2X4 expression among higher grade cases in both benign and tumor spots. Interestingly, P2X4 was also upregulated in prostatic intraepithelial neoplasia. P2X4 expression was elevated in tumors with Erg positivity and PTEN loss. Considerable P2X4 mRNA expression was detected in multiple metastatic tissue sites. We observed P2X4 expression on some $CD66^+$ neutrophils and most $CD68^+$ macrophages in the prostate. There was a positive correlation between CD68 and P2X4 mRNA expression in both primary and metastatic tumors. The ATP- and P2X4-specific agonist CTP increased transwell migration and invasion of PC3, CWR22Rv1 and E006AA cells. P2X4 antagonist 5-BDBD resulted in a dose-dependent decrease in cell viability in PC3, LNCaP, CWR22Rv1, TRAMP-C2, Myc-CaP, BMPC1, and BMPC2 cells. Further, 5-BDBD treatment or siRNA knockdown of P2X4 increased cleaved caspase-3 protein expression in PC3 cells.

Conclusions: Multiple P2 receptor antagonists are in clinical trials or are FDA approved for treatment of other diseases. Deciphering a role for P2X4 in PCa may unveil a subset of patients for which targeted therapies are successful.

Expansion of Luminal Progenitor Cells in the Aging Mouse and Human Prostate

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TITLE: Expansion of Luminal Progenitor Cells in the Aging Mouse and Human Prostate

BACKGROUND: Aging represents a significant risk factor for growth-related prostate diseases including benign prostatic hyperplasia (BPH) and prostate cancer (PrCa), yet the effects of aging on prostate epithelial cells remain poorly understood. We sought to characterize age-related changes to prostate epithelial cells isolated from mouse and human prostate.

METHODS: We compared prostate epithelial cells isolated from 3-month-old and 24-month-old C57BL/6 mice. Bulk RNA sequencing was performed to define changes in gene expression. Protein level validation of select gene expression changes was performed using flow cytometry, immunohistochemistry, and Western blot. Functional assays, including the prostate organoid assay, were used to measure changes in *ex vivo* progenitor activity. Human prostate tissues from young healthy men and older men with BPH were utilized to determine if age-related changes observed in the mouse prostate are conserved in the human prostate.

RESULTS: We found that the organoid-forming capacity of basal and luminal cells is maintained with age. This is caused by an age-related expansion of progenitor-enriched luminal cells, identified by expression of Trop2, that exhibit a greater organoid-forming capacity and form larger organoids. Trop2+ luminal cells in the mouse prostate share features with human prostate luminal progenitor cells, including high expression of PSCA. Importantly, we demonstrate an expansion of PSCA+ luminal progenitor cells in aging human prostate and BPH.

CONCLUSIONS: Our study helps explain why aging is associated with increased risk of growth-related prostate disease. Our findings have clinical implications, as they suggest that preventing age-associated luminal progenitor cell expansion may reduce the incidence of BPH and PrCa.

Methylation of SRD5A2 promoter predicts a better outcome for castration-resistant prostate cancer patients undergoing androgen deprivation therapy

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Methylation of *SRD5A2* promoter predicts a better outcome for castration-resistant prostate cancer patients undergoing androgen deprivation therapy

Background: Steroid 5-alpha reductase (SRD5A2) is a critical enzyme for prostatic development and growth. Our objective is to demonstrate if the *SRD5A2* promoter methylation is associated with cancer progression during androgen deprivation therapy (ADT) in castration-resistant prostate cancer (CRPC).

Materials & Methods: 58 CRPC samples and 23 benign prostatic specimens were used for testing. The methylation status of CpG site(s) at *SRD5A2* promoter regions was tested via Targeted Next-Gen Bisulfite Sequencing (tNGBS), and the methylation level was calculated. Data was retrieved on primary and secondary ADT treatment response. Overall survival (OS) was calculated from time of diagnosis to time of death. The protein expression of SRD5A2 was determined with immunohistochemistry (IHC) in 42 CRPC samples. The intensity of positive-staining was determined by integrated optical density (IOD) using Image-Pro Plus 6.0, and then correlated to *SRD5A2* methylation status.

Results: Compared with benign prostatic tissue, CRPC samples demonstrated higher *SRD5A2* methylation in the whole promoter region (MGH samples: $P < 0.0001$; samples from NCT01393730: $P = 0.002$). Higher ratio of methylation was correlated with better OS ($R^2 = 0.11$, $P = 0.032$). Hypermethylation of specific regions (nucleotides -434 to -4 (CpG#: -39 to CpG#: -2)) was associated with a better OS (11.3±5.8 vs 6.4±4.4 years, $P = 0.001$) and progression-free survival (PFS, 8.4±5.4 vs 4.5±3.9 years, $P = 0.005$) with cutoff value of 37.9%. Multivariate analysis showed that *SRD5A2* methylation was associated with OS independently (whole promoter region: $P = 0.035$; specific region: $P = 0.02$). The protein expression of *SRD5A2* in 42 CRPC samples was negatively correlated with the ratio of *SRD5A2* methylation both in the whole promoter region ($R^2 = 0.235$, $P = 0.0011$) and in the specific region (CpG#: -39 to CpG#: -2) ($R^2 = 0.287$, $P = 0.0003$).

Conclusion: Our study demonstrates that *SRD5A2* hypermethylation in specific promoter regions of *SRD5A2*, a condition that favors estrogenic as opposed to an androgenic milieu in the prostate, is significantly associated with better survival in CRPC patients who are treated with ADT. We show that a well-defined subset of prostate cancers with *SRD5A2* methylation, specifically at CpG#: -39 to CpG#: -2, predicts better outcomes. Recognition of epigenetic modifications of *SRD5A2*, which affects the prostatic hormonal environment, may affect the choices and sequence of available therapies for management of CRPC.

Obesity-associated inflammation induces androgenic to estrogenic switch in the prostate gland

Zongwei Wang, PhD, Harvard University

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Obesity-associated inflammation induces androgenic to estrogenic switch in the prostate gland

Background: The steroid 5-α reductase type 2 (SRD5A2) is critical for prostatic development and growth. Strategies to block SRD5A2 using 5-alpha reductase inhibitors (5ARI) remain a mainstay in the treatment of benign prostatic hyperplasia (BPH). However, one-third of men are resistant to 5ARI therapies. We previously have found that body mass index (BMI) can predict increased SRD5A2 promoter methylation level and decreased protein expression level. We have demonstrated that there is an “androgenic to estrogenic switch” when SRD5A2 is absent in the prostate gland. Here we wished to identify whether obesity-associated inflammation contributes to the androgenic to estrogenic switch in human prostate tissue.

METHODS: Human prostate tissue samples came from men undergoing transurethral prostate resection. Obese mice was induced by high fat diet. Primary prostatic stromal cells were isolated from patients undergoing prostate reduction therapy for BPH. Adipocytes and macrophages were differentiated and treated with saturated fatty acids (SFA) to induce inflammation, and SFA-free conditioned media were collected for stromal cell culturing. Protein, genome DNA and mRNA were extracted, *SRD5A2* promoter methylation was tested with MethylCollector kit. The expression of aromatase, estrogen receptor alpha (ERα), and SRD5A2 was determined by Western Blot, ELISA and qPCR.

RESULTS: Obesity induced *SRD5A2* promoter hypermethylation and SRD5A2 low expression in prostate both in human and mice. The levels of inflammatory mediators were elevated in prostatic tissues with obesity. In human prostatic stromal cells inflammatory mediators regulated SRD5A2 promoter methylation and expression, inflammatory mediators and saturated fatty acid synergistically regulate aromatase activity. Obesity promoted an androgenic to estrogenic switch in prostate. Androgen and estrogen levels are modified in obese human prostate tissue upon the treatment with 5-α reductase inhibitors (5ARIs).

CONCLUSIONS: Our study demonstrates for the first time that there is an androgenic to estrogenic switch, which can improve SRD5A2 methylation level and reduce SRD5A2 expression in the prostate glands of obese patients. Associated with body weight, somatic epigenetic silencing of SRD5A2 changes the prostatic hormonal milieu, and may modulate prostatic homeostasis and growth. Targeting the estrogenic signaling may serve as an effective treatment strategy in subset of overweight BPH patients.

Development of CRISPR human Skp2 knock-in in the prostate of mouse and the associated prostate organoids for testing Skp2 targeting agents

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Development of CRISPR human Skp2 knock-in in the prostate of mouse and the associated prostate organoids for testing Skp2 targeting agents

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Background:

S-phase kinase associated protein 2 (Skp2) is a promising drug target as studies have demonstrated that Skp2 is required for spontaneous tumor development that occurs in the retinoblastoma protein (pRb), Pten or p53 deficient mice. We therefore have aimed to establish CRISPR human Skp2 (hSkp2) knock-in in the prostate of mouse to examine its role in prostate carcinogenesis. In addition, Skp2 overexpressing organoids are being developed for convenient and efficient screening of specific Skp2 inhibitors.

Methods:

cDNA of hSkp2 and porcine teschovirus-1 (P2A) was introduced into immediate upstream of mouse probasin coding sequencing using CRISPR knock-in targeting approach to establish transgenic mice specifically overexpressing hSkp2 in the prostate glands. Quantitative PCR, western blot and immunohistochemistry (IHC) were used to identify the expression levels of Skp2 in mice prostate. Histopathological analyses were performed after H&E staining. Prostate glands were dissected and then digested for organoid culture. Morphological features and viability of organoids were evaluated following treatments of potential Skp2 inhibitors.

Results:

Prostate glands and other tissues from mice of three transgenic lines which were genotyped positive were dissected and hSkp2 was detected in ventral, dorsal and lateral prostate lobes, but not in testis, kidney, spleen and liver. Line 10092 was found to have the highest expression of hSkp2 among these three lines. Prostate weight slightly increased in transgenic mice compared to that of wild-type control mice. Hyperplasia, prostate intraepithelia neoplasia (PIN), and carcinoma were noted, which suggested that overexpression of hSkp2 in the prostate of mice promoted proliferation and prostate tumorigenesis. Organoids that were derived from the prostate of hSkp2 transgenic mice recapitulates mouse phenotypes and maintained high expression of hSkp2. A novel Flavokawain A derivative was found to selectively alter the morphology of hSkp2 organoids and decrease the viability.

Conclusions:

Histopathological changes in the prostate of hSkp2 knock-in mice demonstrated a potential role of hSkp2 in early prostate carcinogenesis. Prostate organoids overexpressing hSkp2 has its utility for screening out compounds for targeting Skp2 in prostate cancer.

Lymphocyte Mediated Luminal Progenitor Cell Expansion in the Aging Prostate

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Lymphocyte Mediated Luminal Progenitor Cell Expansion in the Aging Prostate

Background:

Prostate Cancer(PCa) is the second most commonly diagnosed cancer and second deadliest in cancer-related deaths among U.S. men. Notably, PCa is one of the most common **age-associated diseases in men**. 91.3% of PCa cases are diagnosed in men older than 55 years old. However, how age contributes to disease initiation is poorly understood. The prostate is a glandular structure composed of a tubular network made up of stromal cells surrounding a basal layer and luminal cells. There is growing evidence that luminal progenitor cells may act as cells of origin for PCa. We have found age-associated expansion of Trop2+ luminal progenitor cells coinciding with a dramatic increase in inflammation. Given this, further understanding of luminal progenitor cell biology and factors that may contribute to their expansion and cancer initiation may lead to therapeutic or preventative strategies against PCa. Currently, the direct relationships between inflammation, luminal progenitor cell expansion and aging have yet to be deeply studied. We hypothesize that chronic inflammation contributes to the age-associated expansion of Trop2+ luminal cells.

Methods:

We will utilize flow cytometry to define proportional changes in epithelial and non-epithelial compartments in the prostate during aging. We will also use immune cell depletion methods to reveal whether age-associated prostate inflammation is involved in the expansion of luminal progenitor cells.

Results:

We and others have reported that aging is a driver of inflammation in the prostate. This inflammation can be characterized by an increase in the proportion of CD45+ immune cells in the prostate and an increase in the proportion of lymphocytes that make up the immune population. Our data has shown that Trop2+ luminal progenitor cells in aged mice have an enriched inflammatory/immune response signature and their proportion is correlated with an increase in the proportion of lymphocytes in the aging prostate. While an age-associated increase in inflammation was conserved among a variety of adult tissues, the increase in the proportion of T cells and B cells was not observed in all aging tissues. This inflammatory phenotype was observed as early as 6 months of age and occurred concurrently with the increase in Trop2+ luminal cells. Immune cell depletion studies are currently underway.

Conclusions:

Given that Trop2+ luminal progenitor cells are at risk for developing into PCa, understanding if lymphocytes play a role in their expansion may lead to new strategies to prevent or treat PCa initiation.

The Role of UDP-glucuronosyl transferase 2B28 in Prost Cancer

2019 Travel Award Winner

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The Role of UDP-Glucuronosyl Transferase 2B28 in Prostate Cancer

Introduction

Current treatment regimens for prostate cancer (PCa) revolve around androgen ablation which is limited by treatment resistance. Patients with castrate resistant PCa (CRPC) have a median survival of 2 years. Recent studies performed in our laboratory have shown rewired metabolism in advanced prostate cancers centered on elevated levels of the UDP-glucuronosyl transferase (UGT) pathway which glucuronidates androgens and promotes their clearance. UGT2B15 and 17, the only characterized isoforms of the UGT family of enzymes, are known to be regulated by androgen receptor (AR) signaling and their expression levels are correlated with a castration resistant state. One of the most highly upregulated targets in CRPC however is an unstudied isoform of the UGT family, namely UGT2B28. The objective of my project is to understand the role of UGT2B28 in PCa.

Methods

In order to bridge this knowledge gap, we used RNA interference to knock-down (KD) UGT2B28 in androgen-dependent (AD) and CRPC cell lines such as LNCaP and C42 respectively. We studied 3D organoid formation, testosterone levels, tumor growth *in vivo*, and associated signaling mechanisms in response to downregulation of UGT2B28. Additionally, we performed chromatin immunoprecipitation-sequencing (ChIP-seq) to study UGT2B28 regulation by AR. We treated UGT2B28 KD LNCaP cells with synthetic androgen, R1881 and AR-antagonist, enzalutamide to study the effect of UGT2B28 on AR signaling and its mechanism of action in the context of PCa.

Results

Our studies suggest that UGT2B28 KD impairs organoid formation in LNCaP cells, markedly reduces tumor growth in subcutaneous LNCaP xenografts in athymic nude mice, and affects cell adhesion and mitochondrial bioenergetics. ChIP-seq of the UGT2B28 promoter region has revealed binding sites for AR and ARv7 (an AR splice variant that is active in CRPC) suggesting AR regulation of UGT2B28. We have also demonstrated that UGT2B28 reciprocally regulates AR signaling.

Conclusions

These studies provide an understanding of a previously unexplored, highly altered target of rewired PCa metabolism and uncover a novel role for a UGT family member in cell adhesion and mitochondrial bioenergetics. We intend to use this collective information to design novel metabolic markers for non-invasive PCa screening in patient urine and metabolite-based therapeutic strategies for CRPC.

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Nuclear factor I/B increases in prostate cancer to support androgen receptor activation

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SBUR 2019 RESEARCH ABSTRACT

Nuclear factor I/B increases in prostate cancer to support androgen receptor activation

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Background: Our laboratory research is focused on identifying transcription factors that interact with and modify AR and AR-V function. We have previously shown that transcription factor nuclear factor I/B (NFIB) regulates AR activity and is frequently associated with genomic AR and FOXA1 binding sites in an androgen dependent prostate cancer cell line (LNCaP). However, the status of NFIB in prostate cancer and whether it interacts with androgen receptor (AR) and AR splice variants (AR-V) to regulate AR activity was unknown.

Methods: To determine status of NFIB in prostate cancer, we immunostained a tissue microarray for NFIB, AR, and synaptophysin (a marker of neuroendocrine differentiation). The tissue microarray included normal, hyperplastic, prostatic intraepithelial neoplasia, primary prostatic adenocarcinoma, and castration-resistant prostate cancer tissue samples. We analyzed NFIB expression in nuclear and cytoplasmic fractions of prostate cancer cell lines. We used co-immunoprecipitation studies to determine interaction of NFIB with AR and ARVs. We analyzed the effect of NFIB over-expression in LNCaP and C4-2B cells on AR activity via luciferase assays and for any changes in cytokine proteome.

Results: In tissue microarray analysis, we observed NFIB increased in the nucleus and cytoplasm of prostate cancer samples. NFIB increase was independent of Gleason score but positively correlated with AR expression. Based on staining analysis and its adjustment to tumor margin status, a cytoplasmic-to-nuclear NFIB staining ratio could be used to predict earlier biochemical recurrence in prostate cancer. We observed nuclear extracts of prostate cancer lines are predominantly enriched in NFIB whereas cytoplasmic NFIB was increased only in castration-resistant cell lines. We observed NFIB interacts with AR and AR-Vs through co-immunoprecipitation experiments. We observed stable over-expression of 3X-FLAG-NFIB in LNCaP and C4-2B cells did not increase AR expression, but it did increase prostate specific antigen (PSA) production and PSA promoter activity. Over-expression of NFIB was also associated with changed cytokine profile.

Conclusion: These results show the expression pattern of NFIB in prostate cancer and that, NFIB over-expression in AR-dependent prostate cancers leads to increased AR activity, enabling future studies to determine how NFIB promotes tumorigenesis and castration resistance *in vivo.*

Macrophage recruitment, activation and neural cross-talk are associated with development and maintenance of chronic pelvic pain

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Macrophage recruitment, activation and neural cross-talk are associated with development and maintenance of chronic pelvic pain.

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Introduction-- Chronic pelvic pain syndrome (CPPS), the most common form of prostatitis, is a complex multi-symptom syndrome with unknown etiology. Previous reports suggest that inflammatory mediators such as mast cells, T-cells and macrophages contribute to the development of pain responses in CPPS. Using the experimental autoimmune prostatitis (EAP) mouse model of CPPS we demonstrate here that recruitment and activation of macrophages in the spinal cord of affected animals plays a significant role in pain responses. We also show that direct interaction between macrophages and afferent neurons at the level of the prostate may serve to induce development of chronic pain.

Methods—EAP was induced by subcutaneous injection of rat prostate antigen and adjuvant, as previously described (1). Pain responses were assessed by tactile allodynia using Von Frey filament behavioral testing, every 7 days. Animals were sacrificed at day 14 and day 28 following injection and tissues including the prostate and spinal cord at both lumber and sacral levels were collected. Tissues were analyzed by multi-color confocal microscopy using antibodies against activated macrophages and neural markers. Statistical analyses were performed using Microsoft Excel and GraphPad Prism software and imaging analyses in ImageJ.

Results— Examination of prostate tissue at both time-points revealed a striking increase in macrophage recruitment to activated neurons specifically PGP9.5-positive afferent fibers. Increased association of these cell types was also associated with EAP-induced extension of macrophage processes along nerve fibers that was markedly distinct from staining patterns in control animals. To delineate the details of peripheral monocytic recruitment to the spinal cord and their role in the development of EAP-induced hyperalgesia we first determined the level of macrophage recruitment into the spinal cord. Our data demonstrate significant increases in $IBA1^{+}/CD45^{high}$ cells (macrophages) in the L_4-S_2 dorsal horn of EAP mice compared to control counterparts. We further demonstrate that these infiltrating macrophages have increased direct interaction with tissue resident $IBA1^{+}/CD45^{low}$ microglia. These phenomena were only observed at day 28 and not at day 14 suggesting their importance in maintenance of chronic pain.

Conclusions—Taken together these data demonstrate the potential role for macrophage and neural interaction in both induction and maintenance of pain responses associated with CPPS.

Epigenetic modifications drive autoimmunity in CPPS Stephen F Murphy, Ph.D., Department of Urology, Feinberg School of Medicine, Northwestern University *Dr. Anthony J. Schaeffer, MD, Dr. Praveen Thumbikat, DVM,PhD.*

Introduction:
Chronic pelvic pain syndrome (CPPS) is a multi-symptom syndrome with unknown etiology and thus limited effective treatments. To address the need for a better understanding of the immunological responses that may underlie the condition our laboratory performed immune profiling of CPPS patient urine and peripheral blood samples. We have previously reported that the pro-inflammatory T-cell maturation and activation cytokine IL7 is increased in expressed prostatic secretions (EPS) and positively correlated with patient reported pain, urinary and quality of life responses. Extending these findings we hypothesized that CPPS emerges due at least in part to underlying defective T-cell adaptive immune responses that facilitate development of autoimmunity.

Methods:
RNA was extracted from voided bladder 3 (VB3) urine samples collected from 22 CPPS patients and 8 healthy controls. Following cDNA synthesis QRTPCR was performed for CD4 and transcripts associated with T-cell subsets including; Th1 (IFNg and Tbet), Th2 (GATA3), Th17 (RORgT and Th17) and T-reg (FoxP3). Analysis was performed to compare the average of all healthy controls against each CPPS patient individually. Following this we isolated PBMCs from 10 CPPS patients and 10 healthy controls and performed a methylation array of 32 gene regions from 23 specific genes involved in FoxP3 expression and function; including, IL2/4/10, PDL1/2, CTLA4, MYC, ITGAL and FoxP3. Finally we examined the ability of these cells to produce IL10 in response to stimulus.

Results:
QRTPCR profiling demonstrated a decrease in T-reg associated transcripts and increased expression of Th17 associated transcripts in CPPS patients compared to controls. These data indicate an imbalanced immune response characteristic of an autoimmune phenotype. Our methylation array results also signify a loss of T-cell regulation in the periphery of CPPS patients. Here we demonstrate hyper-methylation of the major functional cytokine of T-reg activity, IL10 and hypo-methylation of the promoter of the pro-inflammatory mediator ITGAL/LFA-1. *Ex vivo* expansion of patient PBMC also revealed that IL10 production can be de-repressed by treatment with the demethylase AZA. Furthermore CPPS patient PBMCs are less capable of producing IL10 in response to LPS than their control counterparts.

Conclusions:
Epigenetic modifications in the IL10 and ITGAL promoters in CPPS patients may facilitate development of auto-immunity in CPPS. These novel data give us deeper insight into the etiology of the syndrome and potential therapeutic targets.

Urinary Metabolites for the Risk Stratification of Prostate Cancer

2019 Travel Award Winner

Qin Gao Ph.D., Postdoctoral Researcher, University of Texas at El Paso

Xiaogang Su Ph.D., Professor, University of Texas at El Paso, Heinric Williams, M.D., FACS, Physician, Geisinger Medical Center, Michael Hani Annabi,M.D., Physician, The Clinic Internal Medicine, Wen-Yee Lee Ph.D., Associate Professor, University of Texas at El Paso

Title: Urinary Metabolites for the Risk Stratification of Prostate Cancer

Background: Prostate cancer (PCa) is a heterogeneous disease ranging from indolent to life threatening stages. The choice of watchful waiting or therapies is always depending on the risk level of PCa. To reduce the population of lethal prostate cancer, the importance of early screening and risk stratification of PCa has been highlighted. Our previous study has shown that urinary volatile organic compounds (VOCs) could discriminate PCa patients from controls (AUC 0.92). We hypothesized that urinary VOCs could be further tested for PCa prognostic and cancer risk stratification.

Methods: Urine samples from 89 men who presented for transrectal ultrasound guided prostate biopsy for an elevated PSA or abnormal digital rectal exam were collected. Based on the D'Amico risk scores system, these PCa patients were divided into two groups: low-risk group (indolent PCa, GS = 6, PSA< 10, n=55), and high/intermediate-risk group (clinically significant PCa, GS = 6 and PSA $\geqslant$ 10, or GS > 6 with any PSA values, n=34). Urinary metabolites were analyzed by Gas Chromatography-Mass Spectrometry. A PCa risk stratification model was developed and validated using innovative statistical machine-learning techniques. Additional cohort of patients were recruited as the testing group to validate the PCa risk stratification model.

Results: Using the Wilcoxon rank sum test, 23 VOCs were found to be positively related to high/intermediate−risk PCa and 44 VOCs negatively associated ($p<0.05$). Regularized logistic regression analysis and the Firth method were then applied to develop a PCa Risk Model that contains 11 VOCs. Under cross-validation, the area under the receiver operating characteristic curve (AUC) for the Risk model was 0.86 (sensitivity: 0.85; specificity: 0.80), which indicates a highly promising discrimination power of urinary VOCs in PCa risk assessment. The validation results from testing group will be presented.

Conclusion: The study describes the development and validation of a urinary VOC-based model for PCa risk stratification. The model shows the potential of applying urinary VOCs for PCa prognosis.

Application of Urinary Volatile Organic Compounds for the Diagnosis of Renal Cancer

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Background: Renal cancer accounts for more than 2% of cancer incidence and mortality in the United States. The outcome of renal cell carcinoma (RCC) is usually unpredictable even after a long period of asymptomatically development and progression. Its diagnosis is often incidental through the use of medical imagology and frequently at an advanced stage and metastatic. Therefore, a fast and reliable early screen of RCC enables better treatment outcome and care in patients. However, there is no recommended screening tests for RCC available clinically. This study developed a non-invasive and fast urine based diagnostic tool and evaluated its clinical utility, sensitivity and specificity to screen clear cell RCC (ccRCC, counted for 70–80% of all RCC) in populations.

Methods: Two cohorts were recruited for the study – a training cohort for model development and a testing cohort for model validation. For the training cohort, a total of 108 urine samples were obtained from 71 patients who were undergoing partial or radical nephrectomy and 37 patients ccRCC negative based the imaged renal mass. Volatile organic compounds (VOCs) in urine were analyzed using Stir Bar Sorptive Extraction coupled with Thermal Desorption-Gas Chromatography/Mass Spectrometry. All VOCs were analyzed based on their occurrence and relative quantity in the urine. The significant VOCs were screened by Wilcoxon Rank Sum Test. A VOCs based ccRCC diagnostic model was developed through the logistic regression in training group (57 ccRCC vs 31 controls) and validated in the testing group (14 ccRCC vs 6 controls).

Results: A total of 8,266 VOCs were found in the samples of training set. Using Wilcoxon Rank Sum Test in screening their bivariate association with ccRCC, 79 VOCs were found to be related to urine samples of ccRCC positive patients while 91 VOCs corresponding to RCC negative controls ($p<0.05$). After further selection with l_1 regularization, 15 VOCs were included in the RCC diagnostic logistic model. Via cross-validation, the area under the receiver operating characteristic curve (AUC) was found to be 0.87 and the sensitivity and specificity were 93% and 77% respectively. The VOCs based RCC diagnostic model were then validated in the testing group

and showed a promising diagnostic power with 0.81 AUC, 86% sensitivity and 83% specificity respectively.

Conclusion: This is the first systematic study to demonstrate and validate the clinical utility of a non-invasive urinary VOCs based diagnostic model in ccRCC screening. The VOCs based diagnostic model has the substantial potency and clinical value in RCC screening, and the analytical method was fast and highly translatable.

MAP3K11 drives in vitro enzalutamide resistance in castration-resistant prostate cancer

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MAP3K11 drives *in vitro* enzalutamide resistance in castration-resistant prostate cancer

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Background: Prostate cancer patient tumors treated with androgen deprivation therapy (ADT) progress to a resistant state known as castration-resistant prostate cancer (CRPC). CRPC can be treated with androgen receptor (AR) pathway antagonists such as enzalutamide and abiraterone acetate that target AR signaling and transcriptional activity; however, resistance to these therapies occurs within two years of starting treatment and less tolerable chemotherapy treatments are utilized. The purpose of this study was to evaluate whether a gene we had identified in a short hairpin RNA (shRNA) screen for drivers of enzalutamide resistance in C4-2B cells, *MAP3K11*, was a valid resistance driver. MAP3K11, also known as mixed lineage kinase 3 (MLK3), has been shown to be involved in multiple signaling pathways including the JNK and p38 MAPK pathways. MAP3K11 has also been implicated in regulating AR activity via signaling cascades that lead to changes in phosphorylation of Serine 650 (AR-Ser650).

Methods: To investigate the role of MAP3K11 in enzalutamide resistance, we performed cell survival assays using crystal violet to measure the amount of surviving cells in response to MAP3K11 knockdown or inhibition with the MLK inhibitor CEP-1347 in C4-2B or 22RV1 cells treated with DMSO or enzalutamide. We also used Western blotting to measure changes in MAP3K11, AR, and phosphorylated AR-Ser650.

Results: Our studies show MAP3K11 KD decreased cell survival in CRPC cell lines and further decreased cell survival when KD cells were treated with enzalutamide. Treatment of CRPC cells with CEP-1347 also sensitized C4-2B and 22RV1 cells to enzalutamide treatment and resulted in increased cell death. In order to determine the mechanism of action between MAP3K11 and AR, we investigated the phosphorylation of AR-Ser650. We observed a decrease in phosphorylation of AR-Ser650 but no change in AR protein expression levels in response to MAP3K11 KD. CEP-1347 treatment also resulted in decreased AR-Ser650 phosphorylation but no change in overall AR expression.

Conclusions: This data suggests MAP3K11 plays a role in driving resistance to enzalutamide in CRPC cell lines, and one of the ways in which it achieves this may be by increasing AR transcriptional activity via phosphorylation of AR-Ser650. Importantly, this data shows MAP3K11 pharmacologic inhibition can increase efficacy of enzalutamide treatments *in vitro* and future studies will evaluate whether this is true in pre-clinical models of CRPC.

Therapeutic potential of targeting macrophages in castration resistant prostate cancer

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TITLE: Therapeutic potential of targeting macrophages in castration resistant prostate cancer.

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BACKGROUND:
Prostate cancer patients treated with androgen-deprivation therapy (ADT) eventually relapse with highly aggressive castration-resistant (CRPC), which is commonly metastatic and responsible for the majority of PCa mortality. Identifying mechanisms of castration-resistance is therefore critical for the development of improved therapies, but the majority of studies have focused on androgen signaling and oncogenic tumor cell mutations as a major driver of resistance. Comparatively less attention has been paid to the role of immune cells, but macrophages have been shown to contribute to disease recurrence in a murine model through an unidentified mechanism.
METHODS:
To identify the therapeutic potential of targeting macrophages in castration resistance, we developed an inducible autochthonous and orthoptopic model involving Pten and Trp53 deficiency, with or without expression of the TMPRSS2/ERG fusion protein found in 40-50% of tumors. We first measured macrophage infiltration during different stages of disease. We then used magnetic resonance imaging (MRI) to assess the efficacy of combining ADT with a macrophage-targeted antibody. We also established an in vitro co-culture system of macrophages and tumor cells generated from our unique autochthonous model, to test if macrophages directly contribute to tumor cell proliferation
RESULTS:
We found that macrophages are the predominant immune population in late stage disease. We detected ≥50% reduction in the growth of orthotopic tumors when Lupron (ADT) was combined with macrophage-targeted antibody, indicating an important roles for macrophages in promoting tumor progression during ADT. We also confirmed the growth-promoting ability of macrophages using our established co-culture system.

CONCLUSION: Targeting macrophage may be a promising therapeutic strategy to improve response to ADT.

LSD1-mediated demethylation of FOXA1 regulates AR cistrome in Prostate Cancer

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LSD1-mediated demethylation of FOXA1 regulates AR cistrome in Prostate Cancer

Background: Androgen receptor (AR) cistrome undergoes extensive reprogramming in cooperation with various transcriptional factors during prostate cancer (PCa) progression. FOXA1, a "pioneer" factor, determines cell-lineage and facilitates the access of AR to the regions with compact chromatin. Particularly, recent patient genomic study identified FOXA1 overexpression driven by chromatin rearrangements. Thus, targeting FOXA1 could provide a novel therapeutic strategy in PCa. However, FOXA1 is notoriously undruggable because of lack of targetable domain. We have previously shown that lysine specific demethylase 1 (LSD1) tightly associates with FOXA1 (genomic binding overlap, physical interaction) in PCa. Our genome-wide studies showed that LSD1, still demethylating histone 3 lysine 4, also functions as a major activator in mediating AR-dependent enhancers and facilitates the transcription of androgen-regulated genes. This leads to our hypothesis that inhibition of LSD1 could disrupt the FOXA1-dependent AR cistrome.

Methods: Using ChIP-Seq and RNA-Seq, we interrogated the LSD1 inhibition effect on: 1) FOXA1 global binding, 2) AR-regulated enhancer openness, 3) AR cistrome and transcriptome. Mass-spec analysis together with biochemical assay allowed us to investigate the molecular basis of LSD1 regulation on FOXA1. To provide the clinical implication of targeting LSD1 in PCa, we tested LSD1 inhibitors in an array of PDX models in the castrated mice.

Results: Comprehensive ChIP-seq studies reveal that LSD1 and FOXA1 co-occupy at active enhancers in PCa cells. Before AR recruitment, LSD1 inhibition leads to a rapid and dramatic decrease of global FOXA1 binding, and decreases H3K27ac at AR-dependent enhancers. Under androgen stimulation, inhibiting LSD1 robustly disrupts AR genomic binding and transcriptional output. Importantly, LSD1 inhibition also impairs AR-V7 chromatin binding in CRPC models. Mechanistically, we identify lys270 (located near Forkhead domain) of FOXA1 as a direct target of LSD1. Methylation-deficient FOXA1 mutant (K270R) in PCa cells stabilizes AR chromatin binding and thus renders resistance to AR antagonist. Finally, we show *in vitro* and *in vivo* that LSD1 inhibition dramatically decreases PCa growth alone and in synergy with the AR antagonist treatment in FOXA1-high CRPC models.

Conclusion: LSD1 is an important epigenetic regulator that controls the AR cistrome through directly demethylating pioneer factor FOXA1 to stabilize its chromatin binding and thus increase enhancer availability. This interplay between epigenetic modifiers and transcriptional factors will inform important molecular basis for innovative epigenetic therapies in PCa treatment.

CCL25 neutralization enhances efficacy of Docetaxel in preclinical prostate cancer model

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CCL25 neutralization enhances efficacy of Docetaxel in preclinical prostate cancer model

Background: Docetaxel (DTX), which is one of the standard chemotherapeutic drugs to treat advanced PCa, offers only moderate survival benefits to the patients and it is associated with debilitating toxicities. In this preclinical study, we evaluated the role of CCR9-CCL25 axis on efficacy of DTX.

Method: Mouse xenograft models were developed by subcutaneously challenging immuno-compromised nude mice with PC3 and LNCaP cells. After the establishment of tumor, measured using Vernier Caliper, mice were randomized into treatment and control groups. Treatment group received biological response modifier dose suboptimal (2mg/kg), sub-optimal (4mg/kg) and maximal tolerated dose of DTX (8mg/kg) with or without anti-CCL25 neutralization antibody (2mg/kg). Control group received vehicle. Immunofluorescence staining was used to measure proliferation index, expression and phosphorylation of survival molecules. TUNEL assay was used to measure apoptotic index in tumor sections.

Results: Tumor size was significantly reduced in all treatment groups compared to controls. However, maximum growth inhibition was observed in mice treated with anti-CCL25 and 4mg/kg DTX combination compared to those treated with DTX 4mg/kg alone. Additionally, tumor volume was significantly reduced in PC3 (~1.4-fold) and in LNCaP (~2-fold) xenograft when 4mg/kg or 8mg/kg DTX was combined with CCL25 neutralization compared to DTX alone. However, no significant difference in tumor volume was observed with biological response modifier dose of DTX (2mg/kg) with or without anti-CCL25 antibody. Tumor excised from mice that received DTX and anti-CCL25 showed significant reduction in proliferation and increased apoptosis compared to controls. This was consistent with significant decrease in survivin, phospho-PI3K p85 and phospho-ERK in combination groups versus control groups.

Conclusion: Comparable response to maximum tolerated dose of DTX and sub-optimal dose of DTX combined with CCL25 neutralization strongly implies that blockade of CCR9-CCL25 axis has the potential to enhance the efficacy of DTX in PCa.

Differential role for SIRT1 in prostate cancer development and progression

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Differential role for SIRT1 in prostate cancer development and progression

Shih-Bo Huang, Dinesh Thapa, Amanda R Muñoz, Suleman S. Hussain, Xiaoyu Yang, Roble G Bedolla, Zhao Lai, Yidong Chen, Paul Rivas, Claire Shudde, Pawel Osmulski, Maria Gaczynska, Robert L Reddick, Hiroshi Miyamoto, Rita Ghosh, Addanki Pratap Kumar

Background: NAD+ dependent deacetylase Sirtuin 1 (SIRT1) has been reported to involved in a plethora of physiological and pathological processes through posttranslational modulation of histone or non-histone proteins. Previous studies using mouse models resulted in paradoxical findings regarding both tumor suppressive and oncogenic role warranting comprehensive investigations to decode its role in prostate cancer (PCa) for development for potential targeted therapies.

Methods: Prostate specific PTEN knockout (PTENKO) mouse model was used to study the impact of SIRT1 on the early stage of cancer progression and orthotopic xenograft model to test contribution of SIRT1 to tumor development. Mechanistic investigations were conducted in cell culture models using genetic and pharmacologic approaches and RNA-seq coupled with bioinformatic analysis to identify SIRT1-regulated targets.

Results: Our results for the first time show that early intervention with resveratrol (RES) prevented initiation of high-grade PIN (HGPIN) lesions with no significant impact on progression of established lesions in PTENKO mice and the development of prostate tumors in nude mice. Intriguingly, mice with longer treatment duration of RES significantly developed invasive prostate carcinoma. Orthotopic implantation with SIRT1-silenced LNCaP cells significantly impaired tumor development in nude mice. SIRT1 inhibition in castrate resistant 22Rv1 cells abrogated mechanical properties without altering cell growth. Importantly, we identified significant correlation of elevated nuclear SIRT1 in human prostate tumors with biochemical recurrence. *In silico* analysis further showed strong association of SIRT1-regulated gene signature with malignancy and short progression-free survival in TCGA PCa cohort. Interestingly, following androgen removal, SIRT1 facilitates cell cycle progression possibly through AR but suppresses senescence through negative regulation of p21 by AR. More importantly, modulation of SIRT1 activity with pharmacological inhibitor EX-527 mimicked blockage of AR signaling.

Conclusions: Our findings demonstrate (i) stage-dependent role for SIRT1 in PCa progression; (ii) prognostic potential for nuclear SIRT1 in cancer recurrence; and (iii) potential beneficial effect of precisely targeting SIRT1 pathways in PCa. Supported by CPRIT Training Grant RP 170345 (SH) and CPRIT RP 150166 (APK).

Loss of Androgen Receptor in Prostate Cancer Stroma Inhibits Luminal Epithelial Differentiation

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Loss of Androgen Receptor in Prostate Cancer Stroma Inhibits Luminal Epithelial Differentiation

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Background: Despite the well-known role of AR as an oncogenic driver of prostate cancer (PCa) in the epithelia, AR has a pivotal function within the stroma to drive luminal epithelial differentiation. Stromal AR signaling produces the paracrine factors required for luminal differentiation. However, studies shown that stromal AR expression is decreased in PCa and associated with poor clinical outcomes. Furthermore, stromal AR loss is proportional to higher Gleason scores. The ability of tumor cells to convert the stroma into cancer-associated fibroblasts (CAF) is well documented. But how AR signaling is lost from cancer stroma and how its loss in turn influences epithelial differentiation and oncogenesis unknown. In this study, we set out to determine the mechanism by which tumor cells induce loss of stromal AR.

Method: A new microfluidic-base Prostate-on-Chip model was used to co-culture normal prostate stromal fibroblasts, BHPrS1, with either normal basal prostate epithelial cells or two PCa cell lines, C4-2 and EMP. Using cytokine array profiling, we identified a set of secreted proteins shared by two PCa cell lines, C4-2 and 22RV1. Immunofluorescence imaging and immunoblotting was used to monitor CAF markers, and AR. Levels of AR mRNA expression were analyzed by qRT-PCR.

Results: Coculture of BHPrS1 cells with tumor cells for 12 days, led to tumor-induced CAF conversion as well as downregulation of AR expression. We identified TNF-α, FGF9, and GDNF as top candidate secreted cytokines shared by two PCa cell lines. Treatment of the BHPrS1 with synthetic androgen (R1881) induces the expression and nuclear localization of AR. Treatment of BHPrS1 with TNF-α for 5 days in the presence of R1881 resulted in a loss of AR expression and reduced nuclear localization. Loss of AR occurred in the absence of CAF conversion. Loss of AR protein was detectable within 24 hr after TNF-α treatment and was partially overcome by R1881. Examination of AR mRNA revealed that 1) R1881 induction of AR protein is not due to transcription, and 2) loss of AR protein by TNFα is accompanied by a decrease in mRNA, which is not recovered by R1881.

Conclusion: These results show that there are two mechanisms controlling AR expression, a post transcription mechanism induced by R1881 and a transcriptional repression induced by TNF-α.

Decreased glucose bioavailability and elevated aspartate metabolism in prostate cancer cells undergoing epithelial- mesenchymal transition

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Decreased glucose bioavailability and elevated aspartate metabolism in prostate cancer cells undergoing epithelial-mesenchymal transition

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Background: Prostate cancer (PCa) is a common malignancy with a high tendency for metastasis. Epithelial-mesenchymal transition (EMT) plays a crucial role in PCa metastasis. Metabolic reprogramming offers metabolic advantages for cancer development and could result in the discovery of novel targets for cancer therapy. However, the metabolic features of PCa cells undergoing EMT remain unclear.

Methods: Metabolome and transcriptome analyses were performed to reveal the metabolic alteration of PCa cells undergoing EMT. *In vitro* studies were used to validate the findings by omics analysis.

Results: PCa cells undergoing EMT showed impaired glucose utilization. In vitro studies demonstrated that PCa cells undergoing EMT were less addicted to glucose than epithelial-like PCa cells. However, cells that underwent EMT had higher levels of aspartate and its downstream metabolites, indicative of upregulated aspartate metabolism. PCa cells undergoing EMT were more sensitive to metformin, an anti-diabetic drug that could inhibit aspartate metabolism.

Conclusions: PCa cells undergoing EMT were less addictive to glucose but more dependent on aspartate relative to epithelial-like PCa cells. Therapeutics targeting aspartate metabolism may provide new approach to inhibit PCa metastasis.

Exogenous testosterone and estradiol prolong prostate smooth muscle relaxation via downregulation of MYPT2: a novel utility for genetically encoded calcium receptors Anne E Turco, University of Wisconsin-Madison

Steven R Oakes, Allison Rodgers, Celeste Underriner, Mark Cadena, Richard E Peterson, Laura Hernandez, Tim Hacker, Nathan Tykocki, Chad M Vezina

Exogenous testosterone and estradiol prolong prostate smooth muscle relaxation via downregulation of MYPT2: a novel utility for genetically encoded calcium receptors

Anne E Turco[1], Steven R Oakes, Allison Rodgers, Celeste Underriner, Mark Cadena[5], Richard E Peterson[4], Laura Hernandez, Tim Hacker, Nathan Tykocki, Chad M Vezina[3]

Background: Alpha-adrenergic antagonists are commonly prescribed to men experiencing lower urinary tract symptoms including dribbling and urinary frequency. These drugs relax the prostate smooth muscle surrounding the urethra. It has been suggested that muscle dysfunction causes excessive contraction in some men, but this has never been shown because methods were lacking. Resultantly, we developed a mouse model to visualize and study smooth muscle contraction *in vitro* and *in vivo*. We used this model to test whether hormone induced urinary dysfunction is caused by changes in smooth muscle contraction.

Methods: We bred genetically encoded calcium receptors (GCAMP5g) into prostate smooth muscle using a MYH11 *Cre*. Activation of smooth muscle causes an increase in fluorescence enabling visualization and quantification contraction. We applied graded concentrations of phenylephrine (contracts muscle) and field stimulation (stimulates axons) to study the regionality of contraction, duration of contraction, and duration of relaxation. Additionally, we examined prostate smooth muscle contraction *in vivo* using an intravenous injection of phenylephrine. Lastly, we determined if exposure to exogenous testosterone and estradiol impacts prostate smooth muscle contraction and relaxation. We treated GCaMP5g*MYH11 offspring with 25 mg testosterone and 2.5 mg estradiol for two weeks and stimulated prostate muscle with phenylephrine to measure the duration of smooth muscle contraction and relaxation.

Results: We determined prostate smooth muscle contracts slower than bladder smooth muscle. Further, prostate ducts contract directionally from duct tip to base. Genetically encoded calcium receptor activity in prostate can be measured *in vivo*. Lastly, we found testosterone and estradiol exposure increases the duration of smooth muscle relaxation via downregulation of the myosin phosphatase MYPT2.

Conclusion: Genetically encoded calcium receptors are a novel and quantitative method to study prostate smooth muscle dynamics *in vitro* and *in vivo*. This is the first evidence smooth muscle dysfunction may contribute to symptoms of hormone induced urinary dysfunction.

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Temporal Analysis of Signaling Events Leading to Bladder Remodeling after Spinal Cord Injury

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Temporal Analysis of Signaling Events Leading to Bladder Remodeling after Spinal Cord Injury

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Background: Suprasacral spinal cord injury (SCI) is associated with profound remodeling of the bladder wall and with the development of neurogenic detrusor overactivity (NDO). However the molecular pathways driving remodeling and how these contribute to functional alterations remain incompletely defined. The objective of this study was to use unbiased expression profiling to assess time-dependent transcriptional changes following SCI and to identify signaling pathways contributing to bladder wall remodeling and fibrosis.

Methods: RNAseq analysis was performed on full thickness bladder tissue from rats 2, 8 or 16 weeks after mid-thoracic spinal cord transection or from age-matched, non-injured controls. Differential gene expression and gene pattern clustering were performed using DESeq2 and EdgeR. Validation of gene expression changes was performed using real time RT-PCR and nanoString. In addition, whole transcriptome data from bladder tissues of humans with different urodynamically-defined states of bladder outlet obstruction and primary human bladder smooth muscle cells treated without or with TNF-α were compared to SCI transcriptome profiles in rats.

Results: 207, 1355, and 2493 differentially expressed genes (DEGs) were identified at the 2, 8, and 16 week time-points, respectively, with significant enrichment for gene ontology terms associated with cytoskeletal remodeling, synapse organization, axon guidance and neuromuscular junction activity. Comparative analyses across seven different groups, comprising neurogenic and myogenic obstruction, and cytokine challenge of SMC revealed 4 common regulated pathways, including CDK5-, cAMP-mediated-, Wnt/β-catenin- and PTEN signaling. Comparison of time-dependent changes following SCI revealed an increasing number and connectivity of regulated pathways.

Conclusions: Expression profiling of bladder tissue following SCI reveals perturbations in a variety of physiologically relevant gene clusters including those associated with innervation and cytoskeletal remodeling. The intensity of changes in regulation of pathways increases significantly from 2 to 8 weeks following injury but then plateaus at 16 wk. Comparison across myogenic and neurogenic modes of injury reveals similar intensity of pathway activation but opposite direction of regulation.

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Development of a Whole-Urine, Multiplexed, Next Generation RNA-Sequencing Assay for the Early Detection of Aggressive Prostate Cancer

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Abstract:

Background: Despite advances in biomarker development, early detection of aggressive prostate cancer (PCa) remains challenging. We previously developed the Michigan Prostate Score (MiPS) for individualized risk prediction of aggressive prostate cancer. MiPS uses transcription-mediated amplification to quantify expression of *TMPRSS2:ERG* (*T2:ERG*) and *PCA3* from whole urine obtained after a digital rectal exam (DRE), combined with serum PSA. To improve upon MiPS, herein we describe the pre-clinical development and validation of a targeted next generation RNA sequencing assay (NGS-MiPS).
Methods: We selected patients with available MiPS scores as well as representing a spectrum of disease grade on biopsy (Benign to Grade Group 5). We used 2.5 mL of post-DRE whole urine to asses ~90 PCa transcriptomic biomarkers: including T2:ERG, PCA3, and additional isoforms of common PCa gene fusions, mRNAs, lncRNAs, and expressed mutations.
Results: NGS-MiPS showed a 98% informative sample rate, high technical reproducibility, robustness and concordance with orthogonal methods (TMA and RT-qPCR), and was able to detect expressed *HOXB13* p.G84E variant expression. NGS-MiPS accurately recapitulated clinical MiPS-measured risk scores for presence of PCa or high-grade PCa (Gleason Score >6) on biopsy as determined by clinical MiPS vs. the same model but with NGS-MiPS data. In an extreme design cohort (Benign or Gleason 6 vs. Gleason ≥ 4+3=7 cancer) NGS-MiPS showed expected differences in the levels of *T2:ERG* T1E4 ($p<0.00001$) and *PCA3* ($p=0.02$), with additional T2:ERG splice isoforms and other biomarkers also showing significantly different expression between low vs. high grade cancer. We used a machine learning approach trained on a subset of the extreme design cohort ($n=73$) to generate a 29-transcript model that outperformed MiPS and serum PSA in two validation cohorts: 1. A held-out set from the extreme design cohort $n=36$, (AUC 0.82 vs. 0.73 and 0.69, respectively); and 2. A separate PCa active surveillance cohort n=45, (AUC 0.66 vs. 0.58 and 0.53, respectively).
Conclusions: These results support the potential utility and continued development of our urine based targeted NGS assay to supplement serum PSA for improved early detection of aggressive prostate cancer.

Targeting steroid sulfatase with novel inhibitors suppresses CRPC tumor growth and improves response to enzalutamide

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Targeting steroid sulfatase with novel inhibitors suppresses CRPC tumor growth and improves response to enzalutamide

Background: Steroid sulfatase (STS) catalyzes the hydrolysis of DHEAS to biologically active DHEA, which is further metabolized to active androgens that bind the androgen receptor (AR) leading to cell proliferation. DHEAS is the most abundant steroid in blood circulation and significant concentrations of DHEAS are present in prostate cancer patients even after ketoconazole or abiraterone therapy, suggesting that this may act as a depot for downstream androgen production. Currently the role of STS in AR signaling and CRPC is largely unknown. This study determines the role of STS in AR signaling and explores the potential of targeting STS to overcome castration resistance in prostate cancer.

Methods: Quantitative rt-PCR and Western blots were used to detect expression of STS and AR. STS was downregulated using siRNA specific to STS. Stable cell lines overexpressing STS were generated and characterized. RNA-seq was performed on the stable clones to determine alterations in gene expression instigated by STS expression. The steroid profiles of the cells were analyzed by LC-MS using the Thermo Scientific Vanquish UPLC/AB Sciex Qtrap system. STS activity was determined by 4-Methylumbelliferyl sulfate assay. Eleven potent STS inhibitors (SI) were synthesized and characterized. Prostate cancer cell sensitivity to SI was tested using cell growth assays and clonogenic assays. Efficacy of two SI was tested *in vivo* in castration relapsed VCaP xenograft tumor models.

Results: STS is overexpressed in CRPC patients and resistant prostate cancer cells including VCaP and C4-2B MDVR. Stable STS overexpression in C4-2B and LNCaP cells increases the levels of testosterone and DHT, respectively. This resulted in increased cell growth and PSA expression *in vitro*. Inhibiting STS with siRNA suppresses cell growth and AR signaling. Furthermore, STS overexpression in C4-2B and LNCaP cells promoted resistance to enzalutamide and this could be reversed by STS siRNA. Of the 11 potential novel SI, SI-1 and SI-2 were the most potent inhibitors of STS activity and growth in VCaP cells. They significantly suppressed AR transcriptional activity, suggesting that inhibition of STS activity by SI downregulates AR signaling. SI-1 and SI-2 significantly suppressed the growth of relapsed VCaP cells and tumors and improved enzalutamide treatment *in vitro* and *in vivo*.

Conclusions: STS is involved in castration resistance prostate cancer and inhibition of this enzyme could be a viable strategy to treat CRPC and improve enzalutamide treatment.

Role of mast cells in an uropathogenic Escherichia coli induced model of lower urinary tract symptoms associated with benign prostatic hyperplasia

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Role of mast cells in an uropathogenic *Escherichia coli* induced model of lower urinary tract symptoms associated with benign prostatic hyperplasia

Background: Prostatic inflammation is one of the key contributors to lower urinary tract symptoms (LUTS). Previously we have shown that intraurethral infection with a uropathogenic isolate of *E. coli,* CP1 (Chronic-prostatitis 1), results in clinical signs of benign prostatic hyperplasia (BPH) and LUTS as demonstrated by an increased urinary dysfunction, prostate inflammation and fibrosis. Increased mast cell infiltration and activation has also been reported in prostate tissues from BPH patients. In this study we demonstrate the importance of mast cells and their activity in driving the development of BPH / LUTS upon pathogenic infection and the therapeutic benefit of mast cell neutralization on disease progression.

Methods: Infiltration and activation of mast cells was characterized by toluidine blue staining of prostate tissue sections from naïve and CP1 infected mice at days 14 and 35. To assess more directly the role of mast cell activation on disease pathology, inhibition was performed using a combination therapy of cromolyn sodium salt (mast cell stabilizer) and cetirizine dihydrochloride (histamine 1 receptor inhibitor) following bacterial infection. At experimental endpoint unconscious terminal cystometry was performed to assess urinary function and the therapeutic benefit on prostate inflammation and fibrosis assessed by immune-histochemical staining.

Results: Our data demonstrates that upon CP1 infection, mice develop clinical signs of urinary dysfunction, determined by an increase in voiding frequency as well as a decrease in voided volume. Prostate tissues also show a significant increase in total number as well as activation status of mast cells. Prophylactic treatment with a combination of cromolyn sodium salt and cetirizine dihydrochloride show decreases in both the total number of mast cells and number of activated mast cells in prostate tissues. Furthermore, the prostate of these mice has decreased prostate fibrosis and inflammation as determined by picrosirius red staining. Finally, we observed that this combination treatment can ameliorate the urinary dysfunction previously observed in response to CP1 infection.

Conclusions: These results provide evidence of the role of mast cells and their activation status in the development of BPH and associated LUTS. A combination therapy of mast cell stabilizer and histamine 1 receptor inhibitor seems to be a potential target for therapeutic intervention in men with LUTS associated with BPH.

A Novel Syngeneic Mouse Model of Prostate Cancer Bone Metastasis: Mechanisms of Chemotaxis and Bone Colonization

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Background: Bone metastasis in human prostate cancer (PCa) remains a major clinical problem since no effective therapy exists. The RANKL/RANK pathway plays a predominant role in the interaction between metastasized PCa cells and osteoclasts that increases the bone turnover. The current therapies, including targeting RANKL with denosumab, address the growth of prostate tumor cells that have already colonized the bone, but are largely ineffective in prolonging the survival of human PCa patients with bone metastasis. Further, a major impediment to PCa bone metastasis research is the lack of an animal model that spontaneously recapitulates human PCa bone metastasis in the context of an intact immune system.

Methods: We utilized the intra-cardiac inoculation experimental-metastasis technique to achieve PCa bone metastasis in syngeneic mice.

Results: To overcome this major limitation, we have developed a novel syngeneic mouse model to study PCa bone metastasis. Both the CXCL12/CXCR4 and RANKL/RANK pathways have been reported to be overexpressed / dysregulated in human PCa bone metastatic samples. Data generated utilizing our immune-intact mouse model shows that the CXCL12/CXCR4 and RANKL/RANK pathways co-operate with each other to drive PCa bone metastasis. Studies have shown that targeting the CXCL12/CXCR4 and RANKL/RANK pathways individually affects the immune system, thereby making our syngeneic mouse model an indispensable tool for studying the critical co-operation between these 2 pathways in the manifestation of human PCa bone metastasis. We found that MPC3 mouse PCa cells with RANKL overexpression (MPC3-Luc-GFP-RANKL) develop 70-80% limb and jaw within 4 weeks of intra-cardiac injection in these syngeneic mice. Control MPC3 cells had no bone metastasis. Bone lesions visualized by luciferase imaging and X-ray were confirmed by micro CT and immunohistochemistry. RANKL signaling drove bone and visceral metastases via the downstream CXCL12/CXCR4 signaling axis. MPC3-

Luc-GFP-RANKL cells showed increased CXCR4 protein levels by immunohistochemistry. Metastatic bone marrow flush showed dramatically increased levels of CXCL12 mRNA compared with control mice (MPC3-Luc-GFP-EV).

Conclusions: In sum, *1)* Circulating PCa cells induce a marked CXCL12 elevation after colonizing bone, triggering chemotaxis and recruiting CXCR4-positive PCa cells to migrate to bone; and *2)* Osteomimetic PCa cells with increased RANKL expression interact with osteoclasts to enhance bone resorption and turnover, releasing additional growth factors and chemokines for PCa cell growth and survival in bone.

A genetically defined tumor model characterizes small cell carcinoma of the bladder

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Abstract: A genetically defined tumor model characterizes small cell carcinoma of the bladder

Background: Small cell carcinoma of the bladder (SCCB) is a rare and lethal phenotype of bladder cancer. The understanding of its pathogenesis and molecular features is largely impeded by a lack of preclinical model.

Method: We established a genetically engineered model by transforming normal human urothelial cells into tumors using a set of defined genetic factors. We compared our model with clinical SCCB samples at histological, molecular, and transcriptional levels to show that it recapitulates clinical SCCB features.

Results: Our model shows that urothelial cell-derived tumors have a mixture of histological phenotypes including small cell carcinoma, urothelial carcinoma, and squamous cell carcinoma. The small cell carcinoma portion of the tumors expresses neuroendocrine markers that are used to diagnose SCCB in clinical. Tumor-derived single-cell clones also give rise to both small cell carcinoma and urothelial carcinoma in xenografts. We found that clinical SCCB samples have a distinct transcriptional profile and a unique transcriptional regulatory network from urothelial carcinoma samples. Using transcriptional analyses, we identified cell surface proteins (CSPs) associated with the SCCB phenotype that can be further evaluated as potential therapeutic targets. We demonstrated that our genetically engineered tumor model is a representative tool for investigating CSPs in SCCB by showing that it shares a similar CSP profile with clinical samples and expresses SCCB-upregulated CSPs at both the mRNA and protein levels.

Conclusion: we provided functional evidence demonstrating that SCCB and urothelial carcinoma shares the urothelial origin and demonstrated that our tumor model as a representative tool to study SCCB.

Identification of a Novel PRC2 Complex as a Therapeutic Target in Castration Resistant Prostate Cancer

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IDENTIFICATION OF A NOVEL PRC2 COMPLEX AS A THERAPEUTIC TARGET IN CASTRATION RESISTANT PROSTATE CANCER

Background: Metastatic castration-resistant prostate cancer (CRPC) is a lethal disease. Understanding the molecular drivers of CRPC will guide the development of targeted therapies. EZH2, the enzymatic subunit of Polycomb repressive complex 2 (PRC2), is one of the most upregulated genes in CRPC. EZH2 catalyzes trimethylation of Histone H3 at Lysine 27 (H3K27me3), a modification that represses target gene expression. A plethora of tumor suppressor genes has been reportedly to be targets of EZH2, and yet how EZH2 is recruited to specific genomic loci in mammals remain unclear.

Methods: Co-IP and size-exclusive chromatography assays were utilized to study protein-protein interactions. Co-occupancy of histone marks on the chromatin were assessed by biochemical and ChIP-Seq assays. Reprogramming of PRC2 cistromes along with EZH2 transcriptome in human prostate cancer models were examined by next-generation sequencing techniques.

Results: By proteomic profiling of EZH2-containing complex in prostate cancer cells, we identified a novel PRC2 sub-complex containing PALI1, a recently identified PRC2-associated protein, and G9a, an H3K9me2 demethylase. Further biochemical experiments revealed that PALI1 utilizes different domains to interact with PRC2 and G9a, acting as a scaffold protein that bridges PRC2 with G9a. Importantly, PALI1 is overexpressed in CRPC and this enhances PRC2-G9a interaction. We further demonstrate that PALI1 and G9a are components of a unique PRC2 sub-complex (named PRC2.3), which is distinct from previously reported EPOP-containing PRC2.1 and JARID2-mediated PRC2.2. Genome-wide co-localization of PRC2-catalyzed H3K27me3 and G9a-catalyzed H3K9me2 were detected at the chromatin. PALI1 promotes reprogramming of PRC2 at G9a co-occupied sites, leading to enhanced H3K27me3 modifications and stronger gene repression. Functionally, these genes are critical for cellular development and differentiation. Their repression, in turn, contribute to prostate cancer de-differentiation and oncogenic progression.

Conclusions: We report a novel PRC2 sub-complex (PRC2.3) in prostate cancer cells, which contains PALI1 and G9a, but not JARID2 and EPOP. PALI is a critical mediator of PRC2 and G9a interaction and its overexpression in CRPC favors PRC2.3 assembly, resulting in a crosstalk between H3K27me3 and H3K9me2 at the chromatin and enhanced epigenetic silencing of cell differentiation genes. Our data suggest PALI1 and G9a as critical regulators of CRPC and promising targets for therapeutic interventions.

Fibroblasts accumulate and produce collagen in dogs prone to prostate related urinary dysfunction Hannah Ruetten, University of Wisconsin- Madison

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Background: Prostatic enlargement contributes to lower urinary tract symptoms (LUTS) in aging men but holds a weak correlation to symptom severity. Prostatic inflammation and collagen accumulation hold strong correlations to LUTS severity. We previously used canine prostates to discover that intact males accumulate collagen with age in the distal glandular regions and within the prostate fibromuscular capsule. Here we leveraged the same canine specimens to test if myofibroblasts expand and produce collagen in the regions of the canine prostate prone to aging related collagen accumulation.

Methods: Transverse sections of canine prostate from young intact males were stained with combinations of antibodies to identify and describe the regional distribution of luminal epithelial cells (KRT8/18+, KRT5-, SYN-), basal epithelial cells (KRT8/18-, KRT5+, SYN-), neuroendocrine cells (SYN+, periductal, nucleated cells), myofibroblasts (VIM+, ACTA2+, S100A4+), fibroblasts (VIM+, ACTA2-, S100A4+), and leukocytes (CD45+). Transverse sections of young, old, intact, and castrate canine prostate were stained with either VIM, ACTA2, and S100A4 or VIM, ACTA2, and CD45 to identify and quantify changes in the relative proportions of myofibroblasts, fibroblasts, and leukocytes with age and castration. Transverse sections of old intact male canine prostate were stained with ACTA2, S100A4, and ProCol1 to determine if fibroblasts and/or myofibroblasts were producing collagen.

Results: We found that fibroblasts and myofibroblasts are equally distributed in the urethra, proximal and peripheral glandular, and capsule regions in young intact males. We also found that the density of fibroblasts increases with age in intact but not castrate animals. Analysis of epithelial cell and leukocyte distribution, and collagen production is ongoing.

Conclusions: Our results suggest that prostatic fibroblasts and not myofibroblasts (as in other tissues) may expand and produce collagen leading to collagen accumulation and urinary dysfunction in aging male intact dogs.

A Retrospective Medical Record Review of Benign Prostatic Hyperplasia in a Well-Defined Population of Client-Owned Dogs: Clinical Presentation, Prevalence of Concurrent Bacterial Infection, and Response to Treatment Hannah Ruetten, University of Wisconsin- Madison

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A Retrospective Medical Record Review of Benign Prostatic Hyperplasia in a Well-Defined Population of Client-Owned Dogs: Clinical Presentation, Prevalence of Concurrent Bacterial Infection, and Response to Treatment.

Background: In men, benign prostatic hyperplasia (BPH) screening, diagnosis, and therapy is routine and standardized. This has led to large cohorts of men for clinical studies and resulted in the development of cutting-edge diagnostics and therapeutics, despite the heterogenicity of the disease. In contrast, current screening, diagnosis and treatment for BPH in dogs is highly variable due to elimination of the digital rectal exam from routine physical exams and a lacking awareness of the disease due to high neuter prevalence. Current treatment options for canine BPH haven't changed in nearly a century. And, despite their prolonged use, a lack of understanding remains around their efficacy and side-effects. This retrospective medical record review aims to described canine BPH, in a well-defined clinical population of client-owned dogs, to include the prevalence of concurrent bacterial prostatitis, and determine the response to medical versus surgical treatment for a minimum of 3 months following the clinical diagnosis of BPH.

Methods: Diagnostic reports prepared between January 1st, 2014 and December 2nd, 2018 were queried for key terms "benign prostatic hyperplasia" and yielded 275 reports belonging to 185 patients. Based on inclusion and exclusion criteria this population was narrowed to 30 patients. We describe the signalment at time of diagnosis, clinical features and severity using the "canine BPH severity score" developed by Zambelli et. al., prostatic volume and echogenicity, prevalence of bacterial prostatitis, and response to treatment.

Results: Data collection and analysis for this clinical study is in-progress. Preliminarily, we have identified two populations of patients: those with urinary dysfunction and those without but we have yet to determine if any other clinical factors delineate these two populations.

Conclusions: We are eager to complete our results and generate an updated clinical definition of BPH in the dog. This updated information will not only help the veterinary field but allow for correlations between men and intact males dogs improving use of dogs as a large animal model.

Estrogen mediated racial disparity in men with benign prostatic hyperplasia

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Background: Benign prostatic hyperplasia (BPH) is a disease associated with aging with >210 million cases worldwide; nearly all men will encounter some clinical lower urinary tract symptoms (LUTS) in their lifetime. African American (AA) men have a higher incidence of BPH with increased incidence of non-surgical treatment failure, larger prostates at time of surgery, and surgery occurring at a younger age. AA men have higher levels of circulating estrogens as compared to Caucasian Americans (CA) leading to an increased prenatal exposure to estrogens. Estrogen exposure has been shown to alter the epigenetic landscape of genes, and this prenatal exposure to estrogens could sensitize the AA men to altered steroid homeostasis as a product of aging, leading to an increased susceptibility to BPH. In this study, we examine the changes in estrogen receptors alpha and beta (ERα, ERβ) and steroid metabolism genes due to race and disease.

Methods: To examine the impact of race on BPH, we examined prostate tissue from 58 men. We had 21 normal transition zone controls and 37 BPH samples divided between CA and AA. Using multispectral quantitative multiplex IHC, we examined the steroid hormone related protein expression of ERα, ERβ, CYP7B1, AKR1C1, COX2, and E-cadherin on one FFPE tissue section. Using InForm® software, we spectrally unmixed each fluorophore and quantified optical density for each protein of interest. In addition to spectral unmixing, we also performed cell and tissue segmentation to examine protein localization.

Results: Examining the racial difference between the proteins of interest, we see a significant upregulation of ERα, ERβ, AKR1C1, and CYP7B1 in AA men compared to CA with a downregulation of COX2. When we examine the same proteins between BPH and normal, not accounting for race, we observed no significant differences. However, when we separated based upon race and disease, we found significant differences in AKR1C1 and CYP7B1, with AA men overexpressing these two steroid enzyme genes in both BPH and normal, above that of CA men. Interestingly, while there is a difference in expression in CA men in all of the markers between BPH and normal, this alteration does not exist or is opposite in AA men between BPH and normal.

Conclusions: Our study shows that there is a racial difference in steroid metabolism enzymes affecting the expression of ERα and ERβ between normal and BPH. While more in depth analysis remains, our study suggests that targeting of the estrogen pathway in treating BPH may be complicated by underlying racial differences in protein expression.

Inhibition of EZH2 enhances the antitumor efficacy of metformin in prostate cancer
Yifan Kong

ABSTRACT

Background: Enhancer of zeste homolog 2 (EZH2), a histone methyltransferase subunit of a Polycomb Repressor Complex 2 (PRC2), plays an important role in prostate cancer (PCa) development and progression. Upregulation of EZH2 is associated with advanced stage and poor prognosis of PCa, it is therefore likely to be a promising therapeutic target. Metformin, a drug that has been used to treat type II diabetes, was found to have anti-neoplastic activity in different cancers.

Method: Prostate cancer cell lines (LNCaP and 22Rv1), normal prostate epithelial cells (RWPE-1), and patient-derived prostate tumor explants were treated with metformin and/or EZH2 inhibitor GSK126. Proliferation and apoptosis were assessed.

Results: Herein, we report that the combination of metformin and EZH2 inhibitor GSK126 exerts synergistic inhibition on PCa cell growth, both *in vitro* and *in vivo*. Mechanistically, we identify that metformin can reduce EZH2's expression through upregulating miR-26a-5p, which is mediated by androgen receptor (AR). Furthermore, we show that AR binds to the promoter of miR-26a-5p and suppresses its transcription. Although metformin can remove AR from the miR-26a-5p promoter, the interaction between AR and EZH2, which usually exists in androgen-refractory PCa cells, strongly impedes the removal. However, GSK126 can inhibit the methyltransferase-dependent interaction between AR and EZH2, thus restoring metformin's efficacy in androgen-refractory PCa cells.

Conclusion: Collectively, our finding suggests that the combination of metformin and GSK126 would be an effective approach for future PCa therapy, and particularly effective for AR-positive CRPC.

Screening of histone post-translational modifications in castration resistant prostate cancer reveals CHD1 gene deficiency engenders a distinct epigenetic profile Joseph Gawdzik PhD, University of Wisconsin

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Screening of histone post-translational modifications in castration resistant prostate cancer reveals CHD1 gene deficiency engenders a distinct epigenetic profile

Category
Big Data as Engines for Discovery

Background
Epigenetic dysregulation contributes towards the development and progression of castration-resistant prostate cancer (CRPC). Moreover, genes encoding epigenetic modifiers are frequently altered in CRPC. Linking a tumors epigenetic phenotype to its genotype can inform therapies targeting a specific histone post-translational modification (PTM) profile. To interrogate this hypothesis, we examined a series of hormone-sensitive prostate cancer (HSPC) patient-derived xenografts (PDX) before and after their transition to CRPC using a novel high-throughput analysis of specific histone PTMs. Furthermore, we examined a unique histone profile linked to a Chromodomain-helicase-binding 1 (CHD1) deficiency, an epigenetic regulator that is deleted commonly (8-15%) in PC.

Methods
Levels of 48 unique histone PTMs were measured in paired HSPC and CRPC PDXs (n=12) using tandem LC-MS/MS. RNA-seq data was examined to characterize expression levels of lysine methyltransferases and demethylases (KDMs, KMTs) in these tumors. Co-expression of CHD1 and KDM/KMTs was examined using HSPC and CRPC patient tissue microarrays (n=70). Histone PTMs and KDM/KMT expression were also measured in CRISPR-mediated *CHD1*-KO CRPC cell lines (DU145, 22Rv1).

Results
A comparison of all PDX tumors revealed increased H3K14 acetylation in CRPC tumors relative to HSPC ($p < 0.05$). *CHD1*-deficient CRPC tumors showed a decrease in H3.3K36me2 ($p < 0.05$) and increases in H3.3K27me2 PTMs ($p < 0.05$). Expression of specific KMT/KDMs was assessed using RNA-seq data and a decrease in expression of the H3K36 KMT *NSD2/MMSET* in *CHD1*-deficient CRPC tumors was revealed ($p < 0.05$). Human PC tumor microarrays showed increased co-expression of CHD1 and NSD2 in CRPC versus HSPC samples (60% vs 32% $p < 0.05$). *CHD1*-KO CRPC cell lines show similar trends in H3.3 methylation; increasing H3.3K27me2 levels, decreasing H3.3K36me2 levels, and a 1.6-fold decrease in *NSD2* expression ($p < 0.05$).

Conclusions
We have identified a unique CRPC epigenetic profile in *CHD1*-deficient PC tumors and have implicated a reduction in *NSD2* expression in this profile's formation. This research raises the possibility of a relationship between CHD1 and NSD2 in supporting epigenetic programs in PC tumors. Finally, this profile provides potentially targetable epigenetic processes for therapeutic intervention of CRPC.

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Methoxychalcone derivative as a potent inhibitor of aggressive prostate cancer through glycolytic targeting
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Methoxychalcone derivative as a potent inhibitor of aggressive prostate cancer through glycolytic targeting

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Introduction & Objective

Patients treated for aggressive, potentially metastatic prostate cancer (PC) ultimately relapse to incurable hormone refractory, or castration resistant PC (CRPC). Standard of care therapies include second-generation anti-androgens (Enzalutamide-Enz, Abiraterone-Abi) or chemotherapy which modestly increase patient lifespan. We sought to generate new therapeutic strategies to improve patient survival in CRPC.

Methods

We generated a compound library of chalcones, natural products observed in many plants. SSMDL-0086 was selected based on viability screen of PC cells, exhibiting high potency and minimal toxicity. SSMDL-0086 impaired clonogenic activity, proliferation, migration and invasion of aggressive 22RV1, C4-2 and DU145 androgen-independent PC cells. These cell lines were implanted as s.c. xenografts in NSG mice (treated i.p. 50mg/kg SSMDL-0086 daily.) To identify targets of SSMDL-0086, liquid chromatography/mass spectrometry (LC/MSMS) was performed on C4-2 and DU145 with SSMDL-0086 treatment. Targets were validated with western blot and immunohistochemistry of treated xenografts. Glycolysis pathway validated by Seahorse glycolytic stress assay, and DESI-MS of treated xenografts. In vitro colony formation, and in vivo xenografts were performed combining SSMDL-0086 with Enz or Abi. LuCaP136 and 147 cells were embedded in Matrigel, grown 15 days in SSMDL-0086, or serially transplanted in mice. Nine compounds with alterations of SSMDL-0086 were further assayed for improvements in biochemical activity.

Results

We identified SSMDL-0086, a novel potent inhibitor of aggressive PC cells in vitro (IC50 1uM) and in vivo, decreasing cell and tumor growth of androgen-independent PC. MS identified impairment of glycolysis through decreased PGK1 and decreased ECAR levels by Seahorse assay. SSMDL-0086 synergized with Enz or Abi to abrogate colony formation, and significantly impaired tumor growth in either combination compared to independent treatments. SSMDL-0086 decreased metastatic colonization in C4-2 and DU145 intracardiac injected mice. SSMDL-0086 treatment inhibited tumorsphere growth of LuCaP 136 and 147 PDX lines, and proliferation of ex vivo treated patient samples. New derivatives of SSMDL-0086 exhibit lower IC50 values and have promising in vitro efficacy.

Conclusions

SSMDL-0086 and derivatives are novel methoxychalcone inhibitors of aggressive PC which possess great potential for treatment as single agents and in combination with current standard of care treatments for CRPC, Enz and Abi, making it a candidate for improvement of CRPC therapy.

Development of Lidocaine-Eluting Catheter Catheter- Induced Bladder Pain

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SBUR ABSTRACT

Development of Lidocaine-Eluting Catheter Catheter-Induced Bladder Pain.

Authors: Eun Bi Jang[1], Ji Young Lee[2], Young Eun Yoon[2], Sung Yul Park[2], Hong Sang Moon[2*]

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Introduction and Objective

The ureter catheter and Foley catheter stimulate the bladder and cause pain. In this study, we developed a lidocaine-eluting catheter and tried to determine if it could reduce catheter-induced bladder pain in a rat cystitis model with silicon catheter in urinary bladder.

Methods

A play lactic-co-glycolic acid (PLGA) sheet containing lidocaine was obtained through electrospraying. The PLGA sheet was wound around the 5Fr silicon catheter and place in an oven to be adhered to the catheter. Five catheter-induced bladder pain model groups were generated in 8-week-old female Sprague-Dawley rats (160±30g). To maximize the bladder pain due to the catheter, we developed cyclophosphamide (CYP)-induced cystitis model. Five groups were (A) no catheter in bladder, (B) catheter in bladder, (C) catheter in CYP-induced cystitis bladder, (D) lidocaine-eluting catheter, and (E) lidocaine-eluting catheter in cystitis bladder. Continuous cytometry (CMG) and behavioral assessment were performed to estimate the bladder pain.

Results

The average thickness or the sheets was 51.8±2.4 μm regardless of the mass ration of PLGA and lidocaine. The sheet exhibited a sustained lidocaine release property, where the lidocaine was released continuously and stably for up to 7 days. In a rat experiments, the lidocaine-eluting catheter diminished frequent bladder contracture and bladder instability caused by cystitis and indwelled catheter (Figure

1). The mean intercontractile intervals (seconds) of each group were (A) 223, (B) 151 (C) 127, (D) 178, and (E) 151.5 seconds. The amplitude (max-min) pressure of groups were (A) 20.7, (B) 32.8, (C) 45.6, (D) 29.9, and (E) 35.6 mmH_20. Similarly, the silicon catheter and CYP-induced cystitis caused pain behavior (eye closing and hypolocomotion) and lidocaine-eluting catheter reduced it.

Conclusions

Lidocaine-eluting silicone catheters are effective in reducing catheter-induced bladder pain and stabilizing bladder function in rat cystitis models.

Keywords: catheter, lidocaine, PLGA

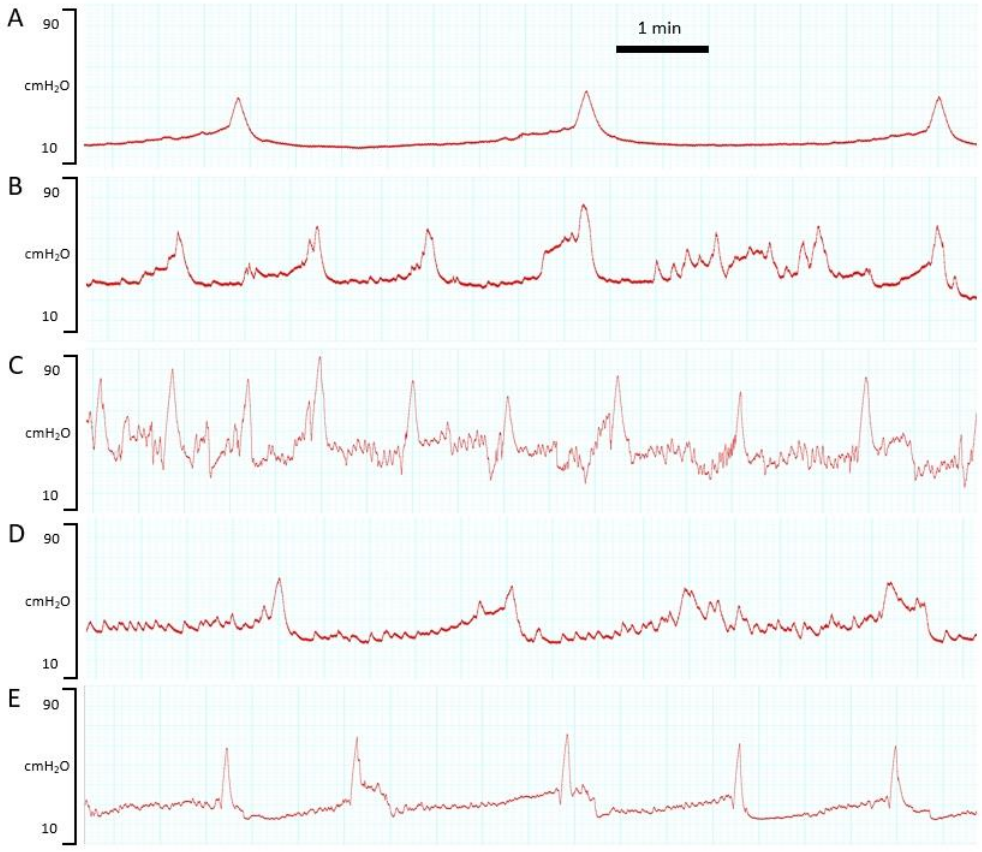

Figure 1. Raw traces of representative cystometric pressure recording (cmH2O) of each group.

The impact of ginsenoside and everolimus use on renal cell carcinoma

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Dae Keun Kim, Sung Yul Park, Young Eun Yoon, Department of Urology, Hanyang University College of Medicine, Seoul, Korea

SBUR ABSTRACT

The impact of ginsenoside and everolimus use on renal cell carcinoma

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Introduction and Objective

Ginsenoside Rg3 and Rh2 from ginseng have been known to inhibit cancer growth and metastasis. However, the anticancer effects of Rg3 and Rh2 in kidney cancers have not been evaluated. In this study, we investigate the anticancer effect of ginsenoside Rg3 and Rh2 on clear cell kidney cancer and the possible synergic effect of ginsenoside and mTOR inhibitor.

Methods

We investigated the efficacy of Rg3 and Rh2 alone in human renal cell carcinoma cell lines (Caki-1, ACHN, A498). Cell viability was measured by CCK8 assay. To evaluate synergic effect of ginsenoside and mTOR inhibitor, everolimus was simultaneously treated with Rg3 or Rh2, followed by estimation of combination index to confirm the simultaneous treatment effect of the two substances. A fundamental pathway analysis was performed through cell cycle analysis and cDNA microarray.

Results

Ginsenoside Rg3 and Rh2 showed concentration-dependent inhibitory effects on Caki-1, ACHN, A498 cell growth in CCK8 assays. Everolimus also showed concentration-dependent anticancer effect on same cell lines. In combination treatment tests, ginsenoside Rg3 and everolimus showed synergic effects (combination index, CI 0.3 - 0.85), while combination of ginsenoside Rh2 and everolimus showed only additive effects. Gene expression alteration in the PI3K-Akt signaling pathway (32 genes), Rap1 signaling pathway (26 genes), Ras signaling pathway (22 genes) and HIF-1 signaling pathway (11 genes) was analyzed in cDNA microarray.

Conclusions

Ginsenoside Rg3 and Rh2 showed anticancer effect in clear cell kidney cancer cell lines. Especially, ginsenoside Rg3 and everolimus showed strong synergic effect when treated in same time. These results suggest a new alternative to kidney cancer treatment.

Keywords: Ginsenoside, Rg3, Rh2, RCC, Everolimus

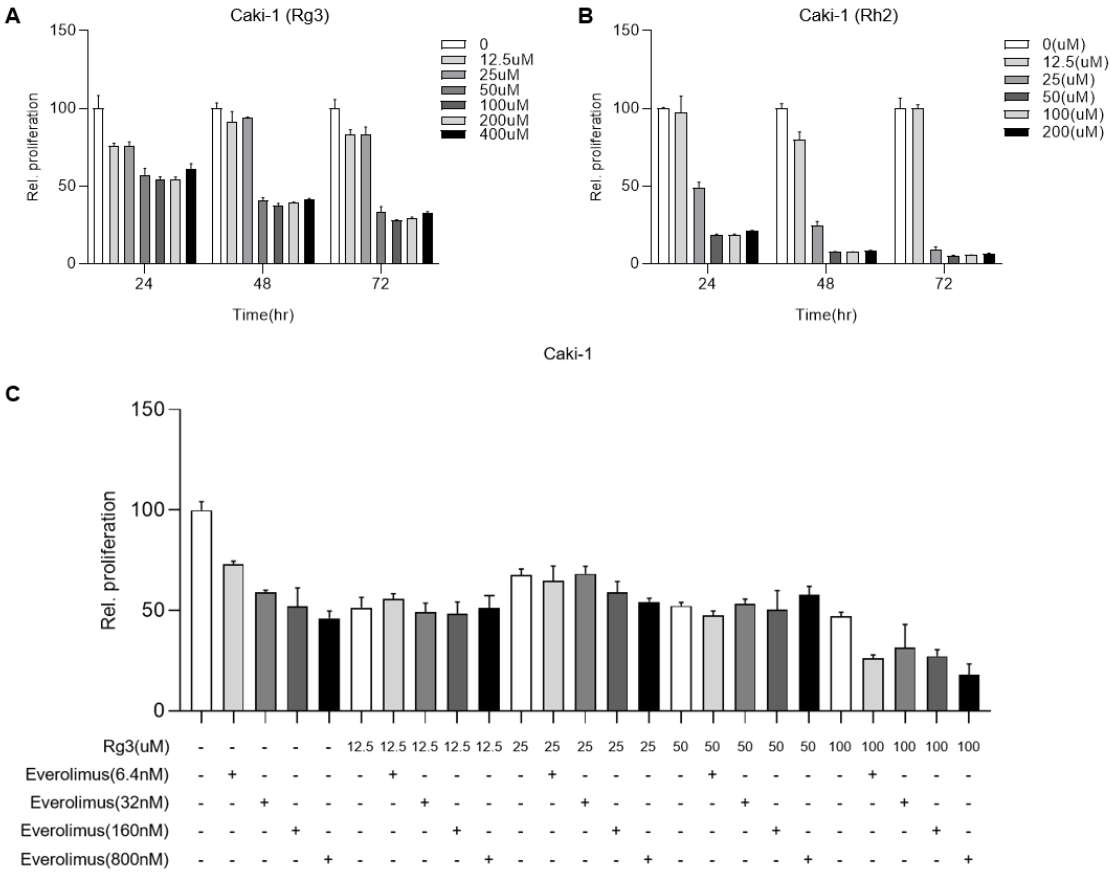

Figure 1. Measurement of cell viability when Rg3 and Rh2 are treated alone or in combination. Cell viability was decreased in a dose-dependent manner with Rg3 and Rh2, and cell proliferation was inhibited at high concentrations when Rg3 and Everolimus were treated together.

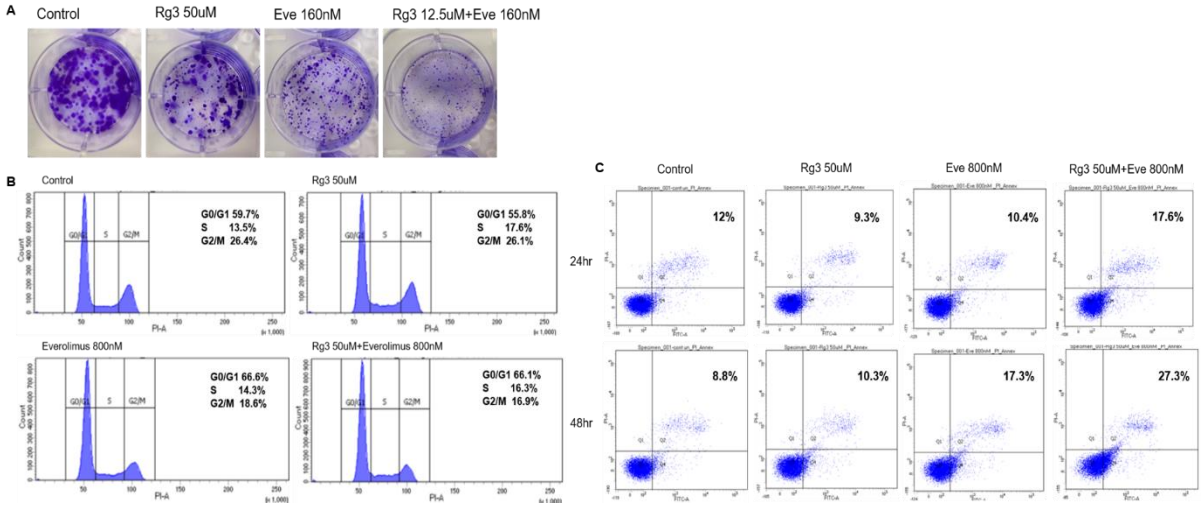

Figure 2. Rg3 induces apoptosis rather than controlling the cell cycle. The inhibition of Rg3-induced cell proliferation was confirmed by apoptosis rather than regulation of the cell cycle.

The Anticancer Effect of Ginsenoside Rg3 and Rh2 in Renal Cell Carcinoma

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SBUR ABSTRACT

The Anticancer Effect of Ginsenoside Rg3 and Rh2 in Renal Cell Carcinoma

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Introduction and Objective

Ginsenoside Rg3 and Rh2 from ginseng have been known to inhibit cancer growth and metastasis. However, the anticancer effects of Rg3 and Rh2 in kidney cancers have not been evaluated. In this study, we investigate the anticancer effect of ginsenoside Rg3 and Rh2 on clear cell kidney cancer.

Methods

We investigated the efficacy of Rg3 and Rh2 alone in human renal cell carcinoma cell lines (Caki-1, ACHN, A498). Cell viability was measured by CCK8 assay. To evaluate effect of ginsenoside was treated with Rg3 or Rh2, followed by estimation of combination index to confirm the simultaneous treatment effect of the two substances.

Results

Ginsenoside Rg3 and Rh2 showed concentration-dependent inhibitory effects on Caki-1, ACHN, A498 cell growth in CCK8 assays (Figure 1 and 2).

Conclusions

Ginsenoside Rg3 and Rh2 showed anticancer effect in clear cell kidney cancer cell lines. These results suggest a new alternative to kidney cancer treatment.

Keywords: Ginsenoside, Rg3, Rh2, RCC

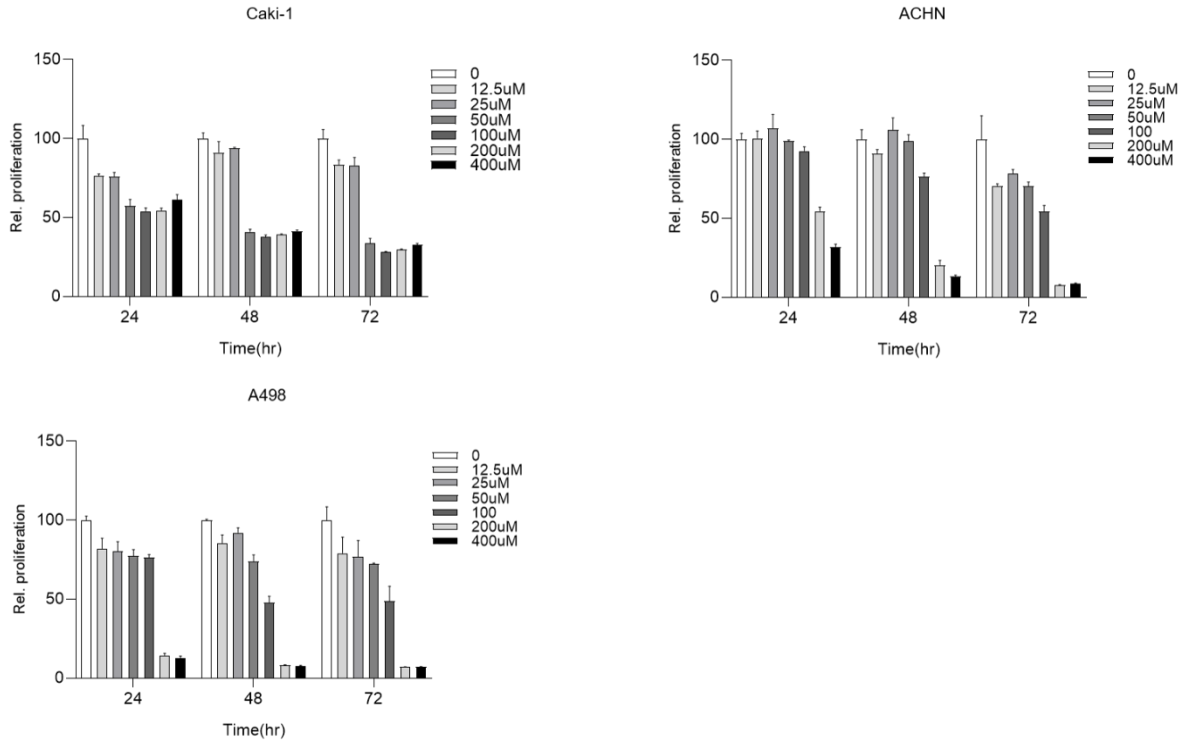

Figure 1. Ginsenoside Rg3의 viability assay

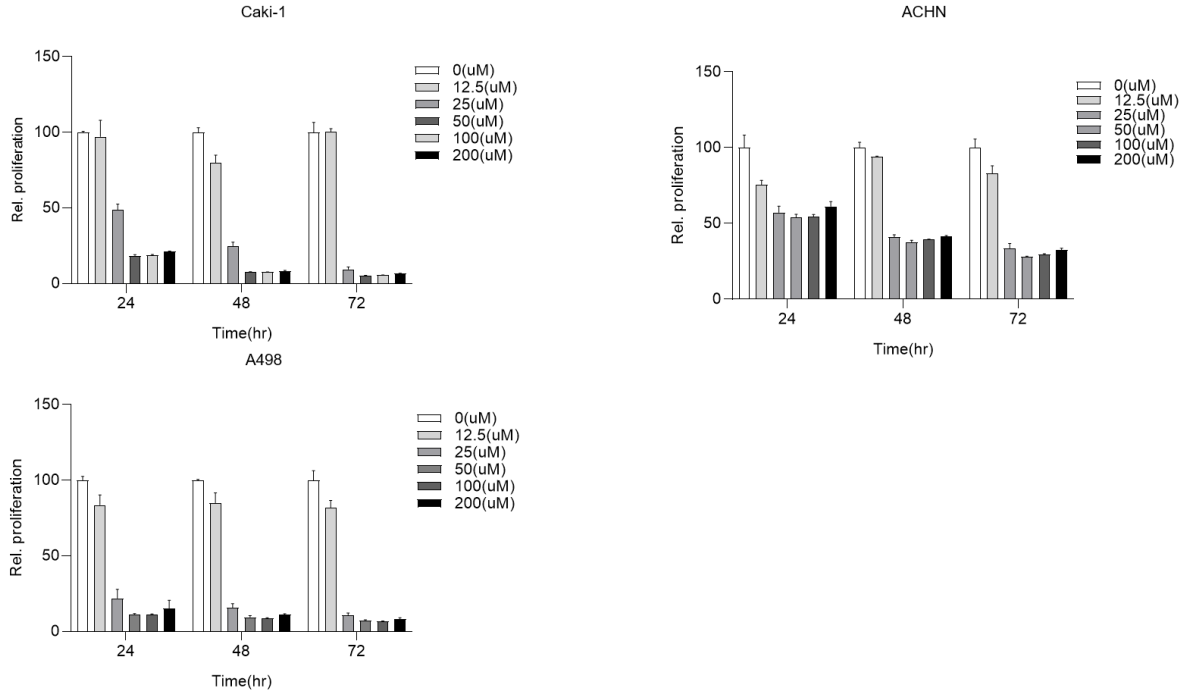

Figure 2. Ginsenoside Rh2의 single drug viability assay

A preclinical study of the combination treatment of high-dose testosterone and CDK4/6 inhibitors in CRPC

2019 Travel Award Winner

Wanting Han, University of Massachusetts Boston *Anthia A Toure, Dong Han, Postdoctoral Fellow, Shuai Gao, Research Assistant, Professor Changmeng Cai, Assistant Professor*

A preclinical study of the combination treatment of high-dose testosterone and CDK4/6 inhibitors in CRPC

Background: High-dose testosterone (high-T) treatment can suppress the growth of castration-resistant prostate cancer (CRPC) in preclinical and clinical trials. While androgen receptor (AR) is known for its transcriptional activation function, it also has repression function on DNA replication and repair genes. Mechanistically, we have demonstrated that AR globally recruits hypophosphorylated retinoblastoma protein (Rb) to DNA replication gene loci and strengthens the activity of Rb-E2F suppressor complex. This finding is consistent with a recent study on castration-resistant LuCaP models, which shows that the most robust molecular phenotype for high-T treatment is the suppression of E2F transcriptional output. It suggests a novel strategy to enhance the efficacy of high-T treatment with CDK4/6 inhibitors, which block Rb hyperphosphorylation. However, recent sequencing studies in CRPC indicate that ~10-15% of tumors have Rb-loss. Therefore, if these patients can be benefitted from high-T treatment or the combination remains to be determined.

Methods: Lenti-viral Rb silencing and CRISPR/Cas9 knock-out cell lines were created in CRPC cells, and the efficacy of the combination treatment of high-T with a CDK4/6 inhibitor, palbociclib were tested in the xenografts derived from these cell lines and also in LuCap CRPC models.

Results: Rb depletion rapidly impaired the AR-mediated transcriptional repression on DNA replication/repair genes and suppression of cell growth. However, high-T treatment still suppresses the expression of DNA replication genes and cell growth even when Rb is completely knocked out, both *in vitro* and *in vivo*. These findings suggest that Rb is contributing but not absolutely required for AR repression activity on DNA replication/repair. Significantly, we found that the interaction of E2F1 and a Rb-like pocket protein p130 was enhanced in Rb knock-out cells and this interaction was dependent on the phosphorylation status of p130, suggesting p130 may function as a substitute of Rb in Rb-loss CRPC cells. For the combination treatment, we found that palbociclib can enhance the efficacy of high-T treatment in cell line and xenograft models. However, this enhancing effect of palbociclib appeared to be dependent on the expression of Rb as it can be only seen in the Rb-positive but not Rb-silenced models.

Conclusions: Our study provides some new insights into the molecular basis of high-T treatment in CRPC. It suggests that high-T treatment can be used even in Rb-null CRPC. However, the synergistic effect of the combination treatment of high-T and CDK4/6 inhibitors is still dependent on the expression of Rb in CRPC cells.

Prostate Neuroendocrine Cell Serotonin Aids to Prevent Microbial Infection

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Jonathan Zhu - UW-Madison Summer Program in Undergraduate Urology Research, Madison, WI; Peiqing Wang - Cardiovascular Research Center, Madison, WI; Celeste Underriner - UW-Madison Molecular and Cellular Pharmacology Graduate Program, Madison, WI; Laura Hernandez - UW-Madison Dept. of Dairy Science, Madison, WI; Nathan Tykocki - University of Vermont Dept. of Pharmacology, Burlington, VT; Tian Shen - University of Columbia Dept. of Medicine, New York City, NY; Jonathan Barasch - University of Columbia Dept. of Medicine, New York City, NY; Michael Romero - Mayo Clinic College of Medicine & Science, Dept. of Physiology & Biomedical Engineering and Nephrology & Hypertension, Rochester, MN; Chad Vezina - UW-Madison Dept. Comparative Biosciences, Madison, WI

Prostate Neuroendocrine Cell Serotonin Secretion Aids to Prevent Microbial Infection

BACKGROUND: The role of prostatic neuroendocrine cells (pNECs) and neuroendocrine differentiation has been characterized in the context of prostate cancer but their role in normal prostate biology is poorly understood. The goal of this study is to test the hypothesis that pNECs secrete serotonin to drive prostatic smooth muscle contraction and protect the prostate from bacterial infection by restricting access to prostatic ducts.

METHODS: We used immunohistochemistry and reporter genes to characterize expression patterns of toll like receptors (TLRs), neuropeptides and their receptors, and cell identity markers in mouse prostatic tissue sections. We deployed genetically encoded calcium sensors in mouse prostatic smooth muscle to examine contractile responses to lipopolysaccharide (LPS) and serotonin receptor agonists *in vitro*. We also used mice deficient in tryptophan hydroxylase 1 (TPH1) to test whether prostatic serotonin production protects mice from *E. coli* infection *in vivo*.

RESULTS: We localized the LPS receptor TLR4 and serotonin to pNECs and the serotonin receptor HTR2B to KIT+ smooth muscle adjacent stromal cells. We found that LPS, serotonin and the HTR2B agonist BW273C86 stimulate mouse prostatic smooth muscle contraction *in vitro*. We also found that TPH1 deficient mice develop more profound prostate infections than control mice in response to intraurethral instillation of uropathogenic *E. coli*.

CONCLUSION: Our findings support the hypothesis pNECs express TLR4 and are thereby capable of responding to bacterial LPS and that pNEC derived serotonin stimulates prostatic muscle contraction and protects the mouse prostate from infection by intraurethral instilled uropathogenic *E. coli*. We therefore propose that pNECs initiate an innate immune response that can protect the prostate from bacterial infection by expelling ascending microbes and blocking access to prostatic ducts.

Lysine-specific demethylase 1A (LSD1) and androgen receptor (AR) against castration-resistant prostate cancer

2019 Travel Award Winner

Victor Pham, University of California, Irvine

Victor Pham, Vinh X Le, Dongjun Fu, Thanh NH Le, Marvin Miller, Matthew Tippin, Liankun Song, Xiaolin Zi

Sanguinarine (SNG) is a novel dual-inhibitor of Lysine-specific demethylase 1A (LSD1) and androgen receptor (AR) against castration-resistant prostate cancer

Victor Pham, Vinh X Le, Dongjun Fu, Thanh NH Le, Marvin Miller, Matthew Tippin, Liankun Song, Xiaolin Zi

Background: Lysine-specific demethylase 1A (LSD1) is considered as a promising target for treatments of castration-resistant prostate cancer ascribed to its interaction with androgen receptor (AR) and critical involvement in neuroendocrine differentiation. However, LSD1 inhibitors currently on clinical trials (i.e. GSK2879552 and ORY-1001) are not highly potent and specific to prostate cancer. Subsequently, we are seeking novel LSD1 inhibitors which can target specific molecular mechanisms that accelerate the progression to castration-resistant prostate cancer and the resistance to anti-androgen therapies, such as Enzalutamide.

Methods: Structural similarity search was performed for a series of analogs of flavin adenine dinucleotide (FAD) which is a co-factor of LSD1, and dihydrotestosterone (DHT) analogs. Molecular docking was used to predict the binding energy within the putative activity pocket of LSD1 and AR. LSD1 enzyme activity was tested using H3K4Me and H3K9Me peptides as substrates. AR transcriptional activity luciferase reporter assay was carried out for screening AR inhibitors. Cellular thermal shift assay (CETSA) was performed to evaluate the target engagement in prostate cancer cell lines. The *in vitro* Surface Plasmon Resonance (SPR) assay was set up for direct binding affinity of inhibitors to LSD1 and/or AR. Cryogenic Electron Microscopy is in progress to obtain the detailed image of binding position (the interacting amino acids and distance) of SNG to LSD1 and AR. Western blotting analysis and quantitative PCR methods were used to detect the substrate modification of LSD1 and the expression of AR target genes. Cell growth inhibition was tested against androgen-sensitive, castration-resistant and neuroendocrine/small cell carcinoma cell lines. Stable LSD1 suppression by short-hairpin RNA (shRNA) and LSD1 overexpression in prostate cancer cell lines were established to determine whether LSD1 is partially a required target for the growth-inhibitory activity of SNG. Patient-derived organoids were used to test the activity of SNG against the heterogeneity of prostate cancer.

Results: A series of naturally occurring polycyclic ammonium ions were identified to inhibit LSD1 enzyme, AR transcription, or both activities. Among the screened compounds, SNG is the most potent compound that equally inhibits both AR and LSD1 activities with an IC_{50} of about $2.13\pm0.18\mu M$ and $2.07\pm0.45\mu M$, respectively. SNG directly engages both LSD1 and AR protein, resulting in the down-regulation towards the expression of AR, AR variant 7 (ARV7), and AR target genes. The binding affinities of SNG to LSD1 and AR are 1.10±0.42μM and 2.54±0.89 μM, respectively based on our SPR results. These effects of SNG are partially dependent on the expression levels of LSD1 as shown in LSD1 knockdown (shRNA) and overexpression experiments. SNG also potently inhibits the growth of several patient-derived organoids.

Conclusion: We provide the strong evidence that SNG can be a structural model as a dual-inhibitor to both LSD1 and AR in prostate cancer. SNG or its derivatives are the newly potential generation of LSD1 inhibitors particularly deserving further investigation for treatment of castration-resistant prostate cancer, including its neuroendocrine subtype.

Characterization of a novel androgen receptor variant, VBl-1, in bladder cancer

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Characterization of a novel androgen receptor variant, VBl-1, in bladder cancer

Background: Bladder cancer is the sixth most common cancer in men and the seventeenth most common cancer in women. A potential mediator for this gender disparity is the androgen receptor (AR), a ligand-dependent transcriptional factor dependent on androgen binding. Cell and tumor cancer models, however, have an increase in AR splice variants, some of which lack a ligand binding domain, but retain their ability to bind DNA and activate transcription. We have isolated and characterized a mutated AR variant, VBl-1, in human bladder cancer cell lines at the RNA and protein levels. **Methods:** qPCR was used to quantify VBl-1 mRNA in the following nine human bladder cancer cell lines: UM-UC-3, T24, J82, TCCSUP, RT4, SW780, HT1376, HT1197, and 5637. Western blot analysis was used to verify full length and low molecular weight AR proteins and AR proteins following knockdowns of total AR and VBl-1. Immunofluorescence was used to localize total AR within the cells. CCK8 and caspase 3/7 assays were used to analyze cell viability and apoptosis respectively, after siRNA-mediated depletion of VBl-1. VBl-1 cloning used 3' RACE followed by cloning into TOPO and pcDNA3(+) plasmid. VBl-1 gene activation was assessed through AR dependent promoter-Luciferase transactivation assays. **Results:** The tested cell lines expressed varying levels of full-length AR and low molecular weight AR proteins. qPCR quantification also verified that UM-UC-3 and TCCSUP cells had the highest expression of AR, followed by T24 and RT4 with medium expression, SW780, HT1376, and 5637 with low expression. J82 and HT1197 cells did not express full length AR, but expressed small amounts of low molecular weight AR. VBl-1 was expressed in 6 out of the 9 cell lines including: UM-UC-3, T24, J82, TCCSUP, HT1197, and HT1376. Localization of AR appeared to be both nuclear and cytoplasmic in the medium to higher expressing total AR cell lines. Immunofluorescence in VBl-1 overexpressing UM-UC-3 clones showed high levels of AR nuclear localization. Knockdown of VBl-1 in UM-UC-3, T24, and TCCSUP cells resulted in significant decreases in cell viability by 6 days as a result of apoptosis (in UM-UC-3 and T24 cells). VBl-1 transactivated the androgen promoter-luciferase construct in a dose dependent manner. **Conclusion:** These results show, for the first time in bladder cancer, a novel mutated AR variant capable of localizing to the nucleus, promoting AR-dependent gene expression, and affecting tumor cell viability and apoptosis. Targeting low molecular weight AR may provide novel therapeutic approaches for bladder cancer treatment.

Molecular determinants for enzalutamide-induced oncogenic transcription in prostate cancer

Fuwen Yuan, Duke University

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Molecular determinants for enzalutamide-induced oncogenic transcription in prostate cancer

Background

Enzalutamide, a second-generation androgen receptor (AR) antagonist, has demonstrated clinical benefit in men with prostate cancer. However, it only results in a temporary response and modest increase in survival, indicating a rapid evolution of resistance. Our previous studies suggest that enzalutamide may function as a partial transcriptional agonist rather than a pure antagonist, but the underlying mechanisms for enzalutamide-induced oncogenic transcription remain poorly understood.

Methods

We performed RNA-seq to determine the impact of enzalutamide treatment on global transcription in prostate cancer cells. We performed chromatin immunoprecipitation (ChIP), quantitative chromosome conformation capture (3C), CRISPR/Cas9-based enhancer deletion, and RT-PCR analyses to study molecular regulation of enzalutamide-activated oncogenes. We used RNA-seq to assess the impact of silencing of GATA2, a cofactor of enzalutamide-liganded AR identified in this study, on enzalutamide-induced global transcription. We performed cell proliferation assays to determine whether the GATA2 inhibitor K7174 can sensitize prostate cancer cells to enzalutamide treatment.

Results

We found that enzalutamide stimulates expression of a novel subset of genes distinct from androgen-responsive genes. Treatment of prostate cancer cells with enzalutamide enhances recruitment of the pioneer factor GATA2, AR, Mediator subunits MED1 and MED14, and RNA Pol II to regulatory elements of enzalutamide-responsive genes. Knockdown of *GATA2*, *MED1* or *MED14* abolished enzalutamide-liganded AR activity. Mechanistically, GATA2 globally directs enzalutamide-induced transcription by facilitating AR, Mediator and Pol II loading to enzalutamide-responsive gene loci. Importantly, the GATA2 inhibitor K7174 counteracts enzalutamide-induced transcription by decreasing binding of the GATA2/AR/Mediator/Pol II transcriptional complex, contributing to sensitization of prostate cancer cells to enzalutamide treatment.

Conclusions

These results establish the concept that GATA2 directs enzalutamide-induced global oncogenic transcription in prostate cancer cells. Our findings provide mechanistic insight into the future combination of GATA2 inhibitors and enzalutamide for improved AR-targeted therapy.

Funding and Keywords

Source of Funding

GM120221, CA217297

Key words

AR, antagonist, GATA2, GATA2 inhibitor, transcription

Big-data analysis reveals a role of opiorphin encoding genes in prostate cancer and possible genetic mechanisms modulating tumor growth and androgen-sensitivity

Dr. Kelvin P. Davies, Albert Einstein College of Medicine *Dr. Amarnath Mukherjee, Mr. Augene Park*

Big-data analysis reveals a role of opiorphin encoding genes in prostate cancer and possible genetic mechanisms modulating tumor growth and androgen-sensitivity.

Background

The opiorphin family of genes (represented in humans by *ProL1, SMR3a* and *SMR3b)* encode peptides which act as a potent neutral endopeptidase (NEP) inhibitors. Because modulated NEP activity is associated with cancer development, several years ago a review postulated that dysregulated opiorphin expression may be involved in oncogenesis. This has recently been supported by reports associating changes in *ProL1 and hSMR3a* expression with breast and oropharyngeal cancer. Based on these reports, we initiated the studies described here to determine if *ProL1* plays a role in prostate cancer (PrCa).

Methods

We screened publicly available data bases (such as GEO and Oncomine) for evidence of an association between PrCa and opiorphin gene expression and then confirmed these finding using PrCa tissue arrays. Cell-lines representing early (LNCaP) and late stage (PC3) PrCa were engineered to overexpress *ProL1* and the growth of xenografted tumors from these lines compared to parent cell lines in male and female nude mice. The changes in global gene expression caused by overexpression of *ProL1* in these cell lines was determined by RNAseq to identify possible genetic mechanisms related to growth and progression of PrCa.

Results

Two datasets available on GEO showed an association between upregulation of genes encoding opiorphin and PrCa, which was confirmed using tissue arrays. Xenografted tumors derived from PC3 engineered to overexpress *ProL1* had an initial growth advantage over parent cell lines whilst tumors derived from LNCaP cells overexpressing *ProL1* had impaired growth in female mice. Global gene expression analysis suggested that overexpression of *ProL1* causes modulated expression of gene involved in signaling, angiogenesis and steroid response pathways. These pathways might be involved in overcoming the hypoxic barrier of the growing tumor and the development of androgen insensitivity.

Conclusions

In conclusion, this is the first report to provide evidence for a role of *ProL1* in PrCa. We demonstrate significantly higher expression levels of *ProL1* in cancerous, compared to non-cancerous, prostate tissue and that overexpression of *ProL1* in PrCa cell-lines modulates tumor growth in mouse xenograft models. We have demonstrated that overexpression of *ProL1* modulates genetic pathways that previous reports have associated with PrCa growth and progression and therefore may represent possible genetic mechanisms for the effect of *ProL1* on tumor development.

Hit-to-Lead Optimization of a First-in-Class FKBP52 inhibitor for the Treatment of Castration Resistant Prostate Cancer

Ashley Nichole Payan - PhD Student, University of Texas at El Paso
Naihsuan C. Guy, Marc B. Cox – PI

Hit-to-Lead Optimization of a First-in-Class FKBP52 inhibitor for the Treatment of Castration Resistant Prostate Cancer

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Preference: Poster

BACKGROUND: The folding, activation, and nuclear translocation of steroid hormone receptors involves no less than twelve proteins and at least four distinct complexes. At least one of these proteins, the FKBP52 cochaperone, is a promising therapeutic target for the disruption of a number of mechanisms important in prostate cancer (PCa). FKBP52 is a positive regulator of androgen (AR), glucocorticoid (GR) and progesterone receptor (PR) hormone binding, nuclear translocation, and transcriptional activity. FKBP52 regulates multiple, distinct steps within the AR signaling pathway, some of which are independent of Hsp90. Our data suggest that the proline-rich loop surface that overhangs the FKBP52 PPIase pocket is important and likely represents an AR interaction surface. Thus, our strategy aims to identify specific PPIase binding molecules that, when docked in the pocket, reorient the proline-rich loop leading to the disruption of FKBP52 interactions. We identified a hit molecule (GMC1) that inhibits FKBP52-mediated AR, GR and PR activity, AR-dependent gene expression in prostate cancer cells, AR-dependent proliferation of prostate cancer cells, and tumor growth in murine models.

METHODS: We screened rationally designed GMC1 modifications with the goal of increasing efficacy, reducing toxicity and ensuring bioavailability. Independent of GMC1, we also used *in silico* structure-based drug design to identify unique FKBP52-specific inhibitors for functional screening.

RESULTS: SAR screening for GMC1 analogs led to a modification library of 97 molecules that were screened at a 25 uM for inhibition of AR activity in AR-mediated luciferase assays. Analogs with 75% inhibition were screened in full dose response curves to determine IC_{50}. Five GMC1 analogs displayed significantly increased potency and were assessed for ADME properties. The two analogs with the most optimal metabolic stability, intestinal permeability and solubility will be moved forward in development as the new lead molecules. The *in silico* structure-based drug design identified 107 molecules, 4 of which displayed inhibition of AR activity in AR-mediated luciferase assays in the low micromolar range, with the most potent displaying an IC_{50} of 2 uM. We are currently testing for FKBP52 specificity in AR-mediated luciferase assays.

CONCLUSION: Based on previous SAR analysis of our first-in-class FKBP52 targeting drug, GMC1, we have identified new lead molecules based on GMC1 as a scaffold that represent unique chemotypes with improved potency.

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Facilitating Biological and Clinical Discoveries Using The Prostate Cancer Transcriptome Atlas

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Facilitating Biological and Clinical Discoveries Using The Prostate Cancer Transcriptome Atlas

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Background: Prostate cancer (PC) is a heterogeneous disease at the genomic level. Transcriptome analysis has proved to be useful for identification of molecular subtypes and disease associated genes. However, access to reported PC subtype information is limited and the paucity of clinical specimens in a single cohort makes it difficult to demonstrate the relevance of experimental findings and to formulate potential hypotheses.
Methods: We developed a web-based tool, the Prostate Cancer Transcriptome Atlas (PCTA), which can help researchers easily access virtually organized large transcriptome data comprised of 2,118 clinical specimens from 38 PC cohorts, as well as 568 PC adenocarcinoma samples from the TCGA. Notably, the PCTA includes subtype information, shown previously to be clinically relevant, that is not available from other databases, as well as Gleason grade and metastasis status. To increase accessibility for laboratory scientist and clinician users, we deployed this novel resource containing a large set of transcriptome profiles to a database linked to a web interface built using Python on top of the Django web framework.
Results: The current version of the PCTA provides applications in 3 major analysis functionalities. First, “Expression View” presents the association of a gene or gene set with PC by displaying the expression or activation score in distinct tumor subtypes or disease categories. Second, “Correlation View” presents correlation measures and plots for association between genes and/or gene sets in the specific disease contexts. Last, “GSEA and MRA” presents the results of gene set enrichment analysis, which shows enrichment scores in specific subgroups of tumors, as well as a list of master regulator candidates and their potential network relationships governing the gene set.
Conclusions: The PCTA facilitates biological and clinical discoveries by allowing user-friendly access to over one thousand human PC specimens. This web-based tool can be publicly accessible at http://www.thepcta.org.

Source of Funding: NIH, Department of Defense PCRP, Urology Care Foundation

Keywords: prostate cancer, data interrogation, database, software, disease association

Upregulation of Androgen Receptor Splice Variants ‚Äì an Inevitable Response to Androgen-Directed Therapies?

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Upregulation of Androgen Receptor Splice Variants – an Inevitable Response to Androgen-Directed Therapies?

Introduction & Objective

Increased expression of constitutively active androgen receptor splice variants (AR-Vs) is indicated as an important mechanism underlying resistance to androgen-directed therapies (ADT). Understanding how AR-Vs are upregulated may open new avenues to improving the therapeutic efficacy of ADT. Androgen-bound full length AR (AR-FL) is known to interact with a region in intron 2 of the AR gene, named AR binding site 2 (ARBS2), to repress the transcription of the AR gene. However, androgen stabilizes the AR-FL protein. Accordingly, we hypothesize that relief from AR-FL negative regulation of the transcription of the AR gene due to ADT inhibition of AR-FL activity leads to enhanced production of AR pre-mRNA and thereby AR-FL and AR-V mRNAs and that increased mRNA expression together with androgen-independent, constitutive stability of AR-V proteins provides a feed-forward mechanism leading to accumulation of AR-V proteins after ADT.

Methods

To test these hypotheses, we first analyzed RNA-seq data of metastatic castration-resistant prostate cancer patient samples for correlation between AR alternative and canonical splicing. We then specifically knocked down AR-FL expression via shRNA or a PROTAC degrader in preclinical models to test the requirement of AR-FL on AR-V induction by ADT. We also generated ARBS2 CRISPR-deleted cells to determine the involvement of ARBS2 in AR-V induction. Lastly, we measured the mRNA and protein stabilities of AR-FL and AR-Vs in response to ADT by mRNA and protein stability assays and pulse-chase analysis.

Results

Our analysis revealed a high correlation between AR alternative and canonical splicing events in clinical samples, supporting a coordinated expression of AR-Vs and AR-FL. This coordinated expression also occurs in preclinical models after ADT. Importantly, we showed the requirement of AR-FL and a critical involvement of ARBS2 in AR-V induction by ADT. We also found that, while unaffected by ADT, the mRNA and protein stabilities of AR-Vs are similar to that of AR-FL after ADT.

Conclusions

These results indicate that, in response to ADT, AR-FL and AR-V mRNAs are coordinately upregulated due to the disruption of the AR negative autoregulatory circuit. Albeit AR-Vs remain a minority of the AR population, there is an increased representation of AR-V proteins after ADT due to diminished AR-FL protein stability. Together, these findings suggest that AR-V upregulation is likely an inevitable response of prostate cancers to ADT.

Funding and Keywords

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Developing New Bone Metastasis Models through Tissue- Engineering and Microfluidics

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Title: Developing New Bone Metastasis Models through Tissue-Engineering and Microfluidics

Background: Metastasis is the primary cause of cancer-related death with bone being the prevalent site for prostate cancer metastasis. Development of therapeutics capable of preventing metastasis or slowing metastatic tumor growth has been hindered by the lack of appropriate tools for studying the metastatic bone microenvironment.

Methods: We have developed several tools to characterize and model the bone microenvironment. Using flow cytometry, we examine the cellular composition of the bone microenvironment with aging, diet-induced obesity, arthritis, and cancer progression. The cellular composition of diseased and healthy bone microenvironments can then be recapitulated *in vivo* in a tissue-engineered bone scaffold or *in vitro* in a microfluidic metastasis model.

Results: We demonstrate that comorbidities in animal models drastically alters the composition of the bone microenvironment, including the number of hematopoietic and osteoblastic stem cell niches, where cancer cells preferentially home during metastasis. Implantation of bone scaffolds in syngeneic mice permits the development of a functional bone microenvironment which can be used to study the homing of prostate cancer cells. Finally, we demonstrate that prostate cancer cells invade towards bone microenvironment stimuli.

Conclusions: By profiling and modeling the bone microenvironments more representative of the patient population, we can identify and test therapeutics aimed at preventing metastasis or slowing metastatic tumor growth.

MicroRNAs in metastatic lymph node as diagnostic tool for prostate cancer

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MicroRNAs in metastatic lymph node as diagnostic tool for prostate cancer.

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Prostate cancer (PCa) is one of the deadliest cancers due to metastatic process. Lymph nodes (LNs) are common sites of metastasis, are critical for initiating antitumor immune responses and considered as main pre-metastatic niche (PMN). Tumoral secreted factors as extracellular vesicles play a major role in PMN development. Pereira showed that metastatic cells from LN are more invasive than those which leaves tumor without passing through the LNs. Molecular changes in tumor cells could include aberrant expression of microRNAs (miRNAs) which shows great stability when they're packed in exosomes. The aim of this study is to determine a molecular signature of miRNAs from metastatic LNs and find it in exosomes of peripheral blood from PCa patients. The signature would work as a non invasive early diagnostic tool in PCa metastasis and contribute to stratify patients with a higher risk of developing metastasis.

Samples were divided into two groups: Group A, tissues of metastatic LN (7), healthy LN (8) and prostate tumor. Group B, blood samples of healthy men (10) and PCa patients (16). Microarrays GeneChip miRNA 4.0 array Affymetrix were used to obtain a miRNA signature of A & B groups. RNA was extracted with miRNeasy FFPE and exoRNeasy kits. Microarray results were analyzed with TAC software Affymetrix ($p<0.05$, fold change ± 2). Search of miRNA targets was performed by TargetScan, miRWalk and mircoT-CDS. Enrichment pathways analysis was performed by KEGG and DAVID. Western blot for CD63, CD81, CD9 was done for exosomes protein.

We obtained 7 miRNAs differentially expressed (DE) with $p<0.05$ in metastatic LN tissue. Two miRNAs (has-miR-23b-3p, has-miR-150-5p) of metastatic LN were identified in exosomes from PCa patients. According to results miRNA targets could be involved in process like osteoclast differentiation (CALCR), histone acetyltransferase activity (ING3), adherents junction, Wnt and TGF-beta pathways. This suggests that miRNAs could affect pathways involved in invasion and bone metastasis. We found the presence of CD63 marker in exosome preparation, indicating the isolation of a population of extracellular vesicles.

For the first time we prove that metastatic LN has a unique signature of miRNAs DE between PCa and healthy LN. We demonstrate that miRNAs of metastatic LN can be found in plasma exosomes of PCa patients. The results suggest that miRNAs could be used as a non-invasive diagnostic tool for patients with a higher risk of metastatic PCa. Further, we need to validate this miRNA signature and evaluate the sensitivity and specificity of this possible tool.

WLS Promotes Cellular Viability and Resistance to Enzalutamide in CRPC

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Title: WLS Promotes Cellular Viability and Resistance to Enzalutamide in CRPC

Background: De-regulation of Wnt signaling pathways has been shown to be associated with progression of castration-resistant prostate cancer and more recently, studies indicate that both canonical and non-canonical Wnt pathways may mediate resistance to anti-androgen therapies such as enzalutamide. However, the mechanisms by which Wnt signaling is altered in prostate cancer remain poorly understood. Wnt pathway function begins with Wnt biogenesis and secretion from Wnt signal sending cells. While previous studies have investigated downstream mechanisms of Wnt pathway alterations in prostate cancer, little is known on the role of Wnt secretion mediating proteins. Wntless (WLS) is thought to be essential for the secretion of all Wnts. In this study, we sought to understand the role of WLS in prostate cancer.

Methods: RNA-seq and gene set enrichment analysis (GSEA) were used to understand expression profile changes in enzalutamide-resistant C4-2B-MDVR (MDVR) cells versus parental C4-2B cells. Quantitative-PCR and western blot were used to confirm RNA-seq data and to assess expression changes of gene targets of interest. Rv1 cells were used as a separate model of enzalutamide-resistant prostate cancer. RNAi was used to inhibit WLS expression. RNA-seq and GSEA were used to assess transcriptional changes in response to WLS inhibition in MDVR cells. Cell viability, colony formation, and PSA ELISA assays were used to assess cell growth and survival.

Results: Transcriptomic profiling revealed enriched Wnt pathway signatures in MDVR versus parental C4-2B cells. We further show that MDVR cells upregulate Wnt signaling and overexpress WLS. Inhibition of WLS decreases Wnt signaling, markedly attenuates prostate cancer cell viability, induces apoptosis, and re-sensitizes enzalutamide-resistant cells to enzalutamide treatment. RNA-seq reveals several HALLMARK gene sets altered by WLS inhibition, including cell cycle related pathways and apoptosis. Notably, we also found the HALLMARK_ANDROGEN_RESPONSE gene set downregulated in response to WLS inhibition. Lastly, we show that inhibition of WLS reduces AR and AR-variants expression and downstream signaling, supporting our RNA-seq data.

Conclusions: Our findings support a role for WLS in the progression of prostate cancer to a treatment-resistant state in part through regulation of AR expression and signaling. Further efforts to understand Wnt signaling pathway alterations in this disease may lead to the development of novel treatments.

Metabolic Re-wiring in African-American Prostate Cancer: A Role for Adenosine-Inosine Axis in Tumor Progression Christy Charles, Graduate Student, Baylor College of Medicine

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Metabolic Re-wiring in African-American Prostate Cancer: A Role for Adenosine-Inosine Axis in Tumor Progression

Introduction: Prostate cancer (PCa) burden is higher in African-American (AA)men compared to European-American (EA) men. To discern the markers of the aggressive tumor, our lab has pioneered the understanding of reprogrammed metabolism, a hallmark of tumor progression, in AA and EA PCa. Analysis of 190 metabolites in AA and EA PCa tissues followed by validation of key pathway components in plasma and urine revealed high inosine to adenosine ratio in AA PCa compared to EA PCa. Consistent with this, the enzyme adenosine deaminase (ADA), which converts adenosine to inosine was also found to be elevated in AA PCa. The current study will address the role of the adenosine-inosine axis in PCa progression and attempt to delineate its significance in the clinical setting.

Methods: Analysis of metabolites in tissues, plasma and urine was done using LC/MS. Tissue microarray analysis (TMA) was done to check the expression of ADA in tissues. To determine the function of ADA in PCa progression, the gene was overexpressed in MDA-PCa-2A (AA) and LNCaP (EA) cells using lentiviral transduction (termed ADA OE). Molecular studies were performed using qPCR, ELISA and western blotting.

Results: Overexpression of the enzyme ADA in PCa cells confers an anchorage-independent growth phenotype (a.k.a. Anoikis Resistance) associated with a reduction in Integrin β1 (ITGB1). Concomitantly, ADA OE cells exhibited higher invasion potential. Anoikis resistance is a key prerequisite for metastasis and a hallmark of circulating tumor cells (CTCs). Molecular analysis revealed downregulation of E-cadherin (epithelial marker) and Bim (pro-apoptotic marker), and upregulation of XIAP (anti-apoptotic marker), all of which support anoikis resistance phenotype in these cells. ADA OE cells had reduced cAMP levels, and addition of external cAMP resulted in reversal of anoikis resistance. ADA OE cells also showed a decrease in Rap1 levels. Mechanistically, it is postulated that high inosine upon ADA OE activates adenosine receptors A1 and A3, which causes a decrease in cAMP levels. Decreased cAMP affects the Epac-Rap1 signaling, a key regulator of integrin-mediated adhesion.

Conclusion: Elevated inosine to adenosine ratio associated with elevated expression of ADA is a metabolic hallmark in PCa, associated with anoikis resistance and possibly increased CTCs. Our findings also support the potential of using inosine to adenosine ratio in plasma/urine as a predictive marker for PCa incidence or progression. Also, ADA could serve as a potential therapeutic target.

Plk1 inhibition enhances the efficacy of BET epigenetic reader blockade in castration-resistant prostate cancer
Fengyi Mao, Graduate student, University of Kentucky Fengyi Mao, Dr. Jie Li, Ruixin Wang, Yifan Kong and Dr. Xiaoqi Liu

PLK1 inhibition enhances the efficacy of BET epigenetic reader blockade in castration-resistant prostate cancer

Background

Polo-like kinase 1 (PLK1), a crucial regulator of cell cycle progression, is overexpressed in multiple types of cancers, and has been proven to be a potent and promising target for cancer treatment. In case of prostate cancer, we once showed that anti-neoplastic activity of PLK1 inhibitor is largely due to inhibition of androgen receptor (AR) signaling. However, we also discovered that PLK1 inhibition causes activation of the β-catenin pathway and increased expression of c-Myc, eventually resulting in resistance to PLK1 inhibition. JQ1, a selective small molecule inhibitor targeting the amino-terminal bromodomains of BRD4, has been shown to dramatically inhibit c-Myc expression and AR signaling, exhibiting anti-proliferative effects in a range of cancers. Since c-Myc and AR signaling are essential for prostate cancer initiation and progression, we aim to test whether targeting PLK1 and BRD4 at the same time is an effective approach to treat prostate cancer.

Methods

In the study, we tested the efficacy of combination therapy between PLK1 inhibitor GSK461364A and BRD4 inhibitor JQ1 both in vitro and in vivo. We detected cell proliferation and apoptosis in CRPC cell lines by flow cytometry and western blot. Besides, effect of this novel therapy was also tested in a human derived CRPC xenograft model, the tumor cell proliferation and apoptosis of which was monitored by immunofluorescence staining. Lastly, we revealed the mechanism under this synergy by western blot in vitro and a 22RV1-derived xenograft model in vivo.

Results

We verified the effects of GSK461364A and JO1 on CRPC cell lines, which could synergistically inhibit cell proliferation, induce cell apoptosis and arrest cell cycle in mitosis. Consistently, the novel combination therapy could dramatically decreases tumor volumes and remarkably induces tumor cell apoptosis in the patient-derived xenograft model. Finally, we determined the mechanism of the combination therapy is due to the co-suppression of c-Myc and AR.

Conclusions

In summary, our in vitro and in vivo data support a strong synergy of PLK1 and BRD4 inhibition in CRPC progression from two aspects: 1) JQ1 suppresses c-Myc expression, thus enhancing the efficacy of PLK1 inhibitor; and 2) PLK1 and BRD4 inhibitors act synergistically in inhibition of AR signaling. Thus, the novel combination strategy can be considered for clinical trials to reduce the resistance and increase the efficiency of JQ1.

Urinary Bacteria Meet Heme Metabolism at the Bladder Surface

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Urinary Bacteria Meet Heme Metabolism at the Bladder Surface

Background: Nutritional immunity describes physical and cellular mechanisms that withhold nutrients from microorganisms. For example, iron is not available to feed bacteria because of its insolubility in phosphate containing solutions. In addition, cells sequester iron by expressing iron transporters and chelators. How these mechanisms confront bacteria invading the urinary system is unknown. We found previously that a protein that captures siderophores, called NGAL is a critical component of the bladder's antimicrobial defense, and now we have identified a novel mechanism involving heme-iron. This mechanism has far-reaching implications for the handling of normal (Addis Count) and abnormal (Hematuria) urinary RBCs.

Methods: To identify acute responses to UTI, we adapted the novel RNA capture method of Cleary and Doe. We utilized a series of cellular knockouts to probe the heme pathway and a series of bacterial knockouts (including HMOX, Uhlemann, *Unpublished*) to determine the significance of heme catabolism in bacterial virulence. In addition, we developed a novel Carbon Monoxide probe.

Results: A search for iron trafficking mechanisms in kidney and bladder revealed high levels of TfR1 and DMT1 in TALH but not in collecting ducts or urothelium. To search for site-specific iron trafficking genes, we developed floxed-uracil-phosphoribosyl-transferase mice and a series of Cre drivers including ATPase6v1b1-Cre and Upk2-Cre (Collecting Ducts and Urothelium) and isolated *nascent* RNA at specific time points after UTI infection. We found transient induction of heme synthetic genes NPAS2 and BMAL, heme transporters HRG1/Slc48a1, heme degradation enzymes HMOX1 and Blvrb, iron storage ferritin, and siderophore capture protein, NGAL. To confirm the data, we utilized the new in situ technology, and found cellular HMOX and NGAL in mouse and human cystitis and pyelonephritis and Slc48a1 in bacterially infected urothelial endosomes. UTI induced a HMOX1 reporter and

activated a novel CO probe, demonstrating that UTI activated heme turnover in bladder and kidney. Heme transport was critical because transport defective ChuA, HMA bacteria (Mulvey and Mobley) were not competitive and CO gas was bacteriostatic.
Conclusions: We have identified a new function of the urothelium. It is a site of nutritional immunity. It uses NGAL to block bacterial siderophores, and it uses heme metabolism to detoxify hematuria and to compete with bacteria that have come to feed on our iron.

Characterization of Inflammatory Cells in Human Benign Prostatic Hyperplasia

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Characterization of Inflammatory Cells in Human Benign Prostatic Hyperplasia

Renee E. Vickman, Gregory M. Cresswell, Nadia A. Lanman, Meaghan M. Broman, Omar E. Franco, Brian T. Helfand, Alexander Glaser, Timothy L. Ratliff, and Simon W. Hayward

Abstract

Background: Benign prostatic hyperplasia (BPH) is a common disease in aging men characterized by enlargement of the prostate and often associated with lower urinary tract symptoms. Inflammatory cells are known to be an abundant cell type within BPH tissues, and high-grade inflammation has been demonstrated to limit the success of current therapies. While a variety of immune cells may infiltrate BPH tissue, the mechanisms by which they influence BPH progression are not well understood. The purpose of these studies is to characterize the subpopulations of inflammatory cells within incidental (<50 grams) *versus* surgical (>100 grams) human BPH tissues.

Methods: To define immune cell types throughout BPH progression, the transition zone was isolated from fresh human prostate tissues after robotic-assisted laparoscopic prostatectomy or simple prostatectomy. Samples were minced and digested, followed by cell sorting of viable, CD45+EpCAM-CD200- immune cells. The 10x Chromium System was used to perform single-cell mRNA-sequencing (scRNA-seq) on the isolated CD45+ cells from incidental and surgical BPH tissues. CellRanger and Seurat were used for data analysis, evaluation of cell clusters, and differential pathway analysis.

Results: CD45+ cells from ten incidental and four surgical BPH tissues were evaluated from men ages 61-76. Surgical BPH tissues had significantly increased International Prostate Symptom Score, but no significant differences in age or body mass index. The number of CD45+ cells was significantly increased in surgical *versus* incidental BPH tissues, determined by both flow cytometry and immunohistochemistry. Preliminary analysis using unsupervised clustering identified 12 immune cell clusters among all samples, with slight differences in cytotoxic T cell and plasma cell clusters between incidental and surgical groups. Additionally, pathway analysis identified significantly altered pathways related to cytokine signaling.

Conclusions: Analysis of CD45+ immune cells in incidental and surgical prostate tissues confirmed they are an abundant cell type in human BPH and significantly increased in surgical BPH tissues. scRNA-seq of CD45+ cells identified an array of immune cell subtypes present throughout BPH progression. Functional analysis of these cell types is ongoing. Preliminary pathway analysis suggests alterations in cytokine signaling between surgical and incidental tissue-derived CD45+ cells. These studies will functionally characterize inflammation during BPH progression and identify signaling pathways that contribute to disease progression and may be utilized for therapeutic targeting.

p300 inhibition enhances the efficacy of programmed death-ligand 1 blockade treatment in prostate cancer Dr. Jinghui Liu, University of Kentucky

Dr. Daheng He, Dr. Lijun Cheng, Dr. Karrie Jones, Dr. Dana Napier, Dr. Eun Y. Lee, Dr. Chi Wang and Dr. Xiaoqi Liu

p300 inhibition enhances the efficacy of programmed death-ligand 1 blockade treatment in prostate cancer

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Background

Blockade of programmed death-ligand 1 (PD-L1) by therapeutic antibodies has shown to be a promising strategy in cancer therapy, yet clinical response in many types of cancer, including prostate cancer (PCa), is limited. Tumor cells secrete PD-L1 through exosomes or splice variants, which has been described as a new mechanism for the resistance to PD-L1 blockade therapy in multiple cancers, including PCa. This suggests that cutting off the secretion or expression of PD-L1 might improve the response rate of PD-L1 blockade therapy in PCa treatment.

Methods

The levels of cellular and exosomal PD-L1 were detected by western blot. The surface PD-L1 was detected by flow cytometry or immunohistochemistry staining. The chromatin immuneprecipitation assay was used to detect the enrichment of the proteins on *CD274* (encoding PD-L1) promoter. The immunoprecipitation assay was used to detect the interaction of proteins of interest. The Spearman's Correlation coefficient and its associated 95% confidence interval (CI) were used to quantify the correlation between each pair of gene expressions of interest. Tumor purity and immune infiltrates was analyzed by TIMER. A syngeneic model of PCa was used to detect the response of various treatments.

Results

We found that p300 inhibition by a molecule dramatically enhanced the efficacy of PD-L1 blockade treatment in a syngeneic model of PCa by blocking both the intrinsic and interferon gamma (IFN-γ)-induced PD-L1 expression. Mechanistically, p300 could be recruited to the promoter of *CD274* by the transcription factor IRF-1, which induced the acetylation of Histone H3 at *CD274* promoter followed by the transcription of *CD274*. A485, a p300 inhibitor, abrogated this process and cut off the secretion of exosomal PD-L1 by blocking the transcription of *CD274*, which combined with the anti-PD-L1 antibody to reactivate T cells function for tumor attack.

Conclusions

This finding reports a new mechanism of how cancer cells regulate PD-L1 expression through epigenetic factors and provides a novel therapeutic approach to enhance the efficacy of immune checkpoint inhibitors treatment.

Cranberry's Role in the Prevention of Urinary Tract Infections

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Abstract:

Cranberry's Use in the Prevention of Urinary Tract Infections

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University of Texas Medical Branch

Background Information:

Urinary tract infections (UTIs) are a common bacterial infection and common complaint among women of all age groups in various health states.[5] Cranberry products (capsules or juice) have been a popular alternative to antibiotics as a natural source for prevention of UTIs. Cranberry has oligosaccharides that play a critical role in reducing the adhesive activity of bacteria to uroepithelial cells, preventing bacteria from thriving.[2,3]

Purpose:

The question being considered is: when attempting to prevent urinary tract infections in women, are cranberry products an effective option compared to no preventive measures?

Material and Methods:

A total of 4577 female human subjects, age ranging from 12-84 years old, were included in this study. Search methods included articles published in the last 10 years, human subjects, and terms including cranberry, prophylaxis, and urinary tract infection. Studies included population of women with different health conditions such as recurrent UTIs, long term facility care, and post-operative conditions.

Results:

In summary of the results, 59% of the relevant studies demonstrated greater efficacy in cranberry supplements versus the placebo. In comparison to 41%, who found no statistical significance when comparing the supplemental group to the placebo group.

Conclusion:

Cranberry supplementation has proven to be an effective method of prophylaxis for UTIs in women, showing reductions in UTI episodes, length of time until first UTI, and decrease in urine pH and bacterial adhesion.[1,3,4,6,7] Patients should be aware that in this study it was found that the use of cranberry supplementation prophylactically was not cost efficient in comparison to no prevention.[8]

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Keratinocyte Growth Factor Blocks Radiation-Induced Cystitis

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Background:
Radiation therapy can cause acute urothelial injury. We previously found that Keratinocyte Growth Factor (KGF), a ligand for Fibroblast Growth Factor Receptor 2, blocks cyclophosphamide (CPP)-induced apoptosis in deeper urothelial cells, but not necrosis of outer Superficial cells in mice. Our objective was to determine if KGF prevented urothelial injury from radiation.

Methods:
We treated female C57BL/6 mice with 5 mg/kg KGF or PBS (vehicle) 24 hours before exposure to 10 Gray radiation. We collected bladders at one and three days post-radiation for paraffin sectioning, followed by hematoxylin and eosin (H&E), TUNEL and/or immunostaining.

Results:
One day post-radiation, H&E stains were relatively similar between KGF and PBS-treated mice, with urothelial and stromal tissues appearing intact. Staining for specific urothelial cell markers, including Keratin 5 (Krt5, Basal and Intermediate cells) and Uroplakin3 (Upk3, Intermediate and Superficial cells), was comparable between KGF and PBS-treated mice at one day. However, PBS-treated mice had patchy loss of Keratin 20 (Krt20, mature Superficial cells), vs. no loss in the KGF group. TUNEL staining one day post-injury revealed widespread apoptosis in PBS-treated urothelium (all cell types, including Superficial cells), while KGF-treated mice had virtually no apoptotic urothelial nuclei. Moreover, in comparison to PBS-treated mice, KGF-treated mice had increased urothelial pAKT staining, which is known to block apoptosis. KGF-treated mice also had several $Ki67^{+}$ proliferating urothelial cells, while the PBS group had very few at one day. By three days, H&E stains in PBS-treated mice showed large patches of urothelial sloughing and denuding with accompanying hemorrhage and inflammation, while the KGF group had intact urothelium. While $Krt5^{+}$ and $Upk3^{+}$ cells were seen in both groups at three days, $Krt20^{+}$ Superficial cells were completely absent in the PBS group but still present in the KGF group. Proliferation rates based on Ki67 staining were low in both groups at three days.

Conclusions:
KGF prevents urothelial apoptosis, likely by activating AKT, and thus reduces bladder injury after radiation, similar to CPP injury. One difference is that KGF blocks radiation-induced apoptosis that occurs in all cells, including in Superficial cells, while KGF does not block the CPP-induced Superficial cell necrosis. Finally, KGF therapy leads to a small proliferative burst one day post-injury, which likely drives accelerated urothelial regeneration in both radiation injury and CPP injury.

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The stem cell inhibitor salinomycin decreases colony formation potential and tumor-initiating population in docetaxel-sensitive and docetaxel-resistant prostate cancer cells

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The stem cell inhibitor salinomycin decreases colony formation potential and tumor-initiating population in docetaxel-sensitive and docetaxel-resistant prostate cancer cells

(Martina Gruber, Florian Handle and Zoran Culig)

Introduction: Prostate cancer (PCa) is one of the most frequently diagnosed tumors in men. In general, therapies for localized PCa are curative. However, treatment of advanced PCa is considered palliative since development of therapy resistance occurs rapidly. It has been shown that tumor-initiating cells are likely involved in therapy resistance. These cells are capable of self-renewing and are not eliminated by conventional therapies. Thereby they are responsible for tumor progression and relapse. The aim of this study was to evaluate the effects of the known stem cell inhibitor salinomycin on this critical subpopulation of cells.

Material & Methods: To investigate the three previously described colony types (holo-, mero-, and paraclones) with varying tumor-initiating potential, PCa cell lines and therapy-resistant sublines were seeded at low density and grown for up to 14 days. Colony formation efficiency and distribution of colony types were analyzed in an automated way by the newly developed CATCH-colonies software. Expression of the cell surface markers CD24 and CD44 was determined by immunofluorescence and FACS.

Results: In concordance with the literature, automated high-resolution colony formation analysis consistently identified the various colony types in a broad range of PCa cell lines. Serial clonogenic assays confirmed that holoclones show the highest colony formation potential and maintain their tumor-initiating capacity over multiple rounds. Furthermore, holoclones showed high expression of CD44, while CD24 was not expressed in these clones, thus representing the well-described tumor-initiating $CD24^{-}/CD44^{high}$ population. Treatment of docetaxel-sensitive PC3, DU145 and docetaxel-resistant (DR) PC3-DR and DU145-DR with salinomycin led to a significant reduction in the colony formation potential and the number of colonies with high tumor-initiating potential. Salinomycin additionally decreased the $CD24^{-}/CD44^{high}$ population in both PC3 and PC3-DR.

Conclusion: Taken together, we demonstrated that automated high-resolution colony formation analysis is able to identify treatment-induced changes in the distribution of the different colony types. Furthermore, we found that salinomycin inhibits the tumor-initiating cell population in docetaxel-sensitive and -resistant PCa cells.

FGF-P: A potential mitigator of radiation-induced GI damage

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FGF-P: a potential mitigator for radiation-induced GI syndrome

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Background: Radiation doses high enough to cure prostate cancer often damage intestinal tissue, leading to adverse events. Safe and effective radioprotectors/radiomitigators are lacking in our clinical armamentarium. Although fibroblast growth factor -2 (FGF-2) is a GI potent mitogen, it is severely depleted humans exposed to radiation. Replacement of FGF-2 with a peptide mimetic would mitigate acute radiation-induced gastrointestinal syndrome (GIS) with decreased loss of crypt progenitor cells, improved gut barrier function and reduced bacterial translocation.

Introduction of growth factors, such as FGF-2, into clinical use has been hampered because of the disadvantages of administering a full length, labile protein that may cause an inflammatory reaction when injected into patients. The studies described here show the radiomitigation effects of a synthetic peptide, FGF-P, which we developed based on a 15 amino acid region of the FGF-2 receptor binding domain. It can be synthesized in powder form with high purity and reconstituted in saline for administration, with no observable toxicity.

Methods: NIH Swiss mice were placed in jigs shielding of a hind leg to spare bone marrow and exposed to total body irradiation (17-21.5 Gy) from a dual-source cesium irradiator (sub-TBI). Starting 24 hr after sub-TBI, mice were injected with FGF-P (10 or 20 mg/kg) or saline vehicle alone daily for 1 or 3 days. Human recombinant FGF-2 (hrFGF-2) at 0.3mg/kg was used as a positive control and injected in a single dose. At Day 30, surviving mice were euthanized for tissue collection. Villi cell proliferation and maturation markers were assessed using immunofluorescence confocal microscopy. Mitochondrial biogenesis and cytokine expression was assessed using realtime PCR and multiplex ELISA assays.

Results: Administration of FGF agents provided a survival advantage in both male and female mice of about 1-2 days at the highest radiation doses. Mice treated with FGF agents had reduced GI bleeding, improved stool formation, and improved electrolyte transport compared to vehicle controls.

Irradiation results in a dramatic reduction in the number of small intestine crypt proliferating cells by Day 3. However, in irradiated mice receiving FGF-P, loss of proliferating crypts was reduced compared to vehicle controls, improving survival for at least 30 days. Administration of FGF-P improved mitochondrial function and preserved progenitor cells in the intestinal crypts as well as expression of biomarkers associated with normal barrier and secretory functions of the villi.

Conclusions: Administration of FGF peptides beginning 24 hr after irradiation produced survival benefits in mouse GIS models. Both hrFGF-2 and FGF-P reduced the symptoms of GIS by affecting common survival and mitochondrial pathways, with no observable toxicitiy. These results show that the synthetic FGF-P peptide has potential as a safe and effective mitigation agent for radiation-induced GIS.

PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA double-strand break repair genes

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Title: PRMT5 cooperates with pICln to function as a master epigenetic activator of DNA double-strand break repair genes

Background: Efficient repair of DNA double-strand breaks (DSBs) is critical for cell survival. Upon recognition of DSBs, repair proteins are upregulated and recruited to the sites of damage to facilitate repair. Although the recruitment and action of repair proteins are well characterized, little is known about how their expression is induced upon DNA damage. Targeting repair proteins is a common approach for cancer treatment, therefore proteins responsible for the upregulation of repair proteins are potential therapeutic targets to improve DNA-damaging therapies.

Protein arginine methyltransferase 5 (PRMT5) is an enzyme involved in cancer development and progression. MEP50 is believed to be the obligate cofactor of PRMT5 and required for PRMT5 activity. Here, we determined that targeting PRMT5 sensitizes prostate cancer cells to DNA damage. However, further studies including RNA-seq and analysis of clinical patient data suggested PRMT5 also plays a conserved role in repair of DSBs independently of MEP50 and be a therapeutic target across cancer.

Methods: Clonogenic assays and foci analysis via immunocytochemistry were used to assess if targeting PRMT5 sensitizes prostate cancer cells to DNA damage. To determine if PRMT5, along with interacting proteins, acts as an epigenetic activator of DNA damage response (DDR) genes, we used RNA-seq, qPCR, western blot, and ChIP. To extend our findings, we analyzed clinical cancer patient data from The Cancer Genome Atlas Pan-Cancer analysis.

Results: Here, we characterize pICln as a novel epigenetic cofactor of PRMT5. Under normal conditions, a complex involving PRMT5:pICln maintains basal expression of DDR genes. DNA damage induces both PRMT5 protein upregulation and pICln nuclear translocation which leads to an increase in the PRMT5:pICln interaction in the nucleus. The PRMT5:pICln complex is then recruited to the promoters of genes involved in the DDR where it epigenetically activates expression of target genes. PRMT5:pICln target genes, including repair proteins, are then upregulated at the mRNA and protein level to facilitate the repair of DSBs. In clinical cancer data sets, both PRMT5 and pICln, but not MEP50, correlated positively with DDR genes in almost all cancers independently of any correlation with AR.

Conclusions: PRMT5 cooperates with pICln and independently of its obligate cofactor MEP50 to function as a master epigenetic activator of DDR genes. Targeting PRMT5/pICln may be explored in combination with DNA-damaging therapies for cancer treatment.

KGF reduces injury and accelerates recovery of bladder urothelium after cyclophosphamide
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KGF reduces injury and accelerates recovery of bladder urothelium after cyclophosphamide

Background:
Cyclophosphamide (CPP), a drug used to treat lymphoma and other diseases, can cause severe acute bladder urothelial injury and long-term problems including fibrosis and cancer. Previous work showed that pretreatment with keratinocyte growth factor (KGF) improves bladder outcomes after cyclophosphamide in rats, although mechanisms of action are unknown. We examined roles for KGF in bladder urothelial injury from CPP.

Methods:
We treated female mice with 5mg/kg KGF or PBS (vehicle) 24 hours before intraperitoneal (IP) CPP (150mg/kg). We collected bladders at various timepoints and performed H&E staining, TUNEL assays and immunofluorescence.

Results:
In PBS treated mice, we noted non-apoptotic sloughing of lumenal Superficial cells within two hours post-CPP and apoptosis of deeper Intermediate and Basal cells from 6-24 hours post-CPP, together leading to hemorrhage and inflammation. While KGF-treated mice still lost Superficial cells, they had intact Intermediate and Basal cells with no apoptosis; KGF treatment but not PBS-treatment led to increased pAKT staining, which is known to suppress apoptosis. Three days post-CPP, KGF-pretreated mice had regenerated Superficial cells, while the PBS group had not. We then examined if KGF led to enhanced proliferation to potentially repopulate these cells. Six and 24 hours post-CPP, KGF-treated mice (but not PBS-treated mice) had proliferating and $pERK^+$ Intermediate cells that were likely regenerating Superficial cells. The KGF-driven proliferating cells were primarily $Keratin\ 5^+/Keratin\ 14^-$ (cells not known to have proliferative potential). From one to three days post-CPP, increasing numbers of mostly $Keratin\ 14^+$ Basal cells were proliferating in response to injury in both groups; however, proliferation rates in the KGF group at three days was lower that the PBS-group, likely reflecting reduced overall injury in the former. 10 days post CPP, KGF-treated mice had almost no urothelial proliferation and restoration of normal bladder urothelial layers, while PBS-treated mice had ongoing urothelial proliferation/regeneration.

Conclusion:
KGF-treatment blocks CPP-induced Intermediate and Basal cell apoptosis likely by pAKT. KGF also drives pERK-mediated $Keratin\ 5^+$ ($Keratin\ 14^-$) cell proliferation (a cell not known to have proliferative potential) that may more quickly regenerate Superficial cells. Together these actions lead to much earlier urothelial restoration than in PBS-treated mice.

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Prostate cancer cell phenotypes are stable following PDE5 inhibition in the clinical range but antagonized by supra- physiological concentrations.

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Prostate cancer cell phenotypes are stable following PDE5 inhibition in the clinical range but antagonized by supra-physiological concentrations.

Background

Cyclic guanosine monophosphate (cGMP) is a nucleotide second messenger in multiple cellular signaling pathways. Its accumulation is stimulated therapeutically by PDE5 inhibitors such as sildenafil (Viagra). Widespread PDE5 inhibitor use in male reproductive health has generated epidemiologic evidence of both tumor suppressive and tumor promoting effects. The prostate cancer patient population is enriched for PDE5 inhibitor use, which correlates with decreased incidence but increased recurrence. We initially hypothesized that increased cGMP signaling due to PDE5 inhibition promotes prostate cancer cell growth, colony formation and migration.

Methods

PDE5 expression and cGMP pathway responses to PDE5 inhibition were profiled in multiple prostate cancer cell lines. Cell growth, colony formation and migration phenotypes were monitored at clinically relevant and supra-physiological concentrations. Immunohistochemical analysis of human patient tissues quantified PDE5 expression across the stages of prostate tumorigenesis. Phospho-proteomic and transcriptomic profiling were performed following PDE5 inhibition.

Results

Clinically relevant concentrations of PDE5 inhibitor triggered cGMP accumulation in a subset of prostate cancer cell models but did not modify cell growth, colony formation or migration. Supra-physiological concentrations of PDE5 inhibitor enhanced cGMP accumulation and counteracted cell growth, colony formation and migration in all tested prostate cancer cell models, while increasing phosphorylation and decreasing expression of molecules driving mitotic progression. Immunohistochemistry detected a progressive increase in PDE5 from normal prostate to androgen-dependent prostate cancer to castration-resistant disease.

Conclusions

This evidence supports the safety and continued use of PDE5 inhibitors as adjuvant agents to promote functional recovery after prostatectomy, and indicates the potential for novel application at higher concentrations to counteract prostate tumorigenesis. These tumor cell-autonomous findings do not rule out cancer-promoting effects of PDE5 inhibitors in the more complex environment of the prostate, but are consistent with epidemiological studies that have failed to reproduce the correlation between PDE5 inhibitor use and prostate cancer recurrence that originally motivated this study.

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Pde5 inhibitor
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Overall Survival in Patients with Metastatic Prostate Cancer: Role of Statins and PSA Nadir after Androgen Deprivation Therapy

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Overall Survival in Patients with Metastatic Prostate Cancer: Role of Statins and PSA Nadir after Androgen Deprivation Therapy

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BACKGROUND: Studies have shown that Statin use was associated with decreased risk of localized CaP, less frequent high-grade CaP and lower CaP volume, suggesting a protective effect against localized CaP. However, less is known about a role of statins in metastatic CaP (mCaP). While it is known that hypercholesterolemia is associated with the development of castration resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT) in patients with bone metastasis, it is not known whether the sequence of statin and ADT affects outcome.

METHODS: Data of 162 patients with mCaP treated at the VA Northern California Health Care System (VANCHCS) with ADT and statins between 1992 and 2016 were analyzed. Overall survival (OS) was followed until 12/2016.Time to event outcomes (except for time to Nadir PSA, which was not censored) were analyzed using Cox proportional hazard models. PSA Nadir and time to PSA Nadir were analyzed using linear models, with outcomes log transformed. All models include age at PSA diagnosis and year of metastatic diagnosis as covariates.

RESULTS: Of the 162 total patients, 110 were on ADT at the time of diagnosis with metastasis (ADT before mets, ABM) while 50 were diagnosed with metastasis at the time of ADT (mets before ADT, MBA).41.4% received statins prior to diagnosis with metastasis, and 12.3% started statins after metastasis diagnosis (29% prior to ADT treatment and 24.7% after starting on ADT). There was no effect of statins on OS among those who had a diagnosis of metastasis prior to initial ADT (MBA group). On the other hand, among those who developed metastases while on ADT (ABM group), statin use significantly increased OS (based on the time of metastasis diagnosis). However, within this group, the largest benefit was in the patients that received statins after starting ADT (HR = 0.51, p=0.022). The same group also took longer to reach PSA nadir (R=1.79, p=0.031) and took longer to be diagnosed with mCaP following ADT compared to those who had never been on Statins (HR=0.45, p=0.003).

CONCLUSIONS: Our data indicate that statins treatment initiated after the patient has undergone ADT is beneficial to patients who develop distant metastases while on ADT treatment.

CRISPRi screen of risk-associated cis-regulatory elements reveals 3D genome dependent causal mechanisms in prostate cancer

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CRISPRi screen of risk-associated cis-regulatory elements reveals 3D genome dependent causal mechanisms in prostate cancer

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BACKGROUND: Prostate cancer is one of the most heritable diseases to date. Hundreds of single nucleotide polymorphisms (SNPs) have been identified by genome-wide association studies (GWAS) to confer risk of prostate cancer in men. Most prostate cancer associated risk SNPs do not directly alter gene codons, rather modulate cis-regulatory elements (CREs) such as enhancers. The primary objective of this study was to perform a systematic essentiality screening of prostate cancer risk associated CREs.

METHODS: We previously pinpointed 270 CREs that harbour at least one risk SNP in prostate cancer. In this study, we targeted these CREs using dCas9-KRAB complex (CRISPRi) guided by 5,571 sgRNAs in three prostate cancer cell lines - LNCaP, V16A and 22Rv1.

RESULTS: The screen identified 98 CREs essential for growth of at least one cell line. Interestingly, essential CREs are significantly enriched in the gene desert region of 8q24.21. The most essential CRE is an enhancer harbouring the SNP rs11986220, which increases the risk for prostate cancer by up to 1.8 fold. Suppression of this enhancer significantly reduces cell proliferation and tumor growth in LNCaP and V16A models. RNA-seq analysis identifies MYC, an important oncogene, to be it's primary target gene. However, this enhancer neither confers essentiality nor regulates MYC in 22Rv1 cells, despite having almost identical epigenetic profiles as in LNCaP cells. Further investigation reveals that a CTCF binding site unique to 22Rv1 intervenes the MYC promoter-enhancer interaction in this cell line. We performed 3C, HiC and H3K27ac HiChIP assays to establish that the enhancer interacts with MYC promoter only when this CTCF site is deleted in 22Rv1 cells. Intriguingly, this CTCF site is also found variable among primary prostate cancer patients, and especially, the SNP rs11986220 is an eQTL for MYC only in patients with low deposition of CTCF at this locus.

CONCLUSION: Our study reveals that CRISPRi is an efficient technique to perform systematic functional analysis of CREs. We thus discover that the interaction between MYC promoter and rs11986220-containing enhancer is governed by CTCF-mediated 3D genomic structure, and the causal effect of rs11986220 is variable among patients depending on CTCF binding in this locus. This unveils a novel regulatory mechanism in human genome and may present a paradigm shift for current target-gene analysis of GWAS loci by incorporating 3D genome variability.

GATA-2 and Twist-1 as Targets of CREB-1 in Prostate Cancer Development

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GATA-2 and Twist-1 as Targets of CREB-1 in Prostate Cancer Development

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Background: Dysregulation in the normal process of basal to luminal cell differentiation results in prostate cancer development. Although TMPRSS2-ERG oncogenic fusion, loss of NKx3.1, Myc overexpression, and PTEN loss are known genetic abnormalities that drive human prostate cancer, the mechanisms that perturb differentiation at the molecular level still remains unclear. Through RNA seq analysis we unexpectedly identified two distinct pools of CREB target genes, one involved in luminal cell differentiation and the other in tumor induction. GATA-2 and Twist-1 were identified as potential CREB targets that are overexpressed in cancer cells. The current study was undertaken to experimentally validate whether GATA-2 and Twist-1 are targets of activated CREB-1 in tumor cells.

Method: The human tumorigenic cell line EMP (overexpressing Erg, Myc and shPTEN) was engineered to express Tet-inducible shRNA to CREB-1 or ATF-1. Chromatin immunoprecipitation (ChIP) using phospho-CREB primary antibody followed by qPCR was performed to measure the enrichment of CRE-containing promoter regions in the GATA-2 and Twist-1 promoters.

Result: CHIP analysis showed an approximate 8-fold enrichment of the GATA-2 promoter region spanning the TGACGTCA CRE binding site whereas the coding region of GATA-2 had very little to no enrichment. Similarly, the Twist-1 promoter showed a 5-fold enrichment spanning the ½ CRE site, around which the primer was designed, with no enrichment in the Twist-1 coding region. Upon knock-down of CREB-1 with shRNA using doxycycline, the enrichment of GATA-2 and Twist-1 at their respective loci decreased to a minimum level. This finding establishes that the enrichment of GATA-2 and Twist-1 promoter loci is specific to phospho-CREB. Since the phospho-CREB antibody also recognizes another CREB-related transcription factor, ATF-1, ChIP was also performed in EMP cells after knocking down ATF-1 with shRNA using doxycycline. No change in the percentage enrichment in the ATF-1 knock-down condition was observed in both the GATA-2 and Twist-1 promoters. This finding substantiates the prediction that enrichment of GATA-2 and Twist-1 is specific to phospho-CREB-1.

Conclusion: GATA-2 and Twist-1 are targets of activated CREB-1, but not ATF-1, in the tumorigenic EMP cells. This finding supports the novel connection between activation of the CREB-1 transcription factor and prostate cancer development.

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The critical role of Interleukin-8 chemokine axis in the development of benign prostatic hyperplasia (BPH) Diandra K. Smith, MPH, Research Associate, Augusta University

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Title: **The critical role of Interleukin-8 chemokine axis in the development of benign prostatic hyperplasia (BPH).**

Authors: **Diandra K. Smith, Natasha Venugopal, Martha K. Terris, Vinata B. Lokeshwar and Bal L. Lokeshwar**

Benign prostatic hyperplasia (BPH), a non-malignant proliferative disease of the prostate, has a global incidence of over 210 million men aged 50 years or older and the principal cause of lower urinary track syndrome (LUTS). BPH strongly associates with prostate volume that begins to increase in men aged 40 or higher. We investigated the role of inflammatory chemokine and chemokine receptors in three immortalized cell lines and primary cultures derived from fresh BPH tissues following transurethral resection of the prostate (TURP). We analyzed the levels of chemokines and chemokine receptors secreted in the culture medium and in the mRNA from cells by qPCR, western blotting and Cytokine Arrays. Epithelial identity of primary cultures was established by analyzing cytokeratin expression. Two natural products isolated from Allspice were identified that inhibit the growth of BPH cultures. A cell line identified as the BPH progenitor, a BPH intermediary cell line and a cell line with frank BPH features secreted a large excess of chemokine, predominantly CXCL-VIII (Interleukin-8, IL-8). Unusually high amount of IL-8 was secreted by BPH progenitor cells and intermediate cells as compared to a stable normal prostate cell line (RWPE1) derived from peripheral zone of the prostate, which did not secrete any IL-8 or express its receptors. Investigation found that expression of CXC receptors CXCR1, CXCR2 and an IL-8 inducible CXCR (CXCR7) was absent in progenitor cells and in intermediate cells, but was highly expressed in the cell line derived from BPH tissue and more significantly, in primary epithelial cultures of BPH tissue. Further, primary cultures and progenitor cells expressed high levels of immune stimulatory factors indicating PMN and monocyte infiltration leading to potential chronic inflammation of the transitional zone of the prostate. The natural products from Allspice inhibited all members of the IL-8 axis and proliferation, suggesting their preventative potential for BPH. These results demonstrate how a novel chemokine axis is likely to enhance pathogenesis of BPH and suggest several avenue to control the abnormal growth of prostate in aging male.

Defining the Androgen Receptor-dependent transcriptome in bladder tumor cells

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Background: Bladder cancer displays a striking gender disparity where it is 3-4 times more prevalent in men than in women. While bladder cancer is not traditionally considered a hormone regulated malignancy, multiple studies argue that the androgen receptor (AR) has a role in bladder tumorigenesis. We found that bladder tumor cells express full length and low molecular weight (LMW) AR variant, including a novel variant that we identified Bl-v1.
Methods: To identify the AR-dependent transcriptome, we depleted total AR, Bl-v1 and overexpressed Bl-v1 in UM-UC-3 cells and used RNA-seq to identify transcript changes. AR and Bl-v1 depletion up and down regulated multiple transcripts.
Results: The major pathways associated with AR depletion are pathways in cancer, FoxO signaling, HIF-1 alpha transcription factor network and apoptosis. We identified a cohort of transcripts that were decreased or increased following Bl-v1 depletion and regulated in the reciprocal manner in Bl-v1 overexpressing cells. The major alter molecular functions were oxidoreductase activity, NADH dehydrogenase activity, and structural constituents of ribosome. The alter biological processes were generation of precursor metabolites and energy, ATP metabolic process, ATP synthesis and ribonucleotide metabolic process. The pathways associated with these transcripts included respiratory electron transport, TCA cycle and rRNA processing.
Conclusions: The studies indicate that in this cellular context AR has a role in regulating cells cycle, apoptosis and cellular metabolism.

Snail Promotes eurite ut rowth in Prostate Cancer Cells Gabrielle dwards, Clark tlanta University Janae Sweeney, eronica enderson, alerie dero-Marah

Snail Promotes Neurite Outgrowth in Prostate Cancer Cells

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Neurite outgrowth is a process where developing neurons produce new tentacle-like extensions as they grow in response to guidance cues. The projection can be an axon or a dendrite (nerve fibers). Nerve growth factors, or neurotrophins which are important for survival or growth, are one family of such stimuli that regulate neurite growth. Studies have also proposed that cancer cells stimulate their own innervations. Therefore, the concept of neoneurogenesis includes the development of nerve endings (axons) towards the tumor. Cancer cells are able to secrete neurite outgrowth-promoting molecules and axon guidance molecules that would stimulate and initiate the growth of these new axons to particular areas of the tumor. Neurite outgrowth involves reciprocal signaling interactions between tumor cells and nerves where invading tumor cells have acquired the ability to respond to pro-invasive signals within the peripheral nerve environment. Neurite outgrowth could serve as a possible mechanism leading to invasion of cancer cells into the nerve sheath and subsequent metastasis. Snail1 or Snail is a zinc-finger protein that can down-regulate cell adhesion proteins such as E-cadherin by binding several E-boxes located in the promotor region, thereby inducing the epithelial mesenchymal transition (EMT) process to promote tumor migration and metastasis. Snail has also been shown to promote neuroendocrine differentiation. The hypothesis is that Snail can promote neurite outgrowth. For this study we utilized various prostate cancer cell lines: C42, E006 AA, E006 AA-hT non-silencing controls (NS) or stable Snail knockdowns (Snail shRNA). Conditioned media collected from these cells was cultured with nerve cells (NS20Y) for 48 hours followed by a quantitative neurite outgrowth assay. Nerve growth factor (NGF) was utilized as a positive control. We also included Lanreotide, an inhibitor of neuroendocrine differentiation. After 48 hours, the nerve cells were incubated with Fix/Stain solution containing Cell Viability, and Cell Membrane stain, and nerve extensions quantified by analysis with a plate reader. Our results showed that C42 NS, E006 AA NS and E006 AA-hT NS (Snail-high) conditioned media led to a higher neurite outgrowth compared to the Snail knockdown cells. We also observed that neurite outgrowth was blocked by Lanreotide inhibitor. In conclusion, Snail promotes neurite outgrowth, which can be inhibited by Lanreotide, a drug currently being used to treat patients with neuroendocrine tumors. Therefore, targeting cancer cell interaction with nerve cells may be helpful in halting prostate cancer progression/metastasis.

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nducible prostate luminal epithelial cell-specific deletion of Cdh1 induces murine prostatic hyperplasia and inflammation and bladder overactivity

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Title: Inducible prostate luminal epithelial cell-specific deletion of Cdh1 induces murine prostatic hyperplasia and inflammation and bladder overactivity

Introduction and Objective: Chronic inflammation is thought to contribute to the development of prostatitis and benign prostatic hyperplasia (BPH) as well as lower urinary tract symptoms (LUTS). In the prostate, the epithelial barrier functions to provide a selectively permeable barrier which serves as an interface between the glandular lumen and the underlying tissues. E-cadherin is an important adherens junction that helps to maintain the epithelial barrier in mucous membranes. This study explored the potential impact of reduced barrier function induced by deletion of E-cadherin in the murine prostate.

Methods: The PSA-CreERT2 transgenic mouse strain expressing tamoxifen-inducible CreERT2 recombinase driven by a 6-kb human PSA promoter/enhancer was crossed with the B6.129-Cdh1tm2Kem/J mouse to generate bigenic PSA-CreER^{T2}/*Cdh1*$^{-/-}$ mice. Deletion of E-cadherin was performed by transient administration of tamoxifen when mice reached sexual maturity (7 weeks of age). Void Spot Assays and cystometry were used to assess bladder function. In vivo biotin permeation assays were performed to analyze prostate barrier function. Mice were examined histologically at 150 days of age.

Results: Mice with *Cdh1* deletion had increased proliferation, inflammation, and stromal fibrosis at 150 days of age, as well as changes in bladder voiding. These alterations persisted

suggest that the loss of E-cadherin could contribute to the development of a chronically inflamed prostate and bladder overactivity.

Conclusions: These findings suggest that loss of epithelial barrier integrity in the prostate could result in chronic inflammation and the development of stromal fibrosis, inflammation and benign epithelial hyperplasia characteristic of BPH and prostatitis.

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Foxa1 expression is required for maintenance of superficial umbrella cells in the urothelium Lauren Shuman, MS, Penn State University
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Foxa1 expression is required for maintenance of superficial umbrella cells in the urothelium

Lauren Shuman, Jenna Buckwalter, Thomas Wildermuth, Klaus Kaestner, Cathy Mendelsohn, and David DeGraff

Background

Chronic injury of the urothelium is associated with bladder pain syndrome and voiding dysfunction for which there are presently no cures. Therefore, maintenance of urothelial differentiation and regeneration is important, and identifying the molecular factors that promote these processes could result in novel approaches to enhance healing. Forkhead Box A1 (Foxa1) is a transcription factor important for the regulation of tissue-specific gene expression in urothelium and other cell types. Consistent with this, we have previously reported that inducible *Foxa1* knockout (KO) in adult mice results in hyperplasia, squamous differentiation, and reduced expression of tissue-specific genes in the urothelium. Here, we use a *Upk2Cre* driven KO of *Foxa1* to determine the impact of early *Foxa1* inactivation in urothelial development and differentiation.

Methods

The *Upk2Cre* mouse line (expressed at embryonic day 13) was bred with previously described *Foxa1*$^{loxp/loxp}$ mice. Control and *Upk2Cre/Foxa1*$^{loxp/loxp}$ mice were sacrificed at 3 months of age and bladders were analyzed using hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), and immunofluorescence (IF).

Results

While urothelium from control mice exhibited a normal number of superficial umbrella, intermediate, and basal urothelial cells, the number of umbrella cells within the bladders of a subset of *Upk2Cre/Foxa1*$^{loxp/loxp}$ mice was significantly reduced in number ($p=0.003$; Student's t-test), with IHC confirming KO in remaining superficial urothelial cells. The urothelium of *Foxa1* KO mice also often appeared dysmorphic and umbrella cells were undetectable in a subset of bladders. In addition, while IF confirmed decreases in the number of Upk3^{+}/Krt20^{+}/Tp63^{-} superficial urothelial cells, we also show that *Foxa1* KO bladders have a reduced number of Upk3^{+}/Tp63^{+} intermediate cells.

Conclusions

These results suggest *Foxa1* expression is required for normal urothelial development and/or maintenance of superficial and intermediate urothelial populations. However, it is still unknown if *Foxa1* expression plays a role in the maintenance of basal urothelium, or

if *Foxa1* is required for urothelial regeneration following injury. Therefore, future studies will focus on these and other areas of importance.

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Modeling cisplatin resistance in testis cancer with the zebrafish

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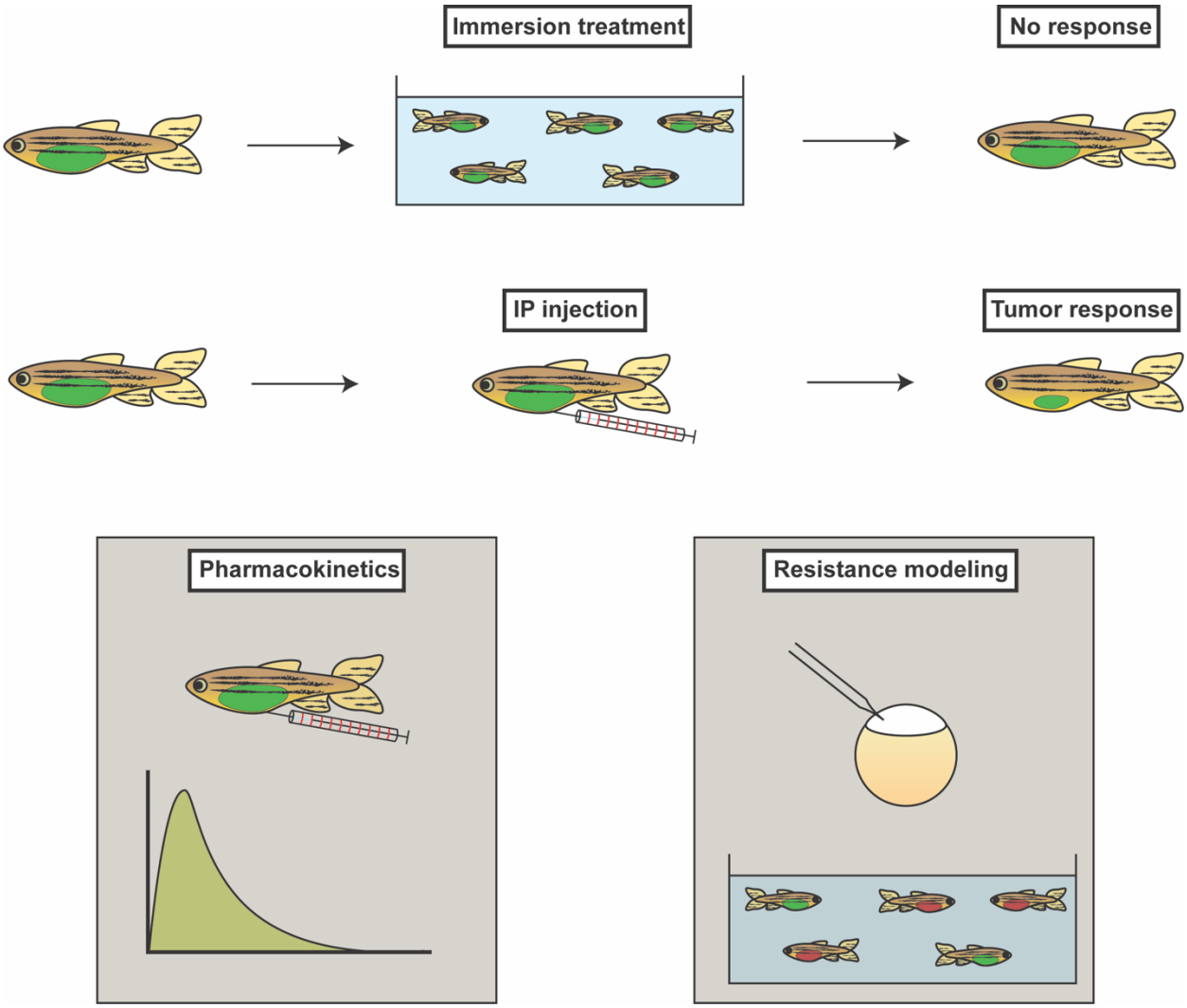
Modeling cisplatin resistance in testis cancer with the zebrafish

Background: Testicular germ cell tumor (TGCT) is the most common solid malignancy in men aged 20-40. Despite exquisite sensitivity to platinum-based therapy, nearly 1 in 5 cases of TGCT exhibit platinum-resistance, with limited salvage options. Recent studies have identified potential molecular mechanisms of cisplatin resistance in TGCT, including MDM2 amplification. However, functional studies in this arena are lacking, due in part to limited available models of TGCT. The *bmpr1bb* zebrafish model of TGCT produces tumors that molecularly and histologically resemble human disease.

Methods: We examined cisplatin response in the *bmpr1bb* zebrafish model of TGCT. We performed dose finding studies by immersing 5 wild-type fish each in tanks with 0, 100, 250, or 1000 µM cisplatin. We then treated tumor-bearing *bmpr1bb* fish ($n \geq 5$ per group) with cisplatin by immersion or intraperitoneal (IP) injection. We measured fluorescence intensity of GFP driven by *piwil1*, a germ cell specific promoter, as a surrogate of tumor size. We also examined tumor concentration of cisplatin by LC-MS/MS for initial pharmacokinetic analysis. To begin to examine the role of MDM2 in cisplatin resistance with this model, we used transposon-based transgenesis to generate fish expressing human MDM2 and mCherry specifically in the germ cells.

Results: The maximum tolerable dose of cisplatin by immersion was 250 µM for 4 hours. Tumor-bearing fish treated at this dose every 3 days for 3 doses did not exhibit a response by 28 days. However, tumor-bearing fish treated with 0.5 mg/mL IP cisplatin exhibited a 58% reduction in relative fluorescence intensity than tumors from saline-treated fish. Initial pharmacokinetic modeling yielded a characteristic concentration-time curve, with estimated C_{max} of 7.7 mg Pt g^{-1} tissue. To prepare for resistance studies, we identified and expanded stable lines of zebrafish with mCherry+ gonads, and confirmed the presence of human MDM2 by RT-PCR.

Conclusions: The *bmpr1bb* zebrafish model of TGCT responds to IP cisplatin, enabling its use as a tool to study resistance. The zebrafish model system benefits from extensive genetic tools available, enabling functional studies that would be challenging in other systems. We used some of these tools to generate transgenic fish to model cisplatin resistance in future studies.


Immersion treatment
No response
IP injection
Tumor response
Pharmacokinetics
Resistance modeling

Bladder cancer metabolomics identifies important differences in lipid metabolites between metastatic and non-metastatic tumors

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Bladder cancer metabolomics identifies important differences in lipid metabolites between metastatic and non-metastatic tumors

Introduction

Bladder cancer is a clinically and biologically heterogenous disease. A standard of care approach for patients with muscle invasive bladder cancer (MIBC) involves neo-adjuvant systemic chemotherapy (NAC) followed by radical cystectomy or trimodal therapy with maximal resection and combination chemo-radiation therapy. Only a minority of patients may actually benefit from treatment intensification and biomarkers to select such patients are critically needed.

Methods

We characterized the global metabolome of 33 high-grade bladder cancers from radical cystectomy specimens. We used targeted and untargeted metabolomic approaches with gas chromatography- time-of-flight mass spectrometry (GC-TOF MS) to profile primary metabolites, hydrophilic interaction liquid chromatography MS (HILIC-QTOF MS) to profile biogenic amines and liquid chromatography charged surface hybrid MS (LC-CSH-QTOF MS) to characterize the complex lipids. The primary outcome was differences in metabolite levels stratified by metastatic disease status. Metabolite levels were also correlated with clinical variables including age, body mass index, smoking status and receipt of neoadjuvant chemotherapy.

Results

We characterized over 1100 metabolites in 33 fresh bladder cancer specimens. When comparing tumors from patients who progressed to metastatic disease (n=23, 68.75%) to those who did not (n=10, 31.25%), most of the differential metabolites where related to cell membrane breakdown and lipid metabolism. Long chain triglycerides were depleted in metastatic samples (3-5X) while several sphingomyelin species showed increased levels. Similarly, tumors from patients treated with NAC (n=21, 63.64%) had 2-3X lower levels of ceramide in addition to triglycerides than patients without prior chemotherapy (n=12, 40.63%). In this case, trilaurate glycerol and octadecylglycerol were most significantly upregulated in patients treated with NAC.

Conclusions

Deregulated lipid metabolism may be important in bladder cancer resistance to chemotherapy and metastases. In this metabolic study of a heterogenous group of bladder cancers, most differences were noted in long chain fatty acids. In particular, it appears that replacement of triglycerides with sphingomyelins is a characteristic of metastatic bladder cancer. Additional studies will be needed to determine whether lipid content of tumor cell membranes can be used to identify patients who may recur vs those who will not.

Androgen deprivation promotes neuroendocrine prostate cancer by activating Wnt/Œ≤-Catenin signaling Siyuan Cheng, Graduate Assistant-Research, LSUHSC- Shreveport

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Androgen deprivation promotes neuroendocrine prostate cancer by activating Wnt/β-Catenin signaling

Background

Prostate cancer (PCa) is the most diagnosed non-cutaneous cancer among American men. Although most patients diagnosed with localized PCa could be treated successfully, when PCa develops into castrate-resistant prostate cancer (CRPCa), the five-year survival rate drops below 30%. During CRPCa development, an aggressive phenotype of PCa, neuroendocrine prostate cancer (NEPCa) provides a mechanism of therapy resistance and promotes cancer aggressiveness. In most cases, NEPCa arises post hormone therapy. Understanding how NEPCa arises after androgen deprivation could reveal novel treatment targets and strategies. Previous evidence showed activation of Wnt/ β-Catenin signaling promotes the progression of PCa to CRPCa with an increased NE phenotype.

Methods

In this study, we analyzed Wnt ligand expression with or without androgen deprivation both in vivo and in vitro by immunohistochemistry (IHC) and quantitative-PCR. We also selected major components contributing to the activation of Wnt/β-Catenin by qPCR and big data mining.

Results

IHC shows Wnt/β-Catenin signaling pathway is activated in NEPCa. Wnt7a, a Wnt ligand, is increased after androgen deprivation. In contrast, DKK1, a Wnt signaling inhibitor, is decreased. Additionally, YES-associated Protein (YAP1), a key transcriptional coactivator modulating Hippo pathway and Wnt/β-Catenin pathway is lost in NEPCa. Our data also support that loss of YAP1 augments Wnt/β-Catenin signaling.

Conclusion

In conclusion, the increase of Wnt7a, decrease of DKK1 and loss of YAP1 expression contribute to the activation of Wnt/β-catenin signaling, promoting NEPCa development after androgen deprivation treatment.

Synergistic anticancer efficacy of simvastatin and metformin on enzalutamide resistant prostate cancer cells Eswar Shankar, Case Western Reserve University

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SBUR Fall Symposium 2019

Synergistic anticancer efficacy of simvastatin and metformin on enzalutamide resistant prostate cancer cells

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Background: Androgen deprivation therapy (ADT) is the prevalent first line of treatment against advanced prostate cancer and as an adjuvant for locally treating the high-risk disease. Cancer cells respond to ADT treatment initially, but subsequently colonize and re-emerge as castration resistant prostate cancer (CRPC). Enzalutamide (ENZU), a second generation androgen receptor (AR) antagonist exhibits survival advantage in CRPC patients, however ~30% of patients develop resistance with ENZU treatment, activating AR in these tumors. These resistant tumors poorly respond to chemotherapy and other treatment modalities. Therefore, identification of an effective low-cost therapeutic alternative with fewer side effects may lead to increased survival and greatly benefit patient quality-of-life. Previous studies from our laboratory (*Mol Cancer Ther. 13:2288-302, 2014*) has demonstrated that synergistic combination of simvastatin (SIM), a drug for the treatment of hypercholesterolemia and metformin (MET), a glucose lowering drug inhibits CRPC growth, invasiveness and migration with minimal effect on normal prostate epithelial cells. Here we investigate whether combination of SIM and MET could be effective in the treatment of ENZU-resistant prostate cancer cells.

Methods: Human CRPC cells C4-2B-ENZU (C4-2B enzalutamide resistant) were generated by growing C4-2B cells in 5-20μM of ENZU over 60 days and maintained in 5μM ENZU in the culture medium for 20 generations. 22Rv1 cells are inherently resistant to ENZU. The cells were treated with SIM (4μM) and MET (2mM) individually and in combination, followed by assessment of cell viability, crystal violet assay, cell cycle analysis, migration, invasion and expression of various target genes by Western blotting.

Results: C4-2B-ENZU and 22Rv1 cells were treated with a combination of SIM and MET at pharmacological dose range (500nM-4μM SIM and 250μM-2mM MET). Combination treatment with 4μM SIM and 2mM MET (SIM+MET) led to significant and synergistic inhibition of cell viability, migration, invasion and cell cycle blockade in both cancer cell lines. The individual treatments of SIM and MET exhibited little or no effect on these cells. Furthermore, SIM+MET combination decreased the expression of AR, AR-V7, p-Akt (Ser473), p-AMPKα_1/α_2 (Ser-485/491) and simultaneously increased p-AMPKα1 (Thr-172) and AMPKα kinase activity in these cells.

Conclusion: Our results suggest that the combination of SIM and MET may be an effective regimen for treatment ENZU-resistant prostate cancer cells.

Interaction between cancer cells and bone microenvironment in the bone metastatic progression of prostate cancer

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Interaction between cancer cells and bone microenvironment in the bone metastatic progression of prostate cancer

Tom Case, Marisol Ramirez-Solano, Alyssa Merkel, Xinchun Zhou, Qi Liu, Julie A. Rhoades and Renjie Jin

Prostate cancer (PC) shows a strong predilection to spread to the bone and almost all patients with advanced PC show histological skeletal involvement. In bone metastases, the interaction with the host organ is favoured if tumor cells gain “osteomimicry”, that is the ability to resemble a resident bone cell, thus intruding in the physiology of the bone. However, detailed mechanism by which cancer cells gain “osteomimicry” and colonize and grow in the bone is not fully understood.

In this study, we demonstrated that long-term treatment with anti-androgens induces PC cell transdifferentiation to neuroendocrine prostate cancer (NEPC) and reprograms PC cells to change their characteristics enabling them to interact with bone marrow-derived macrophages and to grow in the bone microenvironment. We demonstrated that the antiandrogen-resistant or therapy (t) induced NEPC (tNEPC) cells can stimulate bone marrow-derived macrophages (BMM) result in expression of proinflammatory cytokine IL-1α. Further, we demonstrated IL-1α feed-forward loop activates NF-κB signaling and increases osteotropism gene expression in PC cells which benefit cancer cell survival, colonization and growth in the bone microenvironment.

These findings indicate that PC cells initially response to ADT treatment preventing disseminated/circulating tumor cells (DTC/CTC) colonization and growth in the bone microenvironment. However, long-term antiandrogen treatment will reprogram cytokine/chemokine expression profile in PC cells. The cytokine/chemokines secreted from reprogrammed PC cells will stimulate BMM to increase IL-1α expression. Increased IL-1α from macrophages can feed-forward to PC cells to activate NF-κB signaling constitutively and increase osteotropism gene expression in cancer cells thereby contributing to PC cells to survive and grow in the bone microenvironment. Therefore, blocking the interacting loop between cancer cells and the bone microenvironment by targeting IL-1α signaling may be a novel sufficient approach to prevent/treat bone metastatic CRPC.

Extranuclear Nucleolin Induces ITGŒ±6 expression in Prostate Cancer Independent of Androgen Receptor Elsa Merit Reyes-Reyes, University of Arizona

Sara Moore, and Cindy K. Miranti

Extranuclear Nucleolin Induces ITGα6 expression in Prostate Cancer Independent of Androgen Receptor

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Background.
Integrins (ITG) are αβ heterodimeric cell surface receptors that mediate cell survival signaling through adhesion to the extracellular matrix (ECM). ITGα6 is highly expressed in advanced prostate cancer (PCa). Androgen receptor (AR) induces the expression of ITGα6, which preferentially dimerizes with ITGβ1 to form integrin α6β1. Integrin α6β1 binds to laminin, an abundant ECM in PCa, and promotes survival. AR reactivation is the primary mechanism for the incurable lethal castration-resistant prostate cancer (CRPC). ITGα6 expression is enhanced in AR^+ CRPC, and antibodies that block ITGα6 function severely limit PCa tumor growth in mice. Thus, one therapeutic approach for AR^+ CRPC is to block ITGα6. However, we lack a complete understanding of how ITGα6 expression is regulated. The nuclear scaffold protein nucleolin (NCL) is overexpressed and mislocalized into extra-nuclear areas in human cancer tissues relative to normal tissues. Cytoplasmic NCL functions posttranscriptionally to upregulate protein expression by stabilizing or promoting the translation of mRNAs. Because NCL has been implicated in regulating cell adhesion and cell survival, here we sought to determine whether extra-nuclear NCL promotes ITGα6 expression in PCa.

Methods. We modulate NCL activity in AR^+ and AR^- PCa cell lines using siRNA, overexpression of wild-type or NCL ΔNLS mutant, or treat with the extra-nuclear specific NCL antagonist AS1411. ITGα6 expression and adhesion to laminin were evaluated.

Results
We found that NCL RNAi decreases ITGα6 protein independent of AR expression, in LNCaP (AR^+) and PC3 (AR^-) cells and independent of androgen. Knock-down of NCL decreases adhesion to laminin, but not collagen. Moreover, overexpression of extra-nuclear NCL (NCL ΔNLS mutant), but not WT NCL, increases ITGα6 protein. AS1411, an extra-nuclear-specific NCL antagonist, decreases ITGα6 mRNA, but not AR expression.

Conclusions:
Extra-nuclear NCL enhances ITGα6 expression independent of AR and nuclear NCL. These data suggest that in cells where AR is inhibited, post-transcriptional mechanisms may independently drive integrin α6β1 expression to promote survival. Given the importance of ITGα6 as a mediator of AR-dependent survival, these studies suggest that AR-independent post-transcriptional mechanisms, via mislocalized NCL, maybe a bypass mechanism to overcome *AR* suppression to promote CRPC survival.

Mechanism and Targeting the Hippo/YAP and NF-Kappa B/RELA Axis in Prostate Cancer Cells

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Mechanism and Targeting the Hippo/YAP and NF-Kappa B Axis in Prostate Cancer Cells

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Abstract

Background: The Hippo/YAP and NF-kappa B/RELA pathways regulate analogous cellular biologies including cell proliferation, cell survival, cell migration, stem cell biology, and immune responses. Aberrant activation of YAP1/TEAD and RELA plays a critical role in cancer with poor prognosis. This study aims to investigate the interaction between YAP1/TEAD and RELA signaling in prostate cancer cells and to target this mechanism for therapeutic intervention.

Methods: Co-immunoprecipitation and duo link proximity ligation assay were conducted to evaluate the protein-protein interaction between YAP1 (yes associated protein 1) and RELA in the cell. Confocal microscopy was employed to assess the subcellular localization of YAP1 and RELA proteins. Promoter reporter assay was performed to asses NF-Kappa B-responsive promoter-reporter activity. The YAP1/TEAD and RELA ChIP (chromatin immunoprecipitation)-seq (sequencing) datasets were analyzed using bioinformatics tools. Cell growth assay was determined using the CCK-8 kit.

Results: We showed that the RELA and YAP1/TEAD activity overlapped in prostate cancer cell lines and clinical cases. We also showed that endogenous YAP/TEAD and RELA physically interact in the cells. Combined treatment of androgen and RANKL (receptor activator of nuclear factor-kappa B ligand) or SDF1a (stromal cell-derived factor 1 alpha) enhanced YAP1 and RELA colocalization and interaction inside the cells. Moreover, the knockdown of YAP1 or TEAD by siRNA significantly reduced the NF-Kappa B promoter-reporter gene activity. The controlled expression of MST1/STK4, a potent inhibitor of YAP1, attenuated the NF-Kappa B activity. Additionally, our unbiased bioinformatics analysis identified several cancer-associated genes that are likely co-regulated by YAP1/TEAD and NF-Kappa B signaling. Furthermore, we found that disruption of YAP1 activity attenuated the TEAD-RELA interaction and sensitized castration-resistant cells to pimozide.

Conclusions: These findings suggest that molecular and functional interaction between the Hippo/YAP and NF-Kappa B/RELA plays a critical role in human cancer and serves as a prime cancer drug target.

Studying Nanoparticle targeting to Prostate Cancer by using Quantum dot Antibody conjugate

Amarnath Mukherjee, Albert Einstein College of Medicine *Augene Park, Mark Schoenberg & Kelvin Davies*

Background: In spite of several attempts to make targeted nanoparticle (NP) by grafting a tumor homing ligand on its' surface, there has been little success with this approach and thus far the FDA has yet to approve a targeted NP. A major reason for this is the confounding influence of passive NP targeting, which is driven by the Enhanced Permeability and Retention (EPR) effect caused by combination of leaky tumor vasculature and the long circulating half-lives of most NPs. This EPR effect makes it difficult to distinguish actual active NP targeting from passive NP targeting. It has been challenging to develop well-controlled experiments to specifically study active NP targeting because of the complex physio-chemical properties of the macromolecules like Nanoparticles, unavailability of proper tumor model and step-by-step evaluation of targeting. Here we have conjugated a Prostate Specific Membrane Antigen (PSMA) Antibody to a fluorescent Quantum dot NP system and applied to assess ligand-specific active targeting and non-specific passive targeting in a single isogenic tumor model of Prostate cancer.

Methods: We have utilized the commercially available Qdot that fluoresces at near-infrared region (800 nm which do not interfere with body's normal absorption range) and conjugated to monoclonal anti-PSMA antibody J591. The PSMA-specific binding of the Qdot-Ab conjugate were confirmed by microscopy and ELISA-type cell-binding assay. The *in vivo* tumor accumulation were evaluated by intra-venous administration of Qdot-Ab in a bilateral mice model with isogenic PSMA +/- tumor and measuring the fluorescence at 800 nm using Kodak IVIS animal imaging system.

Results: Our results show that the Qdot-Ab binds specifically to the PSMA+ cells (with +/- ratio of 28.1 ± 4.7). However, the tumor accumulation in PSMA+ tumor is marginally better than the control tumor (1.17 ± 0.36).

Conclusions: The *in vitro* binding or specificity of any nanoparticle ligand conjugate might not be the best predictor for *in vivo* outcome. The *in vivo* targeting is probably a complex phenomenon governed by many steps. Step-by-step evaluation of this process is necessary for future success of nanoparticle targeting. Our system can be applied to solve some of the unanswered questions of the field (at least in PSMA targeted area).

Prostate tumor-derived GDF11 accelerates androgen deprivation therapy-induced sarcopenia

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Prostate tumor-derived GDF11 accelerates androgen deprivation therapy-induced sarcopenia

Background:
Most prostate cancers depend on androgens for growth and therefore the mainstay treatment for advanced, recurrent or metastatic prostate cancer is androgen deprivation therapy (ADT). A prominent side effect of ADT is an obese frailty syndrome that includes fat gain and sarcopenia, loss of muscle function and low muscle mass or muscle quality. Prostate cancer bearing mice were examined to gain mechanistic insight into ADT-induced sarcopenic obesity.

Methods:
We castrated adult PbCre4:PTEN$^{fl/fl}$ mice and measured tumor volume (by high-frequency ultrasound imaging), body composition (by qNMR), muscle mass, fat mass, grip strength and expression of TGFß catabolic myokines. Tumor-bearing mice were treated with ActRIIB-Fc or anti-GDF11 neutralizing antibodies to block myokine signaling, and additional tumor-bearing mice were sacrificed biweekly to serially measure myokine levels.

Results:
These mice showed no tumor-induced cachexia. Castration controlled tumor growth but induced both fat gain and skeletal muscle mass and strength loss. As in tumor-free mice, catabolic TGFß-family myokine proteins were induced in muscle prior to strength loss and TGFß-family myokine blockade reversed castration-induced sarcopenia. However, in mice with prostate cancer, castration-induced strength and muscle loss was accelerated. This accelerated sarcopenia was preceeded by an earlier increase of myostatin in muscle. Unique to tumor-bearing mice, GDF11 was also increased in muscle prior to strength loss. We also observed an early surge of GDF11 in prostate tumor and the circulation, suggesting endocrine GDF11 signaling from tumor to muscle may drive the accelerated ADT-induced sarcopenic phenotype. Functionally, blocking GDF11 reduced both the accelerated and overall strength loss.

Conclusions:
Castration side effects in tumor-bearing mice closely resemble the sarcopenic obesity observed in ADT treated prostate cancer patients. ADT-induced sarcopenia may be exacerbated by endocrine catabolic signaling from tumor to muscle. These findings suggest that neutralizing therapies targeting GDF11 may be effective in preventing ADT-induced sarcopenia in prostate cancer patients.

Determining the Roles of DNA Repair Gene Aberrations in Driving the Development and Progression of Prostate Cancer

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Title: Determining the Roles of DNA Repair Gene Aberrations in Driving the Development and Progression of Prostate Cancer

Background: Recent large-scale genomic analyses have revealed that mutations in DNA repair pathway genes (including *BRCA2*) occur in ~20% of metastatic castration-resistant prostate cancers (mCRPC). *CDK12* has been implicating in DNA repair via regulation of splicing and polyadenylation at DNA repair genes and is mutated in ~5% of mCRPC. However, it is unclear if *CDK12* loss in prostate cancers is acting through this DNA repair mechanism. We aim to test whether the loss of *BRCA2* or *CDK12* is sufficient for prostate tumor initiation or progression, and to test how loss of these genes sensitizes cells to targeted therapies.

Methods: To study the role of DNA repair gene loss in prostate cancer, we utilized a combination of genetic mouse models and engineered cell lines. Mice with floxed *Brca2* or *Cdk12* were bred and crossed to Probasin-Cre mice. Floxed cells were isolated and cultured in 2D/3D conditions and recombined *in vitro.* LNCaP cells were engineered with inducible shRNA knockdown of *BRCA2* or *CDK12* and CRISPR-mediated knockout.

Results: Mice with conditional prostate-specific knockout of *Brca2* or *Cdk12* have been bred and are aging for tumor development. Floxed cells were isolated and grown in 2D and 3D culture, but recombination of *Brca2* or *Cdk12* were poorly tolerated. LNCaP-Tet-shCDK12 lines showed a decreased growth rate with *CDK12* knockdown and minimal changes in *ATM*, *BRCA1*, or *BRCA2* mRNA and protein expression. Furthermore, *CDK12* knockdown had minimal effect on PARP inhibitor sensitivity. CRISPR mediated knockout of *CDK12* or *BRCA2* in LNCaP showed decreased fitness in a CRISPR competition assay.

Conclusions: We have developed new models to investigate precisely how mutations in *BRCA2* and *CDK12* affect prostate tumor development and progression. Though *CDK12* knockdown is tolerated, complete loss is poorly tolerated in LNCaP and mouse primary prostate cells. Tumors with *CDK12* mutation likely require compensating alterations. Furthermore, *CDK12* knockdown in LNCaP does not show a strong DNA repair deficiency phenotype, which may represent a difference in prostate epithelium from other cell types. Future experiments are underway to monitor potential tumor development in aging knockout mice and determine which alterations are required for cells to tolerate *BRCA2* or *CDK12* loss. With a better understanding of how mutations in these genes influence prostate cancer development and progression, we strive to exploit these vulnerabilities in the future with targeted therapies.

Synthetic Lethal Metabolic Targeting of Androgen Deprived Prostate Cancer Cells with Metformin

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Synthetic Lethal Metabolic Targeting of Androgen Deprived Prostate Cancer Cells with Metformin

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Introduction & Objective

The initiation of androgen deprivation therapy (ADT) induces susceptibilities in prostate cancer (PC) cells that make them vulnerable to synergistic treatment, resulting in enhanced cell death. Senescence has been shown to result in cell cycle arrest, however cells remain metabolically active. In this study, we investigated the mechanisms by which PC cells undergo senescence in response to ADT and we determined whether the FDA approved antidiabetic drug metformin (met) has a synergistic effect with ADT in PC both *in vitro* and *in vivo*.

Methods

Androgen-dependent PC lines LNCaP and LAPC4 were induced to senescence by ADT (charcoal–stripped serum or bicalutamide) over an 8 day time-course. Senescence was confirmed using SA beta-galactosidase staining as well as increased cell complexity on flow cytometry and increased expression of senescence markers by western blot (WB). Met was added following ADT and cell fate was examined over 72hr. Cell viability was assessed via Hoechst DNA staining and was used to perform a synergistic analysis. The protein expression of the molecules associated with mTOR1 in PC cells following ADT/met alone or in combination were analyzed by WB. A mouse PC model (PDX) was generated via implantation of xenografts derived from aggressive PC patients and subsequently underwent castration for ADT or sham surgery. Tumor growth, senescence, apoptosis and molecular characteristics of the tumors were compared between sham and castration groups +/- met treatment.

Results

Longer-term exposure to ADT induced more robust expression of p16INK4a and/or p27/kip2, markers associated with senescence. The combined effect of PI3K/Akt activation and AMPK inactivation in senescent cells resulted in mTOR activation. In addition, the expression of anti-apoptotic protein XIAP was increased in response to ADT. Subsequently, addition of metformin following ADT demonstrated increased levels of apoptosis, attenuated mTOR activation by ADT, reduced senescent cell number *in vitro,* and inhibited tumor growth in a PC mouse model.

Conclusions

This study suggests that combining ADT and metformin may be a feasible therapeutic approach to remove persistent PC cells after ADT.

Funding

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Targeting activation of AMPK suppresses PCa proliferation by regulating lipogenesis with subsequent inhibition of AR expression and activity

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- **Background:** Most tumors that become resistant to androgen receptor targeting agents continue to be driven by some form of the AR including AR splice variants. These data suggest that additional agents targeting the androgen receptor in novel ways could further prolong life in the patients that become resistant to current AR targeting therapies or in combination with these therapies would be even more effective at prolonging life. We have shown that targeting the tumor metabolome by altering lipogenesis inhibits androgen receptor expression and signaling.
- **Methods:** We screened 700 bumped kinase inhibitors developed inhibitors against castration resistant prostate cancer (CRPC) cell lines that were AR+ or AR-. Fifty compounds inhibited AR+ cell growth at 1um but had no effect on AR- cells up to 50 um. A combination of proteomics, phosphoproteomics, and RNA sequencing, was used to determine the mechanism of action MOA.
- **Results:** Our studies determined that the MOA of our lead compound, BKI1553, was a dephosphorylation of a reepresive serine on the alpha subunit of AMPK that resulted in AMPK activation and suppression of proliferation of AR+ cell lines and AR+ PDX models by phosphorylation of acetyl coenzyme A carboxylase- a downstream effector of AMPK activation. No effect was seen in AR- cell lines or xenografts.
- **Conclusions:** Targeting the lipid metabolome in AR-driven CRPC using small molecules suppress tumor growth. BKI1553 is orally bioavailable and has an excellent toxicity profile making it a translational therapy.

Investigating the Role of Rbl2 in Castration-Resistant Prostate Cancer

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Title: Investigating the Role of Rbl2 in Castration-Resistant Prostate Cancer

Background: At diagnosis, most prostate tumors rely on ligand-mediated androgen receptor (AR) signaling to promote cell proliferation and thus are treated via androgen deprivation therapy (ADT). ADT is typically palliative and prostate cancer cells often adapt to restore AR signaling even when androgen production is low, leading to recurrence as castration-resistant prostate cancer (CRPC). CRPC remains dependent on AR signaling and is treated with androgen pathway inhibitors (APIs). However, prolonged API treatment can lead to the formation of an aggressive AR-indifferent tumor with small cell and neuroendocrine features. This mechanism of resistance is seen in approximately 20-25% of patients following initial treatment with APIs and is known as treatment-induced lineage crisis.

Tumors can sometimes evade therapy by reprogramming to a state that is no longer dependent on the targeted cellular pathway. While Rb1 has recently emerged as a master regulator of lineage plasticity and patient outcome, the role of other Rb family members in CRPC has not been explored. We hypothesize that Rbl2 loss accelerates lineage plasticity, predicts resistance to APIs and is associated with small cell features in CRPC.

Methods: By combining an *in vivo* tumor regeneration assay, immunohistochemistry and metabolomics with bioinformatics analysis of TCGA, CpTAC and ICGC genomic datasets, we will utilize an interdisciplinary approach to study the mechanistic role of Rb family member loss in CRPC.

Results: To model the loss of additional Rb family members, we generated prostate organoids that are p53/Rb1 double knockout and p53/Rb1/Rbl2 triple knockout. We compared the transcriptional profiles of these organoid models and found that the triple knockout has increased gene expression pathways related to axonal guidance and cell cycle regulation, which are associated with neuroendocrine prostate cancer.

Conclusions: Currently, there are no targeted therapies available for the treatment of neuroendocrine prostate cancer and patients typically succumb to the disease within one year. Elucidating the mechanisms underlying lineage plasticity in CRPC could enable targeting of this process to treat advanced disease. By blocking the initiation of neuroendocrine differentiation or reversing its lineage switch, we might restore tumor sensitivity to APIs.

Genetics features of Localized Prostate Cancer in African Americans

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Title: Genetics features of Localized Prostate Cancer in African Americans

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The authors have no financial or other conflict of interest to disclose.

Background:
Prostate cancer of African Americans (AAs) has a poor prognosis and progress rapidly. This study aimed to identify potential genetic risk factors for prostate cancer in AAs from these differences.

Methods:
We used prostate cancer tissue from 61 patients who underwent radical prostatectomy at Rutgers University Cancer Institute New Jersey (CINJ). We compared somatic gene expression in Caucasians (CAs) and AAs using RNA sequences. In order to search for genes which significantly express in AAs and likely to be involved in recurrence, significant genes obtained by comparison analysis in our cohort were examined for gene expression levels and clinical outcomes in TCGA provisional.

Results:
Our cohort included 31 AAs and 30 CAs. There were no significant differences between AAs and CAs in age, GS in surgical specimens, preoperative risk factors assessed using the NCCN risk classification, and pathological stage. From whole transcriptome RNA-seq in prostate cancers from AAs and CAs, we found a significant difference in expression of 45 genes (adj. p. value < 0.05). Comparison of the change RNA expression levels in AAs and CAs showed that the genes specifically up-regulated in CAs were SRMS C1orf95, NKX2-2, and GCG. On the other hand, the top 3 most up-regulate gene in AAs was RASA4B, S1PR3, and CRYBB2.

In TCGA Provisional, RNA sequence data were obtained from 326 patients, of which 41 were AAs and 285 were CAs. RNA expression levels between AAs and CAs in TCGA Provisional showed that the expression level of RNA in genes CRYBB2 and TTC38 was significantly higher in AAs, while the expression level of RNA in genes CDH13, CHML, CHRM3, NAIP, and SLC6A20 was significantly higher in CAs in both our cohort and

TCGA Provisional. Besides, Kaplan-Meier analysis revealed a significant difference in disease-free survival between the high and low expression groups in CHRM3. High-CHRM3 patients had significantly longer disease-free survival (median survival, 77.3 vs. not reached months; P=0.012).

Conclusions:
We revealed genetic differences in AAs and CAs by RNA sequencing. Further studies are needed to determine the biological impact of genetic differences obtained in this study on the clinical outcome of prostate cancer in AAs.

Sulfotransferase SULT2B1b inhibition potently stimulates the expression of immunomodulatory genes in prostate cancer cells

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Sulfotransferase SULT2B1b inhibition potently stimulates the expression of immunomodulatory genes in prostate cancer cells

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Background

SULT2B1b (sulfotransferase family cytosolic 2B member 1b) catalyzes the addition of a sulfate group to cholesterol and its hydroxylated derivatives, oxysterols. SULT2B1b has been detected in both normal prostate epithelium and prostate cancer cells. Our previous studies have demonstrated that SULT2B1b is essential for the survival of prostate cancer cells. The purpose of the current study is to elucidate these underlying mechanisms and to identify novel functions for this enzyme.

Methods

For a comprehensive analysis of pathways regulated by SULT2B1b, RNA-seq was performed with LNCaP and enzalutamide-resistant C4-2R cells after SULT2B1b siRNA transfection. Gene expression was confirmed with real-time PCR. Transcription factor IRF3 and its kinase TBK1 regulate the expression of interferons and other cytokines. To investigate whether IRF3 or TBK1 mediates the function of SULT2B1b, specific inhibitors or siRNAs were used to inhibit these proteins. Markers for DNA damage and apoptosis were analyzed with immunoblot.

Results

We have found that, in both LNCaP and C4-2R cells, interferon response pathways and TNFα signaling pathway were highly enriched with SULT2B1b knockdown (KD). Consistently, genes which belong to these pathways were among the most upregulated after SULT2B1b KD. SULT2B1b-regulated immunomodulatory gene expression may be mediated through TBK1/IRF3, as inhibition of these proteins partially blocked the immunomodulatory gene expression induced by SULT2B1b KD. In addition to the cell death and the unique gene expression pattern, DNA damage was detected in prostate cancer cells after SULT2B1b KD. We then investigated which SULT2B1b products mediate the function of this enzyme. It was found that both cholesterol sulfate (CS) and 25-hydroxycholesterol sulfate (25HCS), a sulfated oxysterol, were able to reverse the DNA damage and cell death induced by SULT2B1b inhibition. However, only 25HCS blocked the induction of immunomodulatory gene expression, indicating that SULT2B1b function may be carried out through both CS and sulfated oxysterols with the latter being more critical in the regulation of immunomodulatory genes.

Conclusions

In summary, these findings suggest that SULT2B1b inhibition may not only directly target cancer cells and induce cell death, it may also alter the behavior of immune cells in the microenvironment through the induction of immunomodulatory genes. Therefore, SULT2B1b targeting may enhance the efficacy of immunotherapy in prostate cancer.

Modulation of the Prostate Tumor Microenvironment by Folate-mediated Targeting of Tumor Resident Myeloid Populations

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Modulation of the Prostate Tumor Microenvironment by Folate-mediated Targeting of Tumor Resident Myeloid Populations

Immunology and Immunotherapy

Introduction and Objective

Prostate cancer is generally known as a "cold" tumor immunologically, characterized by a loss of infiltrating immune cells to the tumor and limited responses to immunotherapies. This makes it difficult to approach immunotherapy in the prostate with current approaches. Despite the lack of robust immune infiltrate there is still a presence of myeloid derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) that suppress any anti-tumor response. Potent inflammatory compounds, such as, TLR7 agonists can re-polarize MDSCs and TAMs but are toxic when given systemically. We present here the ability to deliver highly potent inflammatory compounds to MDSCs and TAMs at the tumor site through their expression of folate receptor beta (FRβ) to drive a re-polarization toward a pro-inflammatory phenotype.

Methods

We utilized the murine prostate cell lines, RM-1, MyC-CaP and TRAMP-C2, to analyze expression of FRβ in MDSCs and TAMs. Functional characterization was carried out using a short-term T cell suppression assay to determine function immediately *ex vivo*. Modulation of the tumor site was done using an orthotopic MyC-CaP model treated with folate targeted TLR7 agonist (FA-TLR7a).

Results

We demonstrated that only tumor MDSCs and TAMs express FRβ+ that can be targeted *in vivo* with folate compounds. Additionally, under tumor specific conditions only FRβ+ MDSCs and TAMs are functional and rely on nitric oxide for suppression. Despite a role for PD-L1 under hypoxia, it was not essential for MDSC and TAM suppression. In orthotopic MyC-CaP tumors, FA-TLR7a was able to increase CD8+ T cells at the tumor site, increase M1/M2 macrophage ratios, and decrease tumor size.

Conclusions

These data demonstrate a unifying marker for suppressive MDSCs and TAMs that rely on nitric oxide production for T cell suppression. This is a unifying observation for the study of immunosuppression within the tumor and for targeting of these cells. Targeting through FRβ with potent immunostimulatory compounds like TLR7 agonists shows promise in reversing the "cold" nature of a prostate tumor by increasing M1 macrophages and CD8+ T cells at the tumor.

Putative tumor suppressor ELL2 is required for proliferation and survival of AR-negative prostate cancer cells
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Title: Putative tumor suppressor ELL2 is required for proliferation and survival of AR-negative prostate cancer cells

Background: Elongation Factor For RNA Polymerase II, 2 (ELL2) was reported as a putative tumor suppressor in the prostate cancer. ELL2 is frequently down-regulated in prostatic adenocarcinoma specimens. Loss of ELL2 induced murine prostatic intraepithelial neoplasia and enhanced AR-positive prostate cancer cell proliferation. However, ELL2 gene was amplified or overexpressed in neuroendocrine prostate tumors, suggesting a potential oncogenic role for ELL2 in AR-negative neuroendocrine prostate cancer cells. In this study, we explored potential function of ELL2 in PC3 and DU145, two AR-negative prostate cancer cells with some neuroendocrine phenotype.

Methods: The role of ELL2 in PC3 and DU145 was studied by determining the impact of ELL2 knockdown. Genes regulated by ELL2 knockdown in PC3 cells was identified using RNA-Seq and bioinformatics. Expression of various genes were confirmed by western blot and quantitative real-time PCR. Cell growth were determined by BrdU, MTT and colony formation assays. The cell death was analyzed by Annexin V/7-AAD staining. Cell cycle was determined by flow cytometry.

Results: ELL2 knockdown inhibited proliferation in PC3 and DU145 cells, in contrast to the enhancement of proliferation in AR-positive LNCaP and C4-2 prostate cancer cells by ELL2 knockdown. RNA-Seq analysis showed an enrichment in genes associated with cell death and survival following ELL2 knockdown. The interferon-γ pathway was identified as the top canonical pathway comprising of 55.6% of the differentially expressed genes. ELL2 knockdown induced an increase in STAT1 and IRF1 mRNA and an induction of total STAT1 and phosphorylated STAT1 protein. Inhibition of cell proliferation by ELL2 knockdown was partly abrogated by siSTAT1 knockdown. Colony formation and MTT assay suggested that ELL2 knockdown could induce profound cell death in both PC3 and DU145 cells. Flow cytometry analysis showed that knockdown ELL2 indeed induced apoptosis and also caused S-phase arrest.

Conclusions: ELL2 knockdown inhibited cell proliferation, induced S-phase arrest, promoted apoptosis in AR-negative PC3 and DU145 prostate cancer cells, which is accompanied by the induction of genes associated with cell death and survival as well as the interferon-γ pathway. This suggests a potential oncogenic role for ELL2 in AR-negative prostate cancer cells. The mechanisms by which ELL2 promotes AR-negative prostate cancer cell proliferation and survival may represent potential targets for the treatment of AR-negative prostate cancer.

Novel Roles for Manganese Superoxide Dismutase Polymorphisms in Prostate Cancer

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NOVEL ROLES FOR MANGANESE SUPEROXIDE DISMUTASE POLYMORPHISMS IN PROSTATE CANCER

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Introduction: Epithelial Mesenchymal Transition (EMT), a key event in prostate cancer metastasis, allows polarized epithelial cells to assume mesenchymal morphologies, enhancing invasiveness and migration. Reactive Oxygen Species (ROS), chemically reactive molecules of cellular metabolism, induce oxidative stress, increasing SNAI1 (Snail) transcription factor and promoting EMT. Manganese superoxide dismutase (MnSOD or SOD2) is a key mitochondrial protein that protects cells from oxidation and is reported to contain potential tumor suppressor and surprisingly, tumor promoting characteristics. Increased susceptibility to aggressive prostate cancer is observed in patients with SOD2 Ala/Ala single nucleotide polymorphism (SNP) compared to Val/Val SNP. Ala/Ala SNP is associated with higher dismutase activity than Val/Val. We hypothesize that increased dismutase activity of SOD2 Ala/Ala genotype may inadvertently increase oxidative stress associated with enhanced EMT.

Methods: Prostate cancer cell lines were analyzed for SOD2 SNPs by pyrosequencing. Site-directed mutagenesis was conducted, creating cDNA with Ala/Ala and Val/Val polymorphisms which were subsequently, transiently and stably transfected into LNCaP prostate cancer cells. Baseline intracellular ROS and EMT marker expression was evaluated in SOD2-overexpressing cells *via* ROS assay and immunoblot analysis. Furthermore, cell migration using Boyden chambers and cell proliferation using MTS assays were performed on various clones.

Results: Pyrosequencing determined that most prostate cancer cell lines were heterozygous for SOD2 (Ala/Val), although several had extra copies, suggesting multiple copy numbers. Only metastatic MDA PCa-2a and -2b contained Ala/Ala homozygous SNPs. LNCaP cells stably transfected with SOD2 Ala/Ala contained higher levels of total ROS and Superoxide as compared to LNCaP Val/Val cells. LNCaP Ala/Ala cells were more migratory, proliferative and associated with higher SOD2 expression and EMT (higher levels of Snail and Vimentin) as compared to SOD2 Val/Val cells.

Conclusion: Our data suggests that increased total ROS in cells containing Ala/Ala SNP may be associated with increased EMT. These studies may uncover the biological basis of different SOD2 SNPs and reveal novel ways to treat or differential responses to treatment in patients carrying different SNPs.

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Neuroendocrine Marker improves the diagnosis of Prostate Cancer

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Neuroendocrine Marker improves the diagnosis of Prostate Cancer

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Prostate cancer (PC) is the most common visceral cancer affecting men. A good prognosis for a PC patient is dependent on early detection. Although the serum PSA screening has improved the early detection of PC, the test is not reliable. Both, positive as well as negative PSA tests need to be confirmed with costly, repetitive and invasive TRUS-guided biopsy. This is because PSA is a natural secretory product of the prostate. Combining PSA test with a cancer- specific biomarker(s) can potentially improve the specificity and precision of PC detection.

We have discovered a novel prostate tumor-specific protein (neuroendocrine marker or NEM). NEM expression and secretion in the prostate seems to be cancer-specific, and its serum levels increase manifolds in cancer. We have also developed a new NEM-PSA combination test that employs an inexpensive, disposable nanosensor chip that can detect sub-picomolar levels of antigens. The assay is label-free and linear over the range of 1-64 pg with a sensitivity of 1 pg/50 µl. With this, one can measure serum NEM levels in as little as 0.1µl serum. We analyzed serum NEM and PSA levels of a cohort of 109 patients (36 healthy donors and 75 positively-confirmed PC patients). NEM levels in non-cancer (controls; Mean ± SEM 2.5±1.286 ng/ml, n=36) were significantly lower than all PC patients (cancer: 12.21±0.6135, n=75) with no overlap ($p<0.0001$, contingency analysis). In contrast, PSA levels of the same cohort displayed significant overlap between non-cancer and cancer patients (Controls: 6.26±0.9, n=36 vs Cancer: 22.85±2.96, n=75). We also analyzed prostate NEM content of some patients by immunohistochemistry of biopsy sections. There was a positive correlation between serum NEM levels and tissue NEM content. We also analyzed 11 “grey zone” PSA samples for NEM (those between serum PSA 4-10 ng/ml). When matched with biopsy pathology, we observed that NEM could separate “grey zone” PSA patients into cancer (TP) and benign disease (TN). These results support a possibility that our “NeoPro” test may improve the specificity of PC diagnosis, specifically in “grey zone” PSA patients, and help reduce the number of diagnostic biopsies.

GWAS and CNV analysis Demonstrate Polygenic Determination of Vesicoureteral Reflux

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GWAS and CNV analysis Demonstrate Polygenic Determination of Vesicoureteral Reflux

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Background: Vesicoureteral reflux (VUR) is a highly familial pediatric disease caused by malfunction of the vesicoureteral junction, resulting in retrograde flow of urine from the bladder into the ureters and kidney. VUR is a major cause of recurrent urinary tract infection (UTI) and pediatric kidney failure worldwide. Very few VUR genetic risk factors have been identified to date.
Methods: We conducted Copy Number Variant (CNV) analysis of 1,737 VUR cases and 24,765 controls and also performed a GWAS of 1,395 unrelated VUR cases of European ancestry and 5,366 matched population controls, under additive, recessive and dominant models. A VUR polygenic risk score (PRS) was computed from GWAS summary statistics, and used to perform a phenome-wide association study (PheWAS) in 98,918 participants in the eMERGE network cohort.
Results: We found a significant excess of known pathogenic CNV in 35 (2.01%) of cases vs. 0.65% of controls (OR = 3.12; 95% CI 2.10-4.54;P = 6.35×10^{-8}). The VUR cases were enriched for 1q21.1 deletion, 16p11.2 deletion, 22q11.21 deletion and duplication, and triple X syndromes. The GWAS identified 3 study-wide significant ($P<7.58 \times 10^{-9}$) and 5 suggestive ($P<1.52 \times 10^{-7}$) loci with large effects (ORs = 1.41 to 3.65). The top SNPs

for each of these associations were within or near genes known to be important in embryonic development and/or kidney disease (*WDPCP, OTX1, BMP5, WNT5A, VANGL1*). In situ hybridization confirmed that the top candidate genes are expressed in the ureter or vesicoureteral junction during mouse genitourinary development. SNP based heritability was estimated to be 15%. A PheWAS using the VUR-PRS in the eMERGE pediatric cohort showed VUR, UTI and pyelonephritis as the most significant phenotypic associations.

Conclusion: This study identifies multiple rare CNV disorders and common variants which impart large effects on the risk of VUR and implicate multiple canonical developmental pathways in the pathogenesis of disease. PRS analysis revealed a polygenic architecture, and also suggested that PRS may be a useful tool for prediction of VUR and UTI in the pediatric population.

SLX4IP is Essential for Telomere Maintenance in Neuroendocrine Prostate Cancer

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SLX4IP is Essential for Telomere Maintenance in Neuroendocrine Prostate Cancer

Background

In advanced prostate cancer (PCa), resistance is acquired through a number of mechanisms such as neuroendocrine (NE) differentiation and loss of AR allowing androgen-independent growth. Therapeutic options remain limited for neuroendocrine prostate cancer (NEPCa) harboring compromised androgenic signaling. To address this therapeutic deficiency, it is essential to uncover regulatory networks propagating NEPCa. SLX4IP, an essential protein for telomere maintenance mechanism (TMM) switching, may fulfill this therapeutic prerequisite. TMMs are responsible for telomere elongation to instill replicative immortality, with the two TMMs available being telomerase or alternative lengthening of telomeres (ALT) pathway. Case studies suggest PCa has the ability to switch from telomerase to ALT with disease progression to perpetuate replication; however, this has not been investigated in the context of NE differentiation. We hypothesize that SLX4IP orchestrates the switch from telomerase to ALT, a transition obligatory for NEPCa survival.

Methods

Stable PCa cell lines were generated with SLX4IP overexpression or knockdown with respective controls followed by TMM characterization and gene expression changes. TMM characterization included telomerase expression via reverse transcription quantitative polymerase chain reaction (RT qPCR), telomerase activity via telomere repeat amplification protocol, visualization of ALT-associated PML bodies (APBs) via immunofluorescence-fluorescence *in situ* hybridization, and visualization of senescent cells via β-galactosidase staining. Expression changes of NE marker ENO2 were determined via RT qPCR and immunoblot.

Results

Our studies identified C4-2B cells, retaining AR and minimal ENO2 expression, utilize telomerase and PC-3 cells, with AR loss and NE markers, utilize ALT. SLX4IP overexpression in C4-2B cells shifted the primary TMM from telomerase to ALT indicated by reduced telomerase activity and appearance of APBs. This was accompanied by a significant increase in ENO2 expression. Conversely, SLX4IP knockdown in PC-3 cells dramatically reduced APB frequency; however, this was not coupled to telomerase utilization or reduced ENO2 expression. Instead, a 12-fold increase in the number of senescent cells compared to control was observed.

Conclusions

These data suggest that SLX4IP governs the ALT TMM required for the propagation of NEPCa. Additionally, inhibition of ALT via SLX4IP interference induces senescence thereby abolishing the immortality of NEPCa *in vitro*.

DNA methylation and DNA methyltransferases contribute to enzalutamide resistance in prostate cancer

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Prostate cancer is the leading diagnosed cancer among men in the United States. The androgen receptor (AR) antagonist enzalutamide is a Food and Drug Administration-approved drug for treatment of patients with late-stage prostate cancer and is currently under clinical study for early-stage prostate cancer treatment. After an initial positive response period, patients will develop drug resistance.

Our preliminary data suggests that DNA methylation and DNMTs may play a role in enzalutamide resistance. In this study, we will use RNA-Seq and bisulfite sequencing to uncover differentially-methylated and differentially-expressed genes that may play a role as markers or contributors to enzalutamide resistance.

Our results indicate that DNMT activity and DNA methylation are over-represented in cell-lines resistant to enzalutamide. DNMT3B is consistently overexpressed in resistant cells. Treatment with the demethylating agent, decitabine, and enzalutamide induces an increase in cleaved-PARP expression and a decrease in cell viability. Furthermore, knockdown of DNMT3B increases the response to enzalutamide in the resistant cells.

These results suggest that DNA methylation and DNMT3B specifically may contribute to enzalutamide resistance in prostate cancer. Targeting these pathways and differentially methylated genes may restore the function of enzalutamide in cells with acquired resistance to the drug. Finally, uncovering DNA methylation patterns in the genome of resistant cells may serve as a prognostic and diagnostic tool.

Inhibition of EphB4 overwhelms enzalutamide resistance by antagonizing the amplification of AR

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Background

Prostate cancer (PCa) heavily relies on androgen receptor (AR) pathway to support their survival. Enzalutamide (MDV3100) is a second-generation antiandrogen drug that was approved by FDA in 2012 to treat castration-resistant prostate cancer (CRPC). Unfortunately, generation of resistance is inevitable, and how to treat the enzalutamide-resistant CRPC has been a major challenge. Erythropoietin-producing human hepatocellular (Eph) receptors have a broad range of functions upon binding to ephrins. Increasing evidence shows that this signaling pathway plays an important role in tumorigenesis. Overexpression of EphB4 has been indicated in multiple types of cancer, which is closely related to proliferation, invasion and metastasis of tumors.

Methods

RNA-seq analysis was performed to showed the significant changes of pathway in cell lines. qPCR, immunoblots, cell viability assay and apoptosis assay was used in experiments. Xenograft experiment was done by inoculating 22Rv1 cells in castrated nude mice. H&E staining and IF staining was performed on tumor slides.

Results

Here we report that enzalutamide-resistant PCa entails active EphB4 pathway to ensue their drug resistant property. Using small kinase inhibitor and RNAi to disrupt the EphB4 activity re-sensitizes enzalutamide-resistant PCa to the treatment both *in vitro* and *in vivo*. Mechanistically, EphB4 positively regulates AR through induction of c-Myc.

Conclusion

Taking together, these results render new insight into the acquisition of drug resistance, thus offering a new approach to enhance the efficacy of enzalutamide.

Immune Cell Interactions in Benign Prostatic Hyperplasia Meaghan M Broman D.V.M., Purdue University
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Immune Cell Interactions in Benign Prostatic Hyperplasia

Background

Benign Prostatic Hyperplasia (BPH) is the most common prostatic disease among older men. While chronic inflammation is frequently associated with BPH, the role of interactions between and within immune cell populations in BPH development and progression is unclear. Single cell RNA Sequencing (scRNA-Seq) is often used to identify cell types and signaling pathways in healthy and diseased tissues and, more recently, identify ligand-receptor interactions within tumors. Here we further develop and apply these methods to identify immune cell ligand-receptor pairs to elucidate the potential roles of immune cell interactions in BPH.

Methods

CD45+ immune cells were isolated from incidental (<50 grams) and surgical (>100 grams) BPH prostate tissue. scRNA-Seq was performed using the 10X Chromium platform. Resulting data were used to identify distinct immune cell clusters based on differential gene expression and subsequently classified based on known immune phenotypes. BPH sample data were combined with previously published scRNA-Seq data from three normal non-BPH prostate samples. Ligand-receptor interaction scores were then calculated based on ligand and receptor gene expression and cell number referencing databases of known ligand-receptor pairs. Median scores were compared between BPH and normal prostate samples to identify significantly different interactions between these sample types.

Results

Unsupervised clustering of scRNA-Seq data from BPH and normal prostates revealed that immune cells segregate into 12 clusters. Within the combined incidental BPH samples, 79,116 immune cell ligand-receptor pairs with a median interaction score greater than zero were identified. Comparison of ligand-receptor interaction scores between incidental BPH and normal prostate samples found 538 ligand-receptor pairs with significantly different interaction scores. These significant differences include ligand-receptor pairs involved in cell adhesion and migration, immune cell recruitment and activation, and tumor immunity.

Conclusions

These results indicate that immune cell interactions are altered in BPH compared to normal prostates, suggesting that these interactions may play a role in the pathogenesis of BPH, and identifies potential immune cell targets for further validation and study. They also highlight the utility of scRNA-Seq and bioinformatic analysis techniques in identification and comparison of cellular interactions in diseased and normal tissues as well as identification of potential disease mechanisms and treatment targets.

Single Cell Analysis of Luminal Epithelial Cells in the Castrate Prostate Reveals a Unique Population of Candidate Luminal Progenitors

Daniel Moline, University of Chicago
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Single Cell Analysis of Luminal Epithelial Cells in the Castrate Prostate Reveals a Unique Population of Candidate Luminal Progenitors

Daniel Moline, Donald Vander Griend

Background: Benign prostate hyperplasia (BPH) is among the most common neoplastic diseases in men, yet the mechanisms underlying the proliferation of tissue emblematic of the disease are largely unknown.[1,2] One hypothesis explaining this unregulated outgrowth of tissue is an 'embryonic reawakening' within the prostate epithelium, where resident epithelial stem cells recapitulate their developmental behaviors in aging males and generate new tissue.[3,4] Here, we investigate the luminal epithelial lineage of the prostate using single cell transcriptome profiling to determine the presence and transcriptome profile of luminal progenitor cells.

Method: To analyze the cell populations of the luminal epithelial lineage of the prostate, we undertook an scRNA-Seq based approach capturing cells from both the intact and castrate mouse prostate. Single cell transcriptomes were analyzed using the Seurat R package and pathway analysis was performed using Integrated Pathway Analysis.[5,6] Biomarkers associated with the candidate luminal progenitor population were then validated in vitro and in vivo using IF microscopy and flow cytometry.

Results: scRNA-Seq analysis of luminal cells in the mouse prostate revealed several unique populations. Among those populations was a unique group of luminal cells expressing numerous unique stem cell markers including Psca, Sox2, and Tacstd2.[7–9] These have been independently reported to mark a unique luminal progenitor population found in both the normal and aging mouse and human prostate.[10] Additionally, IF microscopy and flow cytometry were used to validate the co-expression of markers observed through individual transcriptome analysis, showing that this population of cells indeed expresses the biomarkers assigned to it by dimensional reduction analysis. IF microscopy also corroborates a significant enrichment of candidate luminal stem cells in the castrate condition, consistent with scRNA-Seq analysis. Pathway analyses bioinformatically implicate integrin and Wnt signaling pathways, among others, as potential drivers of the luminal progenitor cell phenotype.

Conclusion: Together, these data create a picture of the biomarkers expressed by luminal progenitor cells in the castrate prostate. Additionally, the suite of biomarkers generated using this methodology allow for further investigation of signaling pathways and other factors that mediate the regenerative phenotype of these candidate luminal progenitor cells.

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African American prostate cancer stroma exhibits higher levels of secreted TGF-beta1 and overexpresses GARP but presents with more infiltration of cytotoxic CD8 (+) T cells Liankun Song, Department of Urology, University of California, Irvine

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African American prostate cancer stroma exhibits higher levels of secreted TGF-β1 and overexpresses GARP but presents with more infiltration of cytotoxic CD8 (+) T cells

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Background: Prostate cancer in African Americans (AA) men is more aggressive, leads to greater treatment failure and has two-three-fold greater mortality compared to prostate cancer of European American (EA) men. However, the mechanism of how race contributes to aggressive prostate cancer in AA patients is not understood.

Methods: Prostate fibroblasts from adjacent to tumor, named carcinoma-associated fibroblasts (CAFs), and paired distant fibroblasts taken from a contralateral region of the prostate glands where tumor was not present, called benign associated fibroblasts (BAFs) have been developed from AA and EA men. Tissue microarrays (TMAs) have also been constructed from corresponding tumor adjacent stroma tissues and contralateral benign stroma tissues. Further, conditioned media have been prepared from culturing CAFs and BAFs. TGF- β1 and 30 chemokines/cytokines and growth factors were measured by ultra-sensitive ELISA and Luminex multiplex protein assay, respectively. TMAs were stained with anti-CD4, anti-CD8, anti-CD3 and anti-glycoprotein A repetitions predominant (GARP) antibodies for immunohistochemistry analysis. The LASER program and single-nucleotide Polymorphisms (SNPs) present in the RNA-Seq was employed to confirm the self-identified ethnicity.

Results: TGF-β1 is secreted at higher levels and cytokines/chemokines known to facilitate T-cell-dependent tumor rejection in microenvironment at lower levels in AA CAFs compared to EA CAFs. AA CAFs decreases CD4 (+) Tim3 (+) T cells. GARP, the docking receptor for the release of active TGFβ1 and immune endurance, is over expressed in the adjacent stroma of AA prostate cancer compared to that of EA prostate cancer ($P = 0.0052$) and AA prostate cancer tissues ($P = 0.036$). Interestingly, the adjacent stroma of AA prostate cancer has more infiltration of cytotoxic CD8 (+) T cells than the distant stroma of AA prostate ($P = 0.0126$), whereas the adjacent stroma of EA prostate cancer has less infiltration of cytotoxic CD8 (+) T cells than the distant stroma of EA prostate cancer ($P = 0.0156$). Cytotoxic CD8 (+) T cells infiltration significantly increased in the adjacent stroma of AA prostate cancer compared to that of EA prostate cancer ($P = 0.0456$).

Conclusion: AA prostate cancer stroma may present increased immune endurance and more chronical inflammation. Creation of many different CAF lineages provides the opportunity to study functional differences and the associated molecular mechanisms of tumor microenvironment between AA and EA prostate cancer in culture.

Mediating EGFR- and ERK-Dependent Enzalutamide- Resistance in Castration-Resistant Prostate Cancer

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Mediating EGFR- and ERK-Dependent Enzalutamide-Resistance in Castration-Resistant Prostate Cancer

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Background:
Androgen receptor (AR) signaling inhibitors (ASI) enzalutamide (enz) and abiraterone acetate (abi) are standard treatments for patients with castration resistant prostate cancer (CRPC). Patients who develop resistance to these therapies have very few options. Epidermal growth factor receptor (EGFR) activity and expression are often upregulated in CRPC, so we explored inhibition of EGFR and its downstream effector ERK in ASI-resistance.

Methods:
Human prostate cancer (PCa) cell lines C4, C4-2B and 22Rv1 were continuously cultured in enz to induce resistance. PCa cell lines were treated with enz, lapatinib (lap, HER2/EGFR inhibitor), erlotinib (erlo, EGFR inhibitor), dacomitinib (daco, pan-ErbB inhibitor), ulixertinib (ulix, ERK inhibitor), or trametinib (tram, MEK inhibitor). Patient-derived-xenografts (PDX) and organoids were prepared from tumor tissues obtained from metastatic lesions of patients resistant to abi.

Results:
Whole genome sequencing revealed that enz-resistant C4-2B and 22Rv1 had elevated expression of genes associated with ErbB signaling when compared to parental lines, including EGFR ligands, resulting in EGFR and ERK phosphorylation. Hence, we investigated whether EGFR and ERK inhibitors prevented enz-resistance. Erlo and daco reduced viability in combination with enz, but lap did not. Combination of daco or erlo with enz reduced growth of abi-resistant PDX and organoids expressing high EGFR, respectively, more than either drug alone. Downstream of EGFR activation, erlo and daco, but not lap, reduced EGF-induced ERK phosphorylation. We observed that enz-resistant C4 cells, similar to parental cells, are still susceptible to erlo, indicating a method of inhibiting ASI-resistant CRPC. Significantly, only EGFR (not ErbB2/ErbB3) knockdown reduced constitutive ERK phosphorylation in PC-346C but not in 22Rv1. However, ulix and tram could alter 22Rv1 ERK phosphorylation levels. Similar to the EGFR inhibitors, ulix or tram reduced viability of enz-resistant lines in combination with enz.

Conclusions:
Enz-resistance from EGFR ligand upregulation, that induces EGFR phosphorylation (Y1068) and ERK phosphorylation, can be effectively reduced by combining enz and an EGFR inhibitor, such as erlo or daco, that successfully decreases ERK phosphorylation. Other forms of enz resistance may be attributed to ERK activity and non-genomic AR signaling. Our results suggest that treatment with enz along with an ERK inhibitor like tram or ulix may help reduce these other forms of enz resistance.

Radiation cystitis modeling: a comparative study of radiation induced bladder fibrosis in different mouse strains

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Radiation cystitis modeling: a comparative study of radiation induced bladder fibrosis in different mouse strains

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Abstract

Background
A subset of patients receiving radiation therapy for pelvic cancer develop radiation cystitis (RC), a complication characterized by mucosal cell death, inflammation, hematuria, and bladder fibrosis. Factors influencing onset and severity of radiation cystitis are not fully known. We aimed to assess the fibrotic response of the bladder to radiation in various genetic mouse strains to determine if genetic background variation may explain differences in risk for patients developing RC after radiation treatment.

Methods
C57BL/6, C3H and Balb/c mice were given a single dose of 40 Gy irradiation using the Small Animal Radiation Research Platform (SARRP) to deliver a gamma-irradiation to the bladder while limiting toxicity to neighboring tissue. Urinary voiding function, bladder shape, histology, collagen composition, and distribution of collagen producing cells were evaluated.

Results
Genetic background profoundly affects the severity of radiation-induced bladder fibrosis and urinary voiding dysfunction. C57BL/6 mice are most susceptible and C3H mice are most resistant. Irradiated C57BL/6 mouse bladders are misshapen and express more abundant collagen I and III proteins than irradiated C3H and BALB/c bladders. We localized *Col1a1* and *Col3a1* mRNAs to FSP1-negative stromal cells in the bladder lamina propria and detrusor. The number of collagen I and collagen III producing cells can predict the average voided volume of a mouse.

Conclusion
Using SARRP, pelvic radiation results in bladder dysfunction in mice modeling symptoms of chronic human RC including hematuria, fibrosis, and micturition frequency. Radiation-induced fibrosis in the bladder is strain dependent, suggesting that there may be a genetic predisposition to radiation sensitivity of the bladder. These results suggest that C57BL/6 may serve as a sensitive model for future RC studies. Lastly, we identified a potential role for FSP1-negative stromal cells in radiation-induced bladder fibrosis.

Low concentration BPA, BPS and BPF exposure: genotoxic effect in prostate cancer.

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Low concentration BPA, BPS and BPF exposure: genotoxic effect in prostate cancer.

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Since the 1970's the incidence of different endocrine cancers has increased, the augmented exposure to environmental endocrine disruptors has been proposed to play an important role in carcinogenesis. Xenoestrogens are endocrine disruptors which mimic structural parts of estrogen compounds consequently they may act or interfere with the physiological effect of estrogens. BPA has been reported to have xenoestrogenic activity also has affinity for the androgen receptor. Due its endocrine activity BPA exposure has been related to different hormone illnesses such as prostate cancer. In spite of BPA well- documented xenoestrogenic activity and probable carcinogenic potential structural analogs have been introduced into the market such as bisphenol S and F (BPS and BPF). Even the endocrine effect of these new molecules have been proved, the genotoxic implications and the DNA damage have been poorly studied. Michalowicz reported that BPA and its structural analogues are capable to induce oxidative DNA damage in peripheral blood mononuclear cells. Also BPA and BPS were reported to induce DNA damage in bronchial cells. Although all this evidence the genotoxic effect of bisphenols in prostate cancer remains unclear. In this work we determined the genotoxic effect of BPA, BPS and BPF in prostate cancer cell lines (LNCaP and PC3).

PC3 and LNCaP cell lines were seeded into 25cm2 flasks. After 24 hours they were hormone depleted with RPMI 1640 media without phenol red and charcoal stripped fetal bovine serum. Cell lines were treated with bisphenols (BPA, BPS and BPF) at low concentration (1nM, 5nM and 10 nM) and compared vs vehicle (ethanol). After exposure we extracted RNA to assess the transcriptomic effect (microarrays) and then we performed cell viability assay (MTT), wound healing assay, micronuclei assay to evaluate genotoxic effect.

Low concentration exposure to bisphenols showed differential gene expression, gene pathways and functional effect over genotoxicity seen as the differential presence of micronuclei, nucleoplasmic bridges and nuclear buds. Also bisphenols have a differential effect over cell viability and migration. We found that the genotoxic effect of BPA, BPS and BPF was different between cell lines LNCaP and PC3. The genotoxic effect was different in two conditions of the disease, androgen sensitivity and androgen resistance.

Bisphenol A and its structural analogues BPS and BPF at low concentration are capable to induce a genotoxic and transcriptomic effect in prostate cancer cell lines in two different stages of the disease.

Combination therapy of cisplatin and siRNA GP130 impacts a DNA repair mechanism in bladder cancer **Darryl T. Martin, PhD, Research Scientist, Yale University** *Shanshan He, MD, Visiting Scholar, Yale University; Gang Li, PhD, Visiting Graduate Student, Yale University; Andreas G. Sch√§tzlein, PhD, Professor of Translational Therapeutics, UCL School of Pharmacy; Robert M. Weiss, MD, Donald Guthrie Professor of Urology, Yale University; and Ijeoma F. Uchegbu, PhD, Professor of Pharmaceutics, UCL School of Pharmacy*

Combination therapy of cisplatin and siRNA GP130 impacts a DNA repair mechanism in bladder cancer

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Background
Glycoprotein-130 (GP130), a signal transducer for interleukin-6 family cytokines, is highly expressed in aggressive bladder cancers (BCa), and silencing GP130 expression decreases tumor growth in a mouse xenograft model. Previously, we showed that IL-6/GP130 is linked to chemotherapy resistance. Cisplatin treatment causes DNA lesions in BCa cells that are recognized, in part, by increased Ku70 expression, a key component in non-homologous end joining. However, it is unknown if overactivity of IL-6/GP130 signaling plays a role in the DNA damage response of BCa.

Methods
Tissue microarrays contained 39 deidentified urothelial cell carcinoma specimens that were collected from 30 patients who underwent transurethral resection of bladder tumor or cystectomy. Two adjacent normal bladder specimens were used as negative controls. An alkaline single cell gel electrophoresis assay was performed to evaluate the impact of DNA strand breaks. A novel EAGC-DOPE lipid hybrid nanoparticle delivery system (EGCDNPs) loaded with GP130 siRNA (siGP130) was prepared using the film evaporation technique. Dye exclusion and agarose gel retardation assays were used to assess the binding of the siRNA - EGCDNPs complex.

Results
A strong association between Ku70 and GP130 expression ($p < .0001$), and a significant correlation between BCa grade and GP130 expression were noted in human BCa. Using drug-resistant UM-UC-3 (UM-UC-3R) cells, there was a greater decrease in cell viability with cisplatin plus SC144, a GP130 inhibitor, compared to SC144 alone (14.24 ± 4.24 %, $p < .01$) or cisplatin alone (12.71 ± 4.24 %, $p < .05$). Also, a higher percentage of tail DNA was observed in UM-UC-3R cells with co-treatment (52.05 ± 2.58 %) when compared to untreated controls (8.91 ± 1.31 %, $p < .0001$), SC144 treatment alone (12.78 ± 1.48 %, $p < .0001$) or cisplatin treatment alone (19.39 ± 1.61 %, $p < .0001$). Cisplatin plus SC144 significantly increased the level of cleaved-caspase3 (2.93 ± 0.38-fold, $p = .0015$) in UM-UC-3R cells. Moreover, our novel EGCDNPs better maintained 108.2 ± 4.3% cell viability in UM-UC-3R cells at the IC50 of Lipofectamine and siGP130 EGCDNPs performed equally well as siGP130 lipofectamine RNAiMAX in knocking down GP130 levels at 33.65 ± 9.38 % and 41.58 ± 9.38 %, respectively ($p = .6857$).

Conclusions
Knocking down GP130 expression in *in vitro* human BCa cells, sensitized the BCa cells to cisplatin by inducing DNA lesions, downregulating Ku70 expression, and promoting apoptosis. Knocking down GP130 in human BCa cells using novel hybrid nanoparticles decreases tumor viability and toxicity compared to lipofectamine, which will allow for effective future drug delivery.

Sprr2f quenches ROS to protect against ischemia- reperfusion injury in the mouse kidney

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Sprr2f quenches ROS to protect against ischemia-reperfusion injury in the mouse kidney

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Background

Renal ischemia is a common cause of acute renal injury during surgery, such as partial nephrectomy, and can lead to chronic renal insufficiency with renal scarring. Ischemia-reperfusion injury (IRI), a model in which ischemia is induced by transient clamping of the renal vessels, produces stereotypical changes in gene expression highly similar to those induced by other forms of renal injury, including renal obstruction. Sprr2f, a member of a highly homologous gene family known to cross-link proteins in the skin during keratinization, is dramatically induced (100-1000-fold) by IRI and obstruction. We characterize Sprr2f expression in the kidney and its role in the response to renal injury.

Methods

We generated 2 mouse lines to study Sprr2f expression and its functional role. A transgenic mouse expressing a construct with the Sprr2f promoter upstream of Cre (Sprr2f-Cre) was crossed with the Ai6-ZsGreen1 reporter mouse to generate a mouse in which cells that express Sprr2f during development were permanently labeled fluorescently. We generated 2 strains of mice in which most of the Sprr2f gene body was deleted (Sprr2f-KO) from the germline (whole body-knockouts). Kidney epithelial and stromal cells were cultured from Sprr2f-wt and Sprr2f-KO mice and exposed to menadione.

Results

Sprr2f was expressed in all epithelial cells of the kidney, but not in the glomerulus. Sprr2f was also expressed in the urothelium, endometrium, ovary, and epididymis. Mice with targeted deletion of Sprr2f showed greater increases in BUN following transient (24 minutes) clamping of the vascular pedicle of both kidneys. For Sprr2f-KO, BUN levels increased to 182.00 ± 9.14 mg/ dL 24 hours later, compared to 132.29 ± 5.55 mg/ dL for Sprr2f-wt ($P<0.05$). Kidney epithelial cells cultured from Sprr2f-KO showed greater death after exposure to menadione, which increases reactive oxygen species (ROS) by mitochondrial decoupling, compared to cells from Sprr2f-wt. Ureteral obstruction for 14 days did not appear to increase renal scarring in Sprr2f-KO compared to Sprr2f-wt mice.

Conclusions

Sprr2f is expressed during development in all kidney epithelial cells, as well as other parts of the urinary and genital system. Sprr2f deletion increases acute injury of kidney epithelial cells due to ROS in both in vitro and in vivo model systems but does not appear to affect renal scarring in an obstruction model of chronic renal damage.

Targeting telomere DNA damage for CRPC therapy Sahn-ho Kim, Assistant Scientist, Henry Ford Health System

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Neuroendocrine Marker improves the diagnosis of Prostate Cancer

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Prostate cancer (PC) is the most common visceral cancer affecting men. A good prognosis for a PC patient is dependent on early detection. Although the serum PSA screening has improved the early detection of PC, the test is not reliable. Both, positive as well as negative PSA tests need to be confirmed with costly, repetitive and invasive TRUS-guided biopsy. This is because PSA is a natural secretory product of the prostate. Combining PSA test with a cancer- specific biomarker(s) can potentially improve the specificity and precision of PC detection.

We have discovered a novel prostate tumor-specific protein (neuroendocrine marker or NEM). NEM expression and secretion in the prostate seems to be cancer-specific, and its serum levels increase manifolds in cancer. We have also developed a new NEM-PSA combination test that employs an inexpensive, disposable nanosensor chip that can detect sub-picomolar levels of antigens. The assay is label-free and linear over the range of 1-64 pg with a sensitivity of 1 pg/50 µl. With this, one can measure serum NEM levels in as little as 0.1µl serum. We analyzed serum NEM and PSA levels of a cohort of 109 patients (36 healthy donors and 75 positively-confirmed PC patients). NEM levels in non-cancer (controls; Mean ± SEM 2.5±1.286 ng/ml, n=36) were significantly lower than all PC patients (cancer: 12.21±0.6135, n=75) with no overlap ($p<0.0001$, contingency analysis). In contrast, PSA levels of the same cohort displayed significant overlap between non-cancer and cancer patients (Controls: 6.26±0.9, n=36 vs Cancer: 22.85±2.96, n=75). We also analyzed prostate NEM content of some patients by immunohistochemistry of biopsy sections. There was a positive correlation between serum NEM levels and tissue NEM content. We also analyzed 11 “grey zone” PSA samples for NEM (those between serum PSA 4-10 ng/ml). When matched with biopsy pathology, we observed that NEM could separate “grey zone” PSA patients into cancer (TP) and benign disease (TN). These results support a possibility that our “NeoPro” test may improve the specificity of PC diagnosis, specifically in “grey zone” PSA patients, and help reduce the number of diagnostic biopsies.

Arsenic Disturbs Prostate Stem-progenitor Cells Homeostasis by Activation of NRF2 Pathway Dan-Ping Hu, MD, University of Illinois

Lishi Xie, PhD; Wen-Yang Hu, PhD, MD; Dan-Ping Hu, MD; Ye Li, BS; Lynn Birch, MS; Gail S. Prins, PhD

Arsenic Disturbs Prostate Stem-progenitor Cells Homeostasis by Activation of NRF2 Pathway

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Background: Inorganic arsenic (iAs) is a ubiquitously distributed environmental toxicant which increases cancer risk, including prostate cancer. However, the underlying biological mechanisms for iAs-induced prostate carcinogenesis are poorly understood. Our previous studies suggested that iAs drives the transformation of human prostate stem-progenitor cells (PrSPCs) through constitutive NRF2 activation caused by autophagic flux blockade. The present study sought to determine the impact of the NRF2 pathway in PrSPCs homeostasis.

Methods: Primary human prostate epithelial cells (PrEC) from disease-free donors were used. Stem-progenitor cells were enriched by 3D prostasphere (PS) culture and their differentiation capability was evaluated by prostate organoid (PO) culture +/- 1 μM iAs. Illumina HumanHT12 Gene Expression Microarray analysis was applied to identify iAs-dysregulated genes, and enriched pathways were revealed by Gene Set Enrichment Analysis (GSEA). Stable gene overexpression and knockdown in PS were achieved by lentiviral infection.

Results: GSEA analysis of iAs-dysregulated genes revealed that iAs activated the NRF2 pathway in both PS and PO groups. Interestingly, we found that the NRF2 pathway was enriched in vehicle control stem-progenitor cells (PS) and decreased during differentiation (PO). This suggests that NRF2 activation may contribute to iAs modulation of prostate stem-progenitor homeostasis. Indeed, we found that PS formation was increased when NRF2 pathway was activated by Oltipraz (an NRF2 inducer) or NRF2 overexpression. On the contrary, NRF2 knockdown by shRNA markedly inhibited PS formation, together suggesting that NRF2 activation increases self-renewal capacity of PrSPCs. Further, qRT-PCR following NRF2 knockdown demonstrated the induction of epithelial differentiation genes, suggesting differentiation may lead to depletion of PrSCs population in PrEC.

Conclusions: NRF2 activation increases prostate stem-progenitor cell self-renewal while NRF2 inhibition drives cell differentiation and stem-progenitor cell depletion, suggesting that NRF2 plays an essential role in maintaining homeostasis of normal PrSPCs. We proposed that iAs may transform normal PrSPCs by disturbing their homeostasis via NRF2 activation.

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Bone-resident neutrophils are mediators of prostate cancer growth in bone

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Bone-resident neutrophils are mediators of prostate cancer growth in bone

Background. In bone, PCa progresses by activating bone remodeling stromal cells, resulting in excessive degradation, and consequent accumulation of bone-sequestered factors, such as transforming growth factor beta (TGFβ), which drive tumor growth. This phenomenon characterized as a "vicious cycle" of progression has been expanded to include bone-resident immune cells. Neutrophils, the most abundant immune cells in bone, have been identified as mediators in tumor progression and their function can be regulated by TGFb. However, despite their abundance in bone, the role of neutrophils in BM-PCa progression is poorly understood. In preliminary findings, we found that: 1) neutrophils heavily infiltrate regions of prostate tumor in bone of BM-PCa patient samples and are recruited towards PCa-derived culture media and 2) BM-PCa media increases neutrophil expression of TGFβ receptor I. Based on this data, we hypothesized that BM-PCa growth in bone is mediated by neutrophils.

Methods. Bone marrow primary neutrophils were cultured *in vitro* with BM-PCa cells, C42B, or with non-metastatic LNCaP, for comparison, and cancer cell growth and apoptosis changes were measured using Trypan Blue exclusion assay, Caspase Glo assay, and Incucyte live cell imaging. Protein array was used to examine neutrophil-mediated phosphokinase signaling in BM-PCa. *In vivo*, luciferase-expressing LNCaP, C42B, or rat BM-PCa PAIII were injected intratibially in mice and treated with neutrophil-depleting antibody, anti-Ly6G (1A8) or an isotype control. Tumor burden was measured via bioluminescence and IHC performed on mouse tibia.

Results. *In vitro,* neutrophils efficiently kill ~50% of PCa and BM-PCa, LNCaP and C42B. Additionally, neutrophils arrested BM-PCa cells in G1/S phase. Analysis of protein kinase phosphorylation revealed that neutrophils significantly inhibit phosphorylation of Stat 5 in C42B, implicating Stat signaling as a critical mediator of BM-PCa. Neutrophil depletion *in vivo* increased C42B and PAIII growth in bone, yet had no impact on LNCaP growth, independently of tumor size suggesting that neutrophils in the tumor-bone microenvironment are predominantly cytotoxic. Stat5 levels were significantly higher in neutrophil-depleted mice compared to controls.

Conclusion. These findings suggest that bone-resident neutrophil BM-PCa progress in bone via evasion of neutrophil-mediated killing. Enhancing neutrophil cytotoxicity in bone may present a novel therapeutic option for treating BM-PCa.

Modulating HSP70/STUB1 machinery by novel small molecules overcomes enzalutamide resistance in lethal prostate cancer

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Modulating HSP70/STUB1 machinery by novel small molecules overcomes enzalutamide resistance in lethal prostate cancer

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Background: Protein homeostasis is altered during prostate cancer progression mediated by a comphrehensive network including the ubiquitin-proteasome-system. This effect is primarily due to the modulation of the HSP70/STUB1 machinery which regulates AR/AR-V7 protein stability in advanced prostate cancer. Emerging evidence suggested that HSP70 protects AR-V7 from degradation induced by STUB1 in anti-androgen resistant prostate cancer cells. HSP70/STUB1 machinery controls cell sensitivity to enzalutamide and abiraterone treatment *via* AR-V7 proteostasis. Modulating HSP70/STUB1 could be a valuable strategy to overcome the resistance.

Methods: Gene **e**xpression was determined by qRT-PCR and western blot. Expression of HSP70 and STUB1 was downregulated using specific siRNA. HSP70/STUB1 and AR/AR-V7 interaction was determined by co-immunoprecipitation and dual immunofluorescence. The gene regulating mechanisms in drug resistant prostate cancer cells was determined by RNA sequencing analyses. The effects of drug treatment on enzalutamide sensitivity were examined *in vitro* and *in vivo*.

Results: In the present study, we discovered that HSP70/STUB1 machinery is involved in AR and AR-V7 protein stabilization and confers anti-androgen resistance. Modulating HSP70/STUB1 by small molecules (HSMs) re-sensitizes the resistant cells or xenograft tumors to anti-androgen treatment. Mechanistically, HSMs inhibit HSP70 and degrade AR-V7 protein through alteration of the ubiquitin-proteasome-system in enzalutamide resistant cells. HSMs promote STUB1 entering into the nuclear and binding to AR-V7. HSMs robustly disrupt HSP70 gene programs and suppress AR/AR-V7 target genes expression. Furthermore, we identified and characterized HSM-7 (one of the small molecules) that has a superior pharmacokinetics profile and is highly effective for the treatment of anti-androgen resistant prostate cancer both *in vitro* and *in vivo*.

Conclusions: Modulating HSP70/STUB1 machinery by HSMs overcomes enzalutamide resistance through AR/AR-V7 regulation. Further development of HSM-7 may yield a novel therapeutics for the treatment of anti-androgen resistant prostate cancer.

The Trained Immunity-like Epigenetic Memory in Urinary Tract Infection

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The Trained Immunity-like Epigenetic Memory of Urinary Tract Infection

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Background: Trained immunity mediates a heightened response to reinfection but its' role in the urinary tract infections (UTIs) remains unknown. Polycomb repressor complex 2 (PRC2) catalyzes histone methylation and epigenetic repression.

Methods: A human strain of uropathogenic *E. coli* (UPEC) was used to infect mice causing acute, repeated or super infection. Outcomes of the infections were examined based urine bacterial counts, histology and inflammatory score, expression of cytokines and chemokines and overall gene expression profiles. The PRC2 mutant mice were used examine its urothelium-specific role in UTIs. We also treated wild type mice with small molecule inhibitors to inhibit PRC2 activity systemically.

Results: We found that a history of UTI induced *Ezh2,* the methyltransferase of PRC2, and exaggerated inflammatory host response and tissue damage upon reinfection. Inactivating PRC2 through bladder urothelium-specific *Eed* knockout improved outcomes of the acute, repeated and superinfections caused by UPEC, including reduction of bacteriuria, attenuation of the *NF-κB* signaling, reduction of inflammatory score, decrease of hyperplasia and enhancement of urothelial cell differentiation. Furthermore, small molecule *Ezh2* inhibitors significantly ameliorated symptom of superinfection and chronic cystitis.

Conclusions: UPEC induces the PRC2-dependent epigenetic changes of bladder urothelium, which result in a trained immunity-like memory and poor disease outcome. The findings further suggest an alternative treatment strategy to ameliorate UTIs by targeting selectively the epigenetic-based trained immunity.

Urine extracellular vesicle GATA2 mRNA alone and in a multigene test predicts initial prostate biopsy result Sandra Santasusagna, PhD, Thomas Jefferson University *Jungreem Woo, Joshua Banks, Ana Dominguez-Andres, Kamlesh Yadav, Raffaella Pippa, Marc Carceles-Cordon, Robert B. Den, Karen E. Knudsen, Lucia Languino, Costas D. Lallas, Grace Luyao, Veronica Rodriguez-Bravo, Ashutosh Tewari, W. Kevin Kelly, Benjamin E. Leiby, Josep Maria Prats7, Leonard Gomella, Josep Domingo-Domenech*

Urine extracellular vesicle GATA2 mRNA alone and in a multigene test predicts initial prostate biopsy result

Background: Prostate specific antigen (PSA) detection in blood is widely used to screen for prostate cancer (PCa). However, PSA has limited utility in discriminating high risk tumors (Gleason score≥7) from indolent-low risk tumors, which leads to unnecessary biopsies and highlights the need to identify better biomarkers for the detection of clinically significant PCa. GATA2 is a pioneer transcription factors which expression is increased in high-risk prostate cancer (PCa), but its utility in discriminating for PCa remains untested.

Methods: To determine whether extracellular vesicle (EV) GATA2 mRNA provides clinically relevant information to distinguish PCa, we prospectively analyzed non-digital rectal exam (DRE) urine EV GATA2 mRNA levels from 165 males with suspicion of PCa prior to biopsy and correlated GATA2 levels alone and combined in a multigene (PCA3 and TMPRSS2) EV mRNA assay to biopsy result.

Results: Prostate origin of GATA2 mRNA detected in urine EVs was confirmed by observing that GATA2 mRNA levels significantly dropped after prostatectomy ($p<0.05$) and positively correlated to PCa tissue GATA2 mRNA levels. GATA2 increased in biopsy-positive PCa patients ($p<0.0001$) and high-grade disease ($p<0.01$). Multivariable analysis showed that GATA2 is an independent predictive factor of any cancer and high-grade PCa. GATA2 alone and combined in a multigene test with PCA3 and TMPRSS2-ERG (GAPT-E) improved discrimination of any cancer (GAPT-E1) and high-grade cancer (GAPT-E2): standard of care (SOC) area under the curve (AUC) of 0.62 and 0.65, SOC plus GATA2 AUC of 0.68 and 0.69, and SOC plus GAPT-E (1-2) AUC of 0.75 and 0.75, respectively. A GAPT-E1 cut-point of 57 would avoid 25.6% of unnecessary prostate biopsies and 13.7% of total biopsies with NPV 50% and missing 9% of PCa. Limitations include lack of PCa volume and imaging assessment of the cohort.

Conclusions: Non-DRE urine EV GATA2 mRNA alone and in combination with other urine EV biomarkers in men with suspicion of PCa provides useful information to distinguish any cancer and high-risk PCa and may reduce the number of biopsies.

Loss of CHD1 promotes chromatin dysregulation leading to heterogeneous mechanisms of resistance to hormone therapy in prostate cancer

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Loss of *CHD1* promotes chromatin dysregulation leading to heterogeneous mechanisms of resistance to hormone therapy in prostate cancer

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BACKGROUND:

Pharmacological targeting of driver alterations in cancer has resulted in many clinical successes but is limited by concurrent or novel genomic alterations. One potential explanation for this heterogeneity is the presence of additional genomic alterations which modify the degree of dependence on the targeted driver mutation. Metastatic prostate cancer (mPCa) serves as a relevant example, where the molecular target is the androgen receptor (AR) which functions as a lineage survival factor of luminal prostate epithelial cells. Next generation AR therapies such as abiraterone, enzalutamide and apalutamide have significantly improved survival of men with mPCa, but resistance remains an issue.

METHODS:

To gain functional insight into the genes impacted by the copy number alterations in mPCa, we screened 4234 short hairpin RNAs (shRNAs) targeting 730 genes often deleted in human prostate

cancer (the prostate cancer deletome) for hairpins that confer *in vivo* resistance to the antiandrogen enzalutamide.

RESULTS:
The chromodomain helicase DNA-binding protein 1 (*CHD1*) emerged as a top candidate, a finding supported by patient data showing that *CHD1* expression is inversely correlated with clinical benefit from next generation antiandrogen therapy. *CHD1* loss led to global changes in open and closed chromatin, indicative of an altered chromatin state, with associated changes in gene expression. Integrative analysis of ATAC- and RNA-seq changes identified 22 transcription factors as candidate drivers of enzalutamide resistance. CRISPR deletion of four of these (*NR3C1, BRN2, NR2F1, TBX2*) restored in vitro enzalutamide sensitivity in *CHD1* deleted cells. Independently derived, enzalutamide-resistant, *CHD1*-deleted subclones expressed elevated levels of 1 or more of these 4 transcription factors. This pattern suggests a state of chromatin plasticity and enhanced heterogeneity, initiated by *CHD1* loss, which enables upregulation of distinct sets of genes in response to selective pressure. This concept is further supported by RNA-seq data from a mCRPC patients cohort, in which we examined the co-association of *CHD1* levels with each of these four TFs across 212 tumors.

CONCLUSIONS:
We demonstrated that loss of the chromodomain gene *CHD1*, a commonly deleted prostate cancer gene, through global effects on chromatin, establishes a state of plasticity that accelerates the development of hormone therapy resistance through heterogeneous activation of downstream effectors.

Single Cell Investigation of Patient-Derived Prostate Organoids Reveals Differentiation Protocol Involving Epithelial Integrin Expression Supported by Stromal Collagen

Tara McCray, University of Illinois at Chicago *Larisa Nonn, PhD*

Single Cell Investigation of Patient-Derived Prostate Organoids Reveals Differentiation Protocol Involving Epithelial Integrin Expression Supported by Stromal Collagen

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Background: Prostate disease is frequent in men, with cancer being a leading cause of death. The ability to model benign and malignant prostate in vitro is essential for understanding underlying biology both of development and disease. Primary prostate epithelial (PrE) cells derived from patient samples can be grown into organoids in Matrigel to mimic prostate tissue and provide a potential route to personalized medicine and drug screening. To improve the model, our group has optimized a protocol to co-culture PrE organoids with primary stromal cells (PrS).

Methods: In order to characterize organoid epithelial populations and their differentiation program, we grew cells derived from 3 separate patients in 3D and collected the organoids at day 8 and 14 for single cell RNA sequencing (scRNAseq). To obverse the influence of stroma over PrE differentiation, scRNAseq was performed on mono-cultured (PrE alone) and co-cultured organoids.

Results: Overtime organoids showed an early expansion of dividing cells followed by an increase in cell adhesion and extracellular structure organization as differentiation occurred. PrS promoted this process by upregulating integrin and laminin expression in PrE cells, most likely in response to PrS collagen secretion.

Conclusion: Stromal cells support the differentiation protocol of PrE that involves upregulation of cell adhesion and extracellular structure organization. Integrins have been shown to be essential in cell polarity and branching morphogenesis in other cell types, and play an important role in prostate cancer metastasis. Future experiments will explore the role of integrins in prostate development and disease.

Vitamin D Inhibits DKK3 To Promote Human Prostate Organoid Differentiation

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Larisa Nonn, PhD, Bethany Baumann, PhD

Vitamin D Inhibits DKK3 To Promote Human Prostate Organoid Differentiation

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Background: The prostate is a hormonally-regulated epithelial gland that harbors cancer in 80% of elderly men. Deficiency in the hormone vitamin D (1,25D) results in aggressive prostate cancer with less-differentiated tumors, but how 1,25D promotes or maintains differentiation in the prostate is not well understood.

Methods: Using benign human primary prostate epithelial (PrE) organoids as a model, the differentiative properties of vitamin D were examined. Flow cytometry, RT-qPCR, immunostaining and western were utilized to identify vitamin D target genes and validate findings. Single cell RNA sequencing (scRNAseq) revealed cell specific expression of genes of interest.

Results: Organoids grown in the presence of 1,25D were strikingly larger than those grown in control and showed earlier $CD49^{Low}$ cell populations via flow cytometry, indicating increased differentiation. DKK3 emerged as a target of 1,25D by RT-qPCR, ChIP-sequencing for vitamin-D-receptor-bound DNA, and western blot. Vitamin D inhibits DKK3 expression, and knockdown of DKK3 enhances its effect. DKK3 was expressed exclusively by non-stem cells by scRNA-sequencing, suggesting a pattern of 1,25D-regulation over differentiated cell types.

Conclusion:
Overall, 1,25D inhibits noncanonical WNT related protein DKK3 in differentiated cells and this protein is not expressed by stem cells. Future experiments will explore the significance of these findings in the context of disease.

Extragonadal androgen biosynthesis associated with variant HSD3B1 (A1245C) allele modulates radiosensitivity in Prostate Cancer cells

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Title: Extragonadal androgen biosynthesis associated with variant *HSD3B1* (A1245C) allele modulates radiosensitivity in Prostate Cancer cells.

Authors: Shinjini Ganguly, Aysegul Balyimez, Zaeem Lone, Aimalie Hardaway, Monaben Patel, Elai Davicioni, Rahul Tendulkar, Eric Klein, Nima Sharifi, Omar Mian

Introduction & Objective
Androgen deprivation therapy (ADT) is the linchpin therapeutic for locally advanced and metastatic PCa. Resistance to ADT has been associated with a gain of function mutation in the 3β-HSD enzyme, which catalyzes extragonadal/intratumoral DHT synthesis. As androgen signaling is known to upregulate the DNA damage response (DDR), we investigated whether variant HSD3B1 modulates DDR and radiosensitivity in PCa via extragonadal androgen biosynthesis.

Methods
We stably knocked down HSD3B1 in LNCaP, C42 and VCaP cell lines (which carry the protein stabilizing variant allele), and overexpressed the variant HSD3B1 allele in LAPC4 (that harbors WT allele which readily undergoes degradation). We examined the proliferative and clonogenic capacity of these cells in presence and absence of substrate, the extragonadal androgen precursors DHEA, followed by treatment with IR (400 cGy, single fraction). We studied DNA DSB formation and resolution kinetics using γH2AX foci formation in response to radiation. We also measured changes in mRNA expression of DDR response genes pre- and post-radiation.

Results
Control shRNA transduced cell lines expressing the variant HSD3B1 allele had increased cell proliferation (3.1289times, $p<0.001$) and clonogenic survival (100times more at 800cGY single fraction radiation,$p<0.001$) in the presence of DHEA compared to corresponding HSD3B1 knockdown LNCaP cells. Variant HSD3B1 cell lines were more radioresistant to IR and exhibited more efficient γH2AX foci resolution at 24 hrs (1foci/nucleus, $p <0.05$) in a DHEA dependent manner. We observe increased mRNA expression of DDR genes from specific repair networks including non-homologous end joining (PRKDC, XRCC4, XRCC5) and homologous recombination (RAD51, RAD54), but not mismatch repair (MSH2, MSH6), in variant HSD3B1 cells at baseline. Transriptional induction of DDR gene expression following radiation and in presence of DHEA, was significantly more pronounced in HSD3B1 variant cells, suggesting a more permissive chromatin context.

Conclusions
Increased intracellular HSD3B1, which converts extra gonadal precursors to DHT, leads to over expression of NHEJ and HR genes, more rapid resolution of γH2AX foci, and radioresistance in prostate cancer. This work has therapeutic implications related to strategies for combined radiation and androgen directed therapy in localized and metastatic prostate cancer. Prospective validation of treatment strategies combining blockade of adrenal steroid precursor synthesis, traditional ADT, and XRT in high risk and low volume metastatic disease are warranted.

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Key words

1. mCRPC
2. Androgen precursor
3. HSD3B1
4. Radioresistance

An orthotopic murine neuroendocrine bladder cancer model offers insights into the phenotypic plasticity of small cell bladder cancer (SCBC)

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Title: An orthotopic murine neuroendocrine bladder cancer model offers insights into the phenotypic plasticity of small cell bladder cancer (SCBC)

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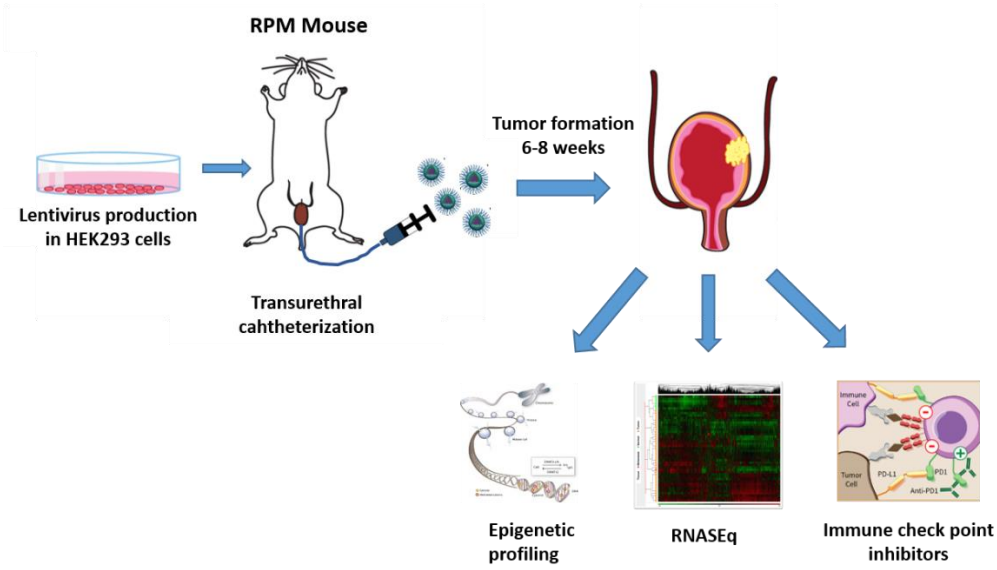
Purpose: SCBC is an aggressive subtype of bladder cancer with high metastatic potential and few effective treatment options. We developed an orthotopic mouse model of SCBC to understand the development of this rare bladder cancer variant and identify epigenetic drivers of neuroendocrine differentiation.

Methods: Lentiviral particles carrying Cre recombinase were produced using Lenti-sgNeo#2/Cre. The bladders of *Rb1*$^{fl/fl}$ *Trp53*$^{fl/fl}$ *Myc*$^{LSL/LSL}$ (RPM) mice was transduced with Cre recombinase expressing lentivirus via transurethral catheterization. Mice were monitored by micro-ultrasound (mUS) and detected tumors were verified by histology. SCBC morphology was confirmed by H&E staining and synaptophysin IHC. Whole transcriptome (RNAseq) analysis was performed to correlate transcriptomic profile of neuroendocrine mouse tumors to a cohort of human SCBC tumors.

Results: Transurethral catheterization successfully transduced the bladder urothelium without evidence of exposure of ectopic (non-urothelial) tissues. RPM mice developed SCBC visible on mUS with a latency of 8-10 weeks. As expected in neuroendocrine tumors, mice developed liver and lung metastases. High grade neuroendocrine morphology and NE markers were confirmed on H&E and IHC, respectively, by a GU pathologist. Western blot analysis confirmed cMyc expression and suppression of TP53 and RB1. Synaptophysin expression was confirmed by IHC. Transcriptomic profiling of both mouse and human SCBC demonstrated concordant gene expression. Gene expression profiling of urothelial and non-urothelial neuroendocrine tumors suggested a phenotypic convergence.

Conclusions: To our knowledge, this is the first genetically engineered murine model of SCBC. Ongoing work seeks to identify epigenetic markers playing role in the development of this aggressive variant of bladder cancer. In addition, we are assessing the activity of immune checkpoint inhibitors in this immunocompetent background.


RPM Mouse
Lentivirus production
in HEK293 cells
Transurethral
cahtheterization
Tumor formation
6-8 weeks
Epigenetic
profiling
RNASEq
Immune check point
inhibitors

NAD+ metabolism as a potential vulnerability in neuroendocrine prostate cancer

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Title: NAD+ metabolism as a potential vulnerability in neuroendocrine prostate cancer

ABSTRACT

Background: In response to therapies targeting the androgen receptor (AR) axis, a subset of advanced prostate cancer (PCa) patients develop resistant tumors with neuroendocrine features. The metabolic differences between adenocarcinoma and neuroendocrine prostate cancers have not been well defined. By characterizing metabolic preferences, we can evaluate potential metabolic vulnerabilities to treat neuroendocrine PCa. A recent bioinformatics study showed that small cell neuroendocrine tumors may be targeted by the drug FK866, which inhibits NAMPT, the rate limiting enzyme in the NAD+ salvage pathway.

Methods: AR positive PCa cell lines have been genetically altered via lentiviral transduction with a constitutive vector containing $p53^{DN}$-shRb1 to disrupt both p53 and Rb. Protein level expression was determined via western blot to validate genetic alterations, lineage markers, and metabolic enzyme expression.

Results: We have demonstrated that the dominant negative form of p53 and knockdown of Rb1 leads to reduced expression of AR target genes and increased expression of neuroendocrine markers including synaptophysin, Brn2 and Ezh2. When comparing the parental AR+ cells and the neuroendocrine-like cells, we observed similar expression of NAMPT. In contrast, expression of the NAD+ synthesis enzyme NAPRT was lower in the $p53^{DN}$-shRb1-expressing neuroendocrine-like cells.

Conclusions: Over-expression of the dominant negative form of p53 and knockdown of Rb1 induce a neuroendocrine phenotype in AR+ PCa cells. By modeling distinct phenotypes, we can study metabolic reprogramming that results from lineage plasticity. Based on differential expression of NAPRT, we propose that NAMPT inhibition may represent a potential metabolic vulnerability in neuroendocrine PCa with mutant p53 and loss of Rb1.

Downregulation of EPHB2 Increases Alterations in Lipid Metabolism Associated with Prostate Cancer Racial Disparities

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DOWNREGULATION OF EPHB2 INCREASES ALTERATIONS IN LIPID METABOLISM ASSOCIATED WITH PROSTATE CANCER RACIAL DISPARITIES

Introduction: African American (AA) men have the highest risk of developing and dying from prostate cancer (PCa). PCa patients with abdominal central obesity, have more aggressive tumors. The Center for Disease Control reports racial disparities (RD) (38.4% AA vs. 28.6% in CA) in adult obesity. Although lipid metabolism is better studied in adipocytes, recent studies show that cancer cells can uptake fatty acids and store in lipid droplets (LDs), formed by a core of neutral lipids containing triacylglycerols (TAG) and cholesterol esters, which can be used to sustain proliferation and migration. Loss of the putative tumor suppressor EPHB2 has been associated with PCa-RD. Here we show the role of aberrant ephrin signaling in lipid metabolism-associated with increased PCa tumorigenesis in AA compared to CA.

Method: LD density (LDD) was assessed in PCa specimens and compared in different racial backgrounds. PCa cells from AA and CA with loss of EPHB2 signaling were engineered using siRNA and CRISPR approaches. LDD and LD size were assessed under basal and obesogenic (Oleic acid, OA) environments. Key molecules involved in triacylglycerol (TAG) metabolism were assessed. The effects of EPHB2 loss on proliferation and motility were tested in vitro.

Results: High grade PCa accumulate significantly more neutral lipid than lower grade tumors. PCa cells from AA showed increased proliferation response to OA compared to CA. Regardless of race, loss of EPHB2 increased the tumorigenicity of PCa cells. However racial differences in LDD and size were observed. Altered EPHB2 led to racial differences in the expression of TAG molecules DGAT1, DGAT2 and ATGL. Targeting key TAG molecules associated with EPHB2-induced tumorigenicity reduced PCa cell proliferation and motility. RD in the regulation of centrosome amplification and microtubule organizing center (MTOC) were associated with lipid metabolic changes.

Conclusions: Loss of EPHB2 commonly associated with PCa-RD exerts aberrant lipid metabolism with excessive accumulation of LD in PCa cells. This lipid-rich energy status may serve as a source to fuel a more aggressive phenotype observed in PCa-RD. Because several TAG inhibitors are currently being evaluated in clinical trials as anti-obesity agents, showing promising results, the potential anti-tumorigenic utility in obese patients with altered ephrin levels requires further study.

Role of polyamine metabolism in prostate cancer therapy resistance

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Sayani Bhattacharjee, Jonathan P. Doan, Jerred P. Pletcher, Rebecca Wynn

Background: Prostate cancer (PCa) is one of the leading causes of cancer-related deaths in the US. Localized PCa is treated with surgery or radiation, while currently approved therapies for castration-resistant prostate cancer (CRPC) include the second generation anti-androgen enzalutamide. Though very effective clinically, the efficacy of enzalutamide is limited by the development of resistance within a short span of time. One of the most pressing questions in PCa management today is how to overcome or circumvent resistance to androgen axis-targeted therapies in CRPC.

PCa is characterized by unique metabolic alterations, which include changes in one carbon metabolism, polyamine metabolism, glucose metabolism etc. Polyamines are small organic molecules that are essential for sustaining cell proliferation and division. An ingenious method of utilizing the dysregulation of polyamine metabolism for therapy is to use polyamine analogs. When taken up by cancer cells, polyamine analogs inhibit the activities of biosynthetic enzymes and increase the activities of catabolic enzymes, leading to the depletion of natural polyamine pools. This, in conjunction with the inability of cancer cells to utilize the analogs, overpowers the cellular machinery and results in cell death. Even though little to no clinical activity was seen as monotherapy, combinations of polyamine analogs have shown more promise more recently. In this study, we hypothesized that dysregulation of polyamine metabolism may underlie the development of resistance to enzalutamide in CRPC.

Methods: We tested the relative expression levels of enzymes of the polyamine metabolism in prostate cancer tissues and in two prostate cancer cell lines C4-2B and 22Rv1 and their enzalutamide-resistant derivatives C4-2B-MDVR and 22Rv1-MDVR. We treated these cells with two polyamine analogs DENSPM and CGC-11047 either singly or in combination with enzalutamide. Cell survival, proliferation, clonogenic ability, and enzyme expression levels were analyzed.

Results: We found that the expression levels of several enzymes of the polyamine metabolism pathway were elevated in prostate cancer tissues as well as in enzalutamide-resistant PCa cells. In addition, co-treatment with polyamine analogs resensitized enzalutamide-resistant PCa cells to enzalutamide treatment.

Conclusions: Polyamine metabolism may represent one of the pathways that are dysregulated during the acquisition of resistance to androgen axis-targeted therapies such as enzalutamide. Co-targeting polyamine metabolism may represent an attractive option to overcome enzalutamide resistance in PCa.

BUB1B is a key component of an AR variant-regulated network in castration-resistant prostate cancer

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BUB1B is a key component of an AR variant-regulated network in castration-resistant prostate cancer

Background

We developed a systems-level gene discovery strategy focused on constitutively active androgen receptor (AR) splice variant-driven pathways, as representative of an intractable mechanism of progression to castration-resistant prostate cancer (CRPC). We identified seven AR variant-regulated genes that also enhance, in a feed forward manner, AR activity and drive PC progression[(1)]. Expression of the seven gene set predicts poor disease-free and overall survival in PC patient cohorts. However, this gene set is not associated with survival metrics in other human cancers. This highly interconnected seven gene network is enriched in cell cycle (G_2/M) related genes (*BUB1*, *BUB1B*, *CCNB1*, *CCNB2*, *KIF20A*, *KIF23* and *TOP2A*).

Methods & Results

Individual depletion of members of the gene set decreased the expression of at least one other member indicative of the interrelationship of these genes. Notably, depletion of the mitotic checkpoint serine/threonine-protein kinase BUB1B (Budding Uninhibited by Benzimidazole-Related 1) decreased expression of the other six genes, suggesting that BUB1B is a key component of the network. Little is known regarding a possible role of BUB1B in PC progression or the mechanisms linking BUB1B to AR signaling. We found that BUB1B depletion more profoundly decreased CRPC cell proliferation compared to androgen dependent PC cells even though expression of BUB1B is comparable. The antiproliferative effects of BUB1B depletion were associated with inhibition of cell cycle but not with induction of apoptosis. Since BUB1B is part of the network driven by the clinically relevant AR variant, AR-V7, and that functionally interacts with AR signaling, we examined the effect of BUB1B knockdown on AR ligand independent transcriptional activity. We examined 22Rv1 CRPC cells, which are largely driven by AR-V7 in the absence of androgen. Depletion of BUB1B decreased expression of specific AR and AR-V7 target genes suggesting that BUB1B depletion may shift the AR/ AR-V7 transcriptome in androgen-depleted conditions. Furthermore, BUB1B knockdown resulted in decreased levels of AR and AR-V7 protein but not mRNA in CRPC cells. In contrast, BUB1B depletion did not affect AR transcriptional activity, AR mRNA or protein levels in androgen dependent PC cells suggesting that BUB1B positive feedback to AR and AR-V7 signaling in CRPC cells might be due to posttranslational regulation.

Conclusions

These results support evaluation of BUB1B, a central member of an AR-variant network, as a possible therapeutic target for CRPC.

Reference

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Roles of retinoid signaling in the developing urothelium Gregory Wiessner, Columbia University Medical Center *Dr. Ekatherina Batourina, Dr. Carolina Rosselot, Dr. Chad Vezina, Kerry Schneider, Dr. Cathy Mendelsohn*

Roles of retinoid signaling in the developing bladder urothelium

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Background: The developing urothelium contains a transient progenitor population, P-cells, that express Foxa2, Isl1, and Shh. Lineage tracing experiments indicate that P-cells produce uroplakin-expressing intermediate and superficial cell daughters between embryonic stages E11.5-E14.5, and Krt5+ and Krt14+ basal cells after E14.5. Retinoic acid (RA) is a metabolite of Vitamin A that regulates expression of target genes through binding to nuclear retinoic acid receptors (RARs). We find that RA signaling is highly active in the developing urothelium from E11.5-E14.5, leading us to hypothesize that RA regulates the behavior of urothelial progenitors.

Methods: To examine the role of RA signaling in urothelial progenitor cells, we produced a mouse line harboring a dominant inhibitory form of RAR inserted in the ROSA26 locus (RaraDN) that is activated in cells expressing Cre recombinase. RaraDN inhibits RA signaling but does not affect signaling via other nuclear receptor family members. We utilized the constitutive and inducible ShhCre lines to drive RaraDN expression in P-cells from the initiation of urothelial development at E11.5.

Results: We showed that depletion of retinoic acid signaling results in loss of intermediate and Superficial cell populations, and induces squamous metaplasia in the urothelium. Interestingly, ureters in mutants fail to insert into the urothelium at the proper integration site, a process that depends on apoptosis of the posterior nephric duct segments. These observations suggest the embryonic urothelium is a source of signals that drive ureter maturation, and that retinoid signaling is required for production of these signals.

Conclusions: Our data indicates that RA signaling in urothelial progenitor cells is required for both proper cell differentiation and morphogenesis of the lower urinary tract. Knowledge of genes and signaling pathways regulated by the RARs may guide future research focused on i) the differentiation of urothelial cells from pluripotent stem cell sources and ii) the mechanisms behind improper ureter insertion in human patients with congenital anomalies of the kidneys and lower urinary tract (CAKUT).

Role of SLCO1B3 Transporter in Prostate Cancer Cell Resistance to Cabazitaxel Chemotherapy

Diane Begemann, University of Kentucky *Natasha Kyprianou, PhD*

Role of SLCO1B3 Transporter in **Prostate Cancer Cell Resistance to Cabazitaxel Chemotherapy**

Diane Begemann[1] and Natasha Kyprianou[2,3]

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Background: Resistance to cabazitaxel chemotherapy (2nd generation FDA-approved taxane) in the treatment of castration resistant prostate cancer (CRPC), leads to lethal disease. Previous work from our group demonstrated that cabazitaxel causes reversal of epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET) in prostate cancer cells. This study examined the impact of androgens on the cabazitaxel mediated EMT conversion to MET therapeutic action of cabazitaxel in both *in vitro* and *in vivo* models of prostate cancer. These studies optimize the therapeutic efficacy of cabazitaxel for restoration of an epithelial phenotype.

Methods: A cabazitaxel resistant cell line (PC3-CR), developed in our lab was used as a model. Cell viability assays established that PC3-CR cells exhibited reduced response to increasing doses of cabazitaxel. RNA-seq analysis (Human Clariom S) was performed to compare PC3-CR transcriptome gene expression to PC3 cells, which identified cellular transport protein SLCO1B3. LC-MSMS to measure intracellular cabazitaxel concentration with and without the presence of dihydrotestosterone (DHT) was performed. *In vivo* therapeutic efficacy by cabazitaxel was investigated using PC3 and PC3-CR xenografts. Tumor-bearing male nude mice were treated with cabazitaxel (3mg/kg) for 14-days. EMT, apoptosis, cell proliferation, and differentiation were evaluated in the excised tumors.

Results: RNA-sequencing identified that PC3-CR cells upon acquisition of the EMT phenotype, lost expression of SLCO1B3 transporter. Preliminary functional analysis of SLCO1B3 knockdown suggest the contribution of this transporter to cabazitaxel resistance. Assessment of the i*n vivo* response to cabazitaxel of PC-3 and PC3-CR prostate xenografts demonstrated reduced sensitivity in PC3-CR derived tumors compared to parental PC3 potentially via EMT-MET conversion. Furthermore mass spectrometry analysis identified that DHT decreases intracellular cabazitaxel concentration.

Conclusions: The effect of cabazitaxel on prostate tumors is modified by the presence of androgens and the transporter protein SLCO1B3, via increasing cell entry/intracellular drug accumulation and enhancing prostate cancer cell response. Our findings have significant significance in optimizing the therapeutic efficacy of cabaxitaxel chemotherapy, via manipulating the transporter, to improve therapeutic response in patients with advanced (hormone naïve and CRPC) prostate cancer.

PB-Csf1 is a novel mouse model for prostate inflammation Li Xin, University of Washington
Ohjoon Kown, Boyu Zhang, Li Zhang, Xing Wei, and Li Xin

PB-Csf1 is a novel mouse model for prostate inflammation

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Inflammation plays a critical role in the initiation and progression of the prostate-related diseases, including both benign prostatic hyperplasia (BPH) and prostate cancer. The density of macrophages has been shown to be increased in both human BPH and prostate cancer specimens than in normal prostate tissues. To evaluate the impact of aseptic inflammation on prostate biology, we generated a PB-Csf1 transgenic mouse model that harbors the mouse colony-stimulating-factor-1 (Csf-1) transgene driven by the rat probasin promoter. PB-Csf1 mice express Csf-1 up to 60-fold higher than that in control littermates. Expression of Csf-1 in the prostate leads to the infiltration of CD45+ leukocytes including macrophages, T cells and neutrophils. The PB-Csf1 mice develop mild prostatic intraepithelial neoplasia at 24 weeks of age. There is an increase in epithelial cell proliferation in the PB-Csf1 model but no difference in apoptotic index is noted compared to the age matched littermate controls. In addition, there is an increased cellular senescence in the prostate tissues of PB-Csf1 mice as determined by the senescence-associated β-galactosidase assay. Increased cellular senescence is often seen in the prostate tissues of ageing men. Our study shows that prostate inflammation can lead to cell senescence as well as none-malignant growth of the prostate epithelial cells. Future study will determine how prostate inflammation affects function of other lower urinary organs, whether senescence serves as a barrier for malignant progression of the mild PIN lesions, and how inflammation impacts progression of prostate cancer.

Adipocyte-dependent lipid/MTOC dysregulation in the prostate tumor microenvironment: A microfluidic approach

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Victoria Gil, Research Associate, John Day, Graduate Student, Univ. of Washington, Omar E. Franco, Research Scientist, Francesca Nardi, Research Scientist, Philip Fitchev, Research Associate, Simon W. Hayward, Director of Cancer Biology, Ashleigh Theberge, Assistant Professor, Univ of Washington, Susan E. Crawford, Professor of Pathology

Introduction & Objective:

Periprostatic fat (PPF) encases the prostate and is an understudied visceral fat depot and stromal component of the prostate tumor microenvironment (TME). Visceral obesity is associated with ectopic lipid accumulation in non-adipocytes and tumor progression; however, the mechanisms responsible for aberrant adipocyte-tumor cell crosstalk are not clear. The PPF stores neutral lipid in the form of lipid droplets (LDs) and secretes a wide array of adipokines and cytokines. In cancer-associated fibroblasts (CAFs), we recently found that increased lipid content promoted centrosomal and microtubule organizing center (MTOC) amplification as well as a proliferative phenotype. Studying the TME has been hampered by limited experimental systems. We used an innovative multicellular open microfluidic platform, the Monorail Device, to study metabolic reprogramming and LD trafficking in a tri-culture of adipocytes, prostate tumor cells, and primary cancer-associated fibroblasts (CAFs).

Methods:

Primary PPF derived from surgical prostatectomy specimens, prostate cancer cell lines, and the 3T3-L1 adipocyte cell line were tested. The cells were cultured in a polystyrene open microfluidic device where only 22uL of medium is required/well. A temperature sensitive agarose was used to partition cells into various wells. The cells were analyzed using live cell imaging, immunofluorescent stains (bodipy, pericentrin, tubulin and metalloproteinases), and oil-red-O stains for neutral lipid.

Results:

We found that co-culture of prostate cancer cells with adipocytes (3T3-L1 or primary PPF) resulted in increased LD area and the addition of CAFs elevated both LD density and area in the tumor cells. Immunofluorescent studies for MTOCs revealed exposure of CAFs or tumor cells to adipocytes in the Monorail Device promoted amplification in centrosomes/MTOCs to two or more per cell. Live cell imaging demonstrated LD trafficking in CAFs and cancer cells via an intact microtubular network and metalloproteinases were located on the surface of the LDs.

Conclusions:

The Monorail Device is an open microfluidic platform to better simulate the complex TME and allows testing of primary human samples where cell number is limited. Adipocytes promote lipid reprogramming and MTOC amplification in both prostate cancer cells and CAFs. In the setting of obesity, higher intracytoplasmic lipid accumulation and centrosomal amplification are potential mechanisms responsible for accelerated prostate tumor growth and progression.

Targeting the WNT5A Receptor, ROR1, in Prostate Cancer **Christina A.M. Jamieson, PhD. Associate Professor, Dept of Urology, University of California, San Diego (UCSD)** *Sanghee Lee, Neurourology Fellow UCSD Urology, Danielle N Burner, Lab Technician, Theresa R Mendoza, MSc student, Michelle T Muldong, Senior Research Associate, Abril Zuniga, Undergraduate Albert Scholar, Catalina Arreola, Lab Technician, Christina N Wu, Senior Project Scientist, John J McDermott, Medical student and Albert Scholar, Rekha S Narasimhan, Medical student and Albert Scholar, SungKu Kang, Visiting Scholar, Catriona HM Jamieson, Professor, Nicholas A Cacalano, Associate Professor, Isaac Y Kim, Professor, Karl Willert, Professor, Terry Gaasterland, Professor, Anna A Kulidjian, Associate Professor, Rana Mckay, Assistant Professor, Christopher J Kane, Professor*

Targeting the WNT5A Receptor, ROR1, in Prostate Cancer

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Background: Prostate cancer preferentially metastasizes to bone and, although some treatments can slow its progression, there is no cure. WNT5A expression is linked to poor prognosis in patients with bone metastatic prostate cancer. WNT5A is expressed in a sub-population of prostate cancer patient tissues and circulating tumor cells (CTCs). The interaction of WNT5A and its receptor, ROR1, is usually restricted to embryonic development but is re-activated in CLL and some solid tumors. In metastatic breast cancer, ROR1 expression increased in patients who developed resistance to paclitaxel. **Cirmtuzumab**, a therapeutic monoclonal antibody that binds to and inhibits ROR1, has successfully passed a Phase 1 trial in CLL. It is currently in clinical trials for CLL patients plus ibrutinib and metastatic breast cancer patients plus paclitaxel. We are characterizing WNT5A/ROR1 expression and function in our patient-derived xenograft (PDX) and patient-derived organoid (PDO) models of bone metastatic prostate cancer and testing Cirmtuzumab to help identify the patient population for a Phase 1B clinical trial in metastatic prostate cancer patients.

Methods: We analyzed WNT5A and ROR1 expression in our PDX and PDO models of bone metastatic prostate cancer using genome-wide expression profiling, qRT-PCR, RNAScope in situ hybridization, flow cytometry, and Western blotting. We are testing Cirmtuzumab alone and in combination with Enzalutamide, Docetaxel and Radiation in our PDX derived PCSD1 and PCSD13 3D organoids and PDX models of bone metastatic prostate cancer.

Results: WNT5A and ROR1 are expressed in the PDX, PCSD1, and the bone metastatic prostate cancer patient samples from which it was derived. ROR1 was expressed in PCSD13 which was confirmed by FACS. Cirmtuzumab treatment decreased the size of PCSD1 3D organoids that were resistant to enzalutamide plus docetaxel treatment. Immunofluorescence analysis of cytokeratins 5 and 8 showed the heterogeneity of cells in 3D organoids and Cirmtuzumab targeted cells. RNASeq and IFC analysis of enzalutamide-resistant PDXs revealed new bone-microenvironment signaling networks.

Conclusions: WNT5A and ROR1 are re-activated in a sub-set of bone-metastatic prostate cancer cells. This may present a unique therapeutic opportunity to use the ROR1-targeting monoclonal antibody, CIRMTUZUMAB, already in Phase 1B clinical trials for CLL and metastatic breast cancer to block WNT5A:ROR1 signaling and prevent growth and survival of metastatic prostate cancer. These pre-clinical studies will support the movement of this drug to the clinic for these patients who have no curative options.

Interactions between prostate hormone levels, African ancestry, and gene expression patterns in stroma and epithelium

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Interactions between prostate hormone levels, African ancestry, and gene expression patterns in stroma and epithelium

Baumann, Bethany, PhD; Pacheco, Julian; Richards, Zachary, PhD; Garcia, Jason; Kittles, Rick, PhD; Nonn, Larisa, PhD

Background: Prostate cancer disproportionately affects African American (AA) men in incidence and mortality. While many systemic factors contribute to this disparity, a biological contribution has been hypothesized due to reported differences in the serum levels of hormones with a known role in PCa, such as vitamin D, androgens and estrogens. For example, AA men have lower serum levels of vitamin D compared with other men due to increased melanin that inhibits its cutaneous synthesis. The goal of this study is to investigate how the prostatic levels of these hormones associate with ancestry and prostatic gene expression.

Methods: We performed a mass-spec analysis of benign prostate tissue from a cohort of AA and European American (EA) men, collecting accurate estimates of prostatic concentrations of vitamin D metabolites (25D and 1,25D) and dihydrotestosterone (DHT). We additionally performed laser-capture microdissection of the stromal and epithelial prostate compartments of 20 men followed by microarray gene expression analysis. African ancestry was determined by SNP analysis.

Results: Our recent publication supports active import rather than passive diffusion of vitamin D into the prostate. We found DHT was higher in prostates, but not serum, of AA men. Larger differences in gene expression were found in the stroma of AA and EA men than epithelium. A differentially expressed gene in stroma was *HSD17B7*, involved in estrogen synthesis. A prostate tissue microarray validated that this enzyme may be higher in AA men. Many genes were correlated with 25D and with % West African ancestry, but DHT had weak associations. Pathway analysis revealed enrichment for estrogen receptor signaling and chromatin remodeling in stroma from AA men. TGF-β signaling was enriched in epithelium of AA men, which is consistent with correlation of TGF-β and % West African ancestry in stroma. RNA splicing was enriched in epithelium of EA men, and associated with higher 25D levels.

Conclusions: Vitamin D may have a large effect on gene expression. However, while 25D is higher in prostates from EA men, 1,25D is higher in prostates from AA men, so any vitamin D contribution to PCa disparities is more complex than a deficiency in circulating levels. The higher level of prostatic DHT in AA men has not been previously reported and may contribute to the disparity of PCa in AA men. Our finding of differential estrogen pathway signaling in stroma from AA men is interesting as recent studies support a central role of estrogens in prostate carcinogenesis and progression.

Resistance to AR Signaling Inhibition Does Not Necessitate Prostate Neuroendocrine Differentiation

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Resistance to AR Signaling Inhibition Does Not Necessitate Prostate Neuroendocrine Differentiation

W. Nathaniel Brennen[1], Yezi Zhu[2], Ilsa Coleman[3], Susan Dalrymple[1], Lizamma Antony[1], Alan Meeker[1,2,4], S. Lilly Zheng[5], Jody E. Hooper[4], Jun Luo[2], Angelo De Marzo[1,2,4], Eva Corey[6], Jianfeng Xu[5], Peter S. Nelson[3,6], William B. Isaacs[2], John T. Isaacs[1,2,4]

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Background: Treatment with 2nd generation androgen receptor signaling inhibitors (ARSi) is suggested to drive lineage plasticity of metastatic castration-resistant prostate cancer (mCRPC) to a therapy-resistant neuroendocrine (NE) phenotype. This emergence of the NE phenotype is accompanied by the lack of AR signaling in addition to mutations/deletions in PTEN, RB1, and TP53 together with overexpression of DNMTs, EZH2, and/or SOX2. Alternatively, AR-negative ARSi-resistant cancers could be derived from tumor-initiating cells that never express either AR or NE markers

Methods: Multiple new serially-transplantable patient-derived xenografts (PDX) models were established from independent mCRPC patients at rapid autopsy, including from bone, liver, skin, and lymph node metastases. Additional PDXs developed previously, including neuroendocrine prostate cancer (NEPC) models from the LuCaP series, were also utilized. Collectively, these models represent the full range of lethal phenotypes observed clinically – AR+ adenocarcinoma, AR- NEPC, AR-/NE- double negative, and AR+/NE+ amphicrine. PDXs were grown and characterized in intact and castrate hosts *in vivo*. The androgen-independent LNCaP95 cell line derived from parental LNCaP via long-term culture in charcoal-stripped media was used to generate AR knockout variants via CRISPR-Cas9 for *in vitro* and *in vivo* analyses. HoxB13 expression confirmed prostatic origin of all models. Immunohistochemistry (IHC), RNAseq, and DNA sequencing performed to characterize all models.

Results: A combination of novel prostate cancer PDX models that recapitulate the proposed genetic background (i.e. AR loss, mutant PTEN, RB1, and TP53, as well as overexpression of DNMT, EZH2, and/or SOX2), in addition to AR-knockout cell lines document that ARSi-resistance does not necessitate acquiring a NE phenotype, but alternatively can occur via emergence of an AR/NE double negative (DN) cancer.

Conclusions: Since the prevalence of such double negative ARSi-resistant cancers is increasing clinically, this highlights the urgent need to develop non-AR targeted therapies.

Acknowledgements: We are grateful to the patients and their families who participated in the Legacy Gift Rapid Autopsy program at Hopkins. The authors would like to acknowledge the following sources of financial support: Movember Foundation Global Action Plan (GAP) program for development of prostate cancer xenografts to model human prostate cancer (JTI); Prostate Cancer Foundation Young Investigator Award (WNB); Patrick C. Walsh Prostate Cancer Research Fund (WNB); Department of Defense Prostate Cancer Research Program, Award No W81XWH-16-1-0410 (JTI), W81XWH-18-1-0348 (JTI); W81XWH-18-1-0347 (PSN), W81XWH-17-1-0415 (PSN), W81XWH-17-1-0528 (WNB), W81XWH-10-2-0045 and

W82XWH-10-2-0056 PCRP Prostate Cancer Biorepository Network (AMD); NIH-Prostate SPORE grant number P50 CA058236 (JTI), (AMD for Pathology Core) and P50 CA097186 (PSN); NIH R01 CA185297 (JL); P01CA163227 (PSN), and the NIH-SKCCC CCSG grant number P30 CA006973, which provides support for the Oncology Tissue Services, Animal Resources, Cell Imaging, Rapid Autopsy Core, and Genetic Resources Core Facilities.

Integrin a6b1 super agonist overcomes drug resistance in castration-resistant prostate cancer by targeting laminin adhesion

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Integrin a6b1 super agonist overcomes drug resistance in castration-resistant prostate cancer by targeting laminin adhesion

Adhesion to matrix has long been recognized as a mechanism by which cells can evade death due to toxic agents, including radiation, chemotherapy, and targeted therapies. Attempts to block matrix adhesion in the clinic have been limited to RGD integrins. However, the majority of epithelial cancers engage laminin and collagen matrices, for which specific targeting has not yet been achieved. In prostate cancer, laminin integrin a6ß1 is the predominant integrin required for prostate cancer invasion and metastases. Our previous studies demonstrated that this integrin is also critical for the survival of prostate cancer and confers resistance to both anti-androgen therapy (ADT) and PI3K inhibitors. We sought to identify the mechanisms that drive this resistance and to find ways to specifically target integrin a6ß1. We identified Bnip3 as a downstream target of androgen and integrin a6ß1 that is specifically elevated in drug-resistant prostate cancer, which confers resistance to PI3K inhibitors. Drug-resistant prostate cancer is associated with an elevated hypoxia signature, where hypoxia induces integrin a6ß1 expression, and integrin a6ß1 is required for HIF1a nuclear translocation and induction of Bnip3. Integrin a6ß1 and Bnip3 promotes the survival of prostate cancer cells through the combined induction of autophagy and mitophagy respectively. Blocking Bnip3 or integrin a6ß1 restores sensitivity to PI3K inhibitors. MTI-101, a peptidic mimetic based on an integrin a6ß1 blocking peptide, potently restores sensitivity to PI3K inhibitors during drug-resistant prostate tumors and blocks tumor growth in vivo. MTI-101 acts as a potent super agonist of integrin a6ß1 signaling, inducing Bnip3, autophagy and mitophagy, but also rapidly depletes Ca+2 stores leading to necroptosis. Despite the potent inhibition of tumor growth with combined MTI-101 and PI3K inhibition, residual tumors were still detectable. Within these tumors, we identified a significant upregulation of PIM kinase, known to promote survival via anti-apoptotic mechanisms. Using a triple combination treatment at sub-optimal doses, aimed at simultaneously hitting integrin a6ß1, PI3K, and PIM, we were able to identify a combination that leads to synergistic tumor cell death. Thus, we have identified an integrin a6ß1-dependent drug resistance pathway that is targetable by a novel new integrin a6ß1-blocking drug that can work in synergy with targeted therapies to overcome drug resistance in prostate cancer. These studies help set the stage for being able to overcome cell adhesion mediated drug resistance.

The Role of IL33 in Microbial Induced Prostate Fibrosis

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Praveen Thumbikat, PhD, DVM

Title: The Role of IL33 in Microbial Induced Prostate Fibrosis

Authors: Ashlee J Bell-Cohn, Praveen Thumbikat

Background: Lower Urinary Tract Symptoms (LUTS) is an umbrella term for a number of symptoms associated with bladder and/or voiding dysfunction. LUTS in men has been linked to prostate tissue remodeling and higher collagen content, in periurethral prostate tissue, during induction of fibrosis. Fibrosis is an aberrant wound healing process that is thought to contribute to LUTS by causing stiffening of the prostate gland and resultant noncompliance of the prostatic urethra. Type 2 cytokine signaling is necessary for fibrosis development in B6 mice after infection with a patient-derived uropathogenic *Escherichia coli* strain, named CP1. IL33 is an alarmin that can be released from damaged epithelial cells and promotes type 2 cytokine production. Therefore, we hypothesize that IL33 could have a role in microbial induced fibrosis.

Methods: After obtaining IRB approval, post-prostatic massage (VB3) fluid was collected from CP patients and controls. Then the fluid was analyzed via QPCR for IL33 mRNA. A benign human prostate epithelial cell line was infected with CP1 for 24, 48, and 72 hours. After which, the supernatant was collected for ELISA analysis for IL33. Day 14 control and CP1-infected IL33 reporter mice prostates were assayed for IL33 via western blot. Control and CP1-infected transgenic and wildtype mice were analyzed for prostate fibrosis via picrosirus red staining.

Results: VB3 fluids from CP patients were found to have elevated mRNA levels of IL33 when compared to healthy controls. Human prostate epithelial cells, after infection with CP1, expressed significantly more IL33 protein. CP1-infected IL33 reporter mice possessed significantly higher levels of IL33. Control and CP1-infected transgenic and wildtype mice were found to have differential fibrosis development

Conclusions: CP patients, whom also sometimes show urinary dysfunction as a symptom, possessed elevated levels of IL33. CP1 infection induces elevated levels of IL33 in both human cells and mouse prostates. CP1 infection is known to cause fibrosis and urinary dysfunction in mice. Elevated levels of IL33 suggest that it could be playing a role in mediating microbial induced fibrosis. Further study into how IL33 could be contributing to fibrosis and subsequent urinary dysfunction development could provide a new target for therapeutic intervention.

Characterization of the Metabolomic Profile of Prostate Cancer by Capillary Electrophoresis Mass Spectrometry of Urine

Andrew Gusev, BA, Massachusetts General Hospital
Alex Buko, PhD, Takushi Oga, PhD, Adam S. Feldman, MD, MPH, Leo L. Cheng, PhD

Abstract Category: Big Data as Engines for Discovery

Title: Characterization of the Metabolomic Profile of Prostate Cancer by Capillary Electrophoresis Mass Spectrometry of Urine

Authors: Andrew Gusev, BA[1], Alex Buko,PhD[2], Takushi Oga, PhD[2], Adam S. Feldman, MD, MPH[1], Leo L. Cheng, PhD[1]

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Background:
Prostate cancer (PCa) pathogenesis is influenced by alterations in cellular metabolism. Metabolomics measures these biochemical changes to create global tissue metabolite profiles. Urinary studies are noninvasive and can potentially identify biomarkers for PCa. We used Capillary Electrophoresis Mass Spectrometry (CE-MS) to analyze urine from men undergoing prostate biopsy for suspicion of PCa to investigate their metabolomic profiles.

Methods:
An analysis of charged metabolites by CE-MS was performed as described (J Proteome Res. 2:488; 2003). Urinary metabolites were extracted from 100 µL urine by mixing with methanol containing 20 µM of internal standards. CE-MS experiments were performed with the Agilent CE system. Screening of potential biomarkers was performed with statistical protocols and pathway analyses. Metabolites with levels below the detection limit in all samples were excluded. Relative abundances of metabolites were normalized to levels of creatinine.

Results:
CE-MS analysis produced thousands of features in the combined anionic and cationic modes. A volcano plot comparing p values against fold change identified 60 metabolites that were statistically different between urine samples of men with PCa and those of men without PCa. Pathway analysis of these using MetaboAnalyst (Metabolites 9:57; 2019) showed high activity in ceramide, short chain fatty acid (SCFA), branched chain amino acid, serine, threonine and tryptophan metabolism. Figure 1: fold change shown by color and vertical scale, number of significant metabolites by size. Figure 2: graphically contrasts metabolomic profiles.

Conclusions:
CE-MS analysis identified several metabolic pathways that were upregulated in urine of men with PCa. These metabolites are involved in steroid, aromatic, microorganism and SCFA processes and warrant targeted studies which are underway in our lab. If validated, they have potential to serve as non-invasive biomarkers for PCa diagnosis and therapeutics.

Figure 1: Overview of Pathway Analysis

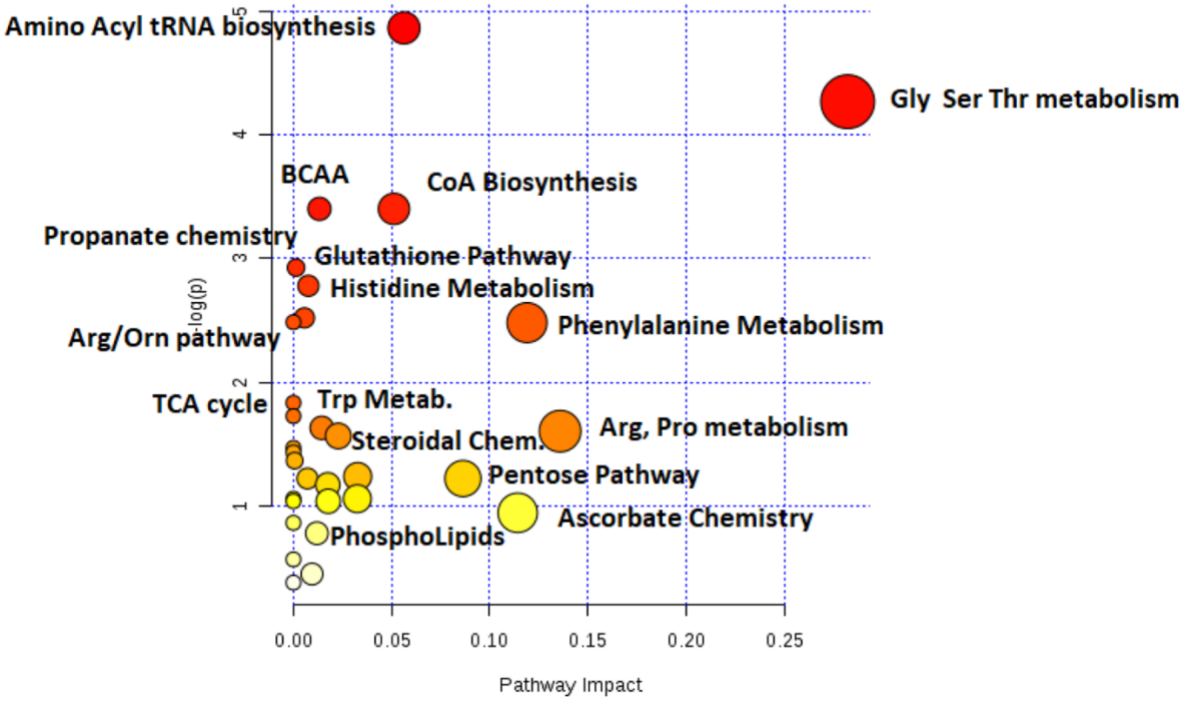
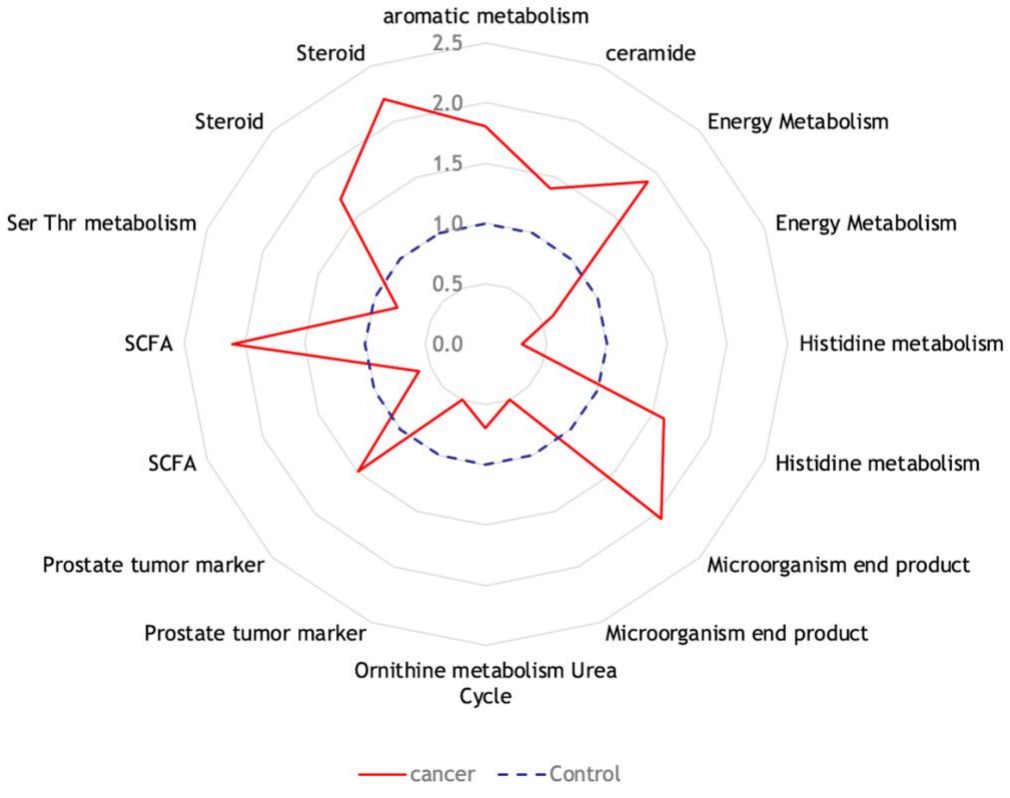


Figure 2: Pathways differentiating metabolomic profiles of men with & without PCa



Effect of the SFRP1 protein on prostate cancer stem cells populations

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Marian Cruz-Burgos, B.D., Sergio Cortes-Ramirez, QFB., Carlos Cruz-Hernanndez, MSc., Mauricio Rodriguez-Dorantes, Ph.D.

Effect of the SFRP1 protein on prostate cancer stem cells populations

Losada-García A., Cruz-Burgos JM., Cortés-Ramírez SA., Cruz-Hernández CD and Rodríguez-Dorantes M.

Prostate cancer ranks second place in incidence and the sixth in deaths from neoplasms in the World. Experimental evidence suggest that the tumors have a hierarchical organization, regulated by the prostate cancer stem cells (PCSC), which are defined as a cell subgroup that has the capacity for self-renewal, can produce the different lineages inside the tumors and are responsible for the cancer progression. This population of cells with stem characteristics has been identified in tumors of advanced prostate cancer. It has also been reported that SFRP1 protein is key in the stromal-epithelial signaling in the prostate, promoting tumor growth. This protein is a regulator of the WNT-β-catenin pathway which can modulate stem characteristics in prostate cancer. The aim of this work was to evaluate the effect of SFRP1 on the activation of WNT/β-catenin pathway and cancer stem cell markers. To this end, an *in vitro* model was established in a 3D culture (prostatospheres), and *in vivo* xenotransplant model. Both models were treated with the SFRP1 protein. After the treatment the sizes of the xenotransplants and prostatospheres was evaluated as well as the levels of expression of the SOX2, NANOG, OCT4, LEF1, FZD4 and CCND1 genes. We observed an increase in the expression of the SOX2, NANOG, OCT4, SOX2, CCND1, LEF1 genes in prostatospheres and xenotransplants treated with SFRP1 with respect to the vehicle. It was also found that treatment with SFRP1 promotes the growth of prostatospheres and xenotransplantation tumors reaching significant differences with respect to the control. The results of the present study show evidence that treatment with SFRP1 is capable of activating the WNT/β-catenin pathway, upregulating the stem cell markers in prostate cancer, thus promoting tumor growth in xenotransplantation and increasing the size of the prostatospheres. These findings, and reports in the literature, give us evidence that SFRP1 participates in the loss of regulation of the WNT/β-catenin pathway in prostate cancer, promoting tumorigenesis by aberrant activation of canonical WNT pathway and their targets genes regulating PCSC populations. Which would be promoting tumor progression, as well as therapeutic failure in advanced prostate cancer. The observation of this effect contributes and provides information to the understanding of the mechanisms that may be involved in the maintenance and progression of the disease, contributing to the identification of new therapeutic targets.

Loss of CDCP1 promotes FAK activation in the detached state

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Title: Loss of CDCP1 promotes FAK activation in the detached state

Background: A major metastasis suppressing mechanism is the rapid apoptotic death of cancer cells upon detachment from extracellular matrix, a process called anoikis. Focal adhesion kinase (FAK) is a key enzyme to overcome anoikis.

Methods: Circulating tumor cells (CTCs) from patients with metastatic castrate resistant prostate cancer (mCRPC) were probed for CDCP1, FAK, CD45 and DAPI expression and an unbiased FAK gene signature was identified in publicly available datasets of CTCs. $CDCP1^{low}$ and $CDCP1^{high}$ xenografts were assessed for tumor volume *in vivo*. Complexes of β1-integrin, CDK5 and PIP5K were identified via co-immunopreipitation experiments in prostate cancer cell lines and the mechanism of suspension-activation of FAK by PtdIns(4,5)P2 (PI(4,5)P2) was discovered via mutants and suspension in human plasma.

Results: Here we show that loss of the Cub-domain containing protein – 1 (CDCP1), which leads to inactivation of β1-integrin, paradoxically stimulates FAK activation in CTCs from patients with metastatic, castration resistant prostate cancer (mCRCP). Detachment-activation of FAK through local production of PI(4,5)P2 by PIP5K1c sensitizes PC cells to FAK inhibitors. In the detached state, the PIP5K1c-201 splicing isoform is stimulated by loss of CDCP1. In $CDCP1^{low}$ PC cells, PIP5K1c activation is triggered by human plasma, SRC phosphorylation and Talin binding.

Conclusions: Patients with mCRPC can be stratified for more effective treatment outcomes based on CDCP1 expression levels in CTCs: $CDCP1^{low}$ CTCs characterized by active FAK-pY397 and high TMPRSS2 or $CDCP1^{high}$ CTCs which express the PC oncogenes: AR-v7, ONECUT2 and HOXB13. The two groups differ in networks of cytoplasmic kinases and targetable transcription factors, which bears important therapeutic implications.

Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2- TSP1 pathway in prostate cancers

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Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2-TSP1 pathway in prostate cancers

Background: The incidence of aggressive neuroendocrine prostate cancers (NEPC) related to androgen-deprivation therapy (ADT) is rising. NEPC is still poorly understood with no effective treatment. Elevated levels of neuroendocrine differentiation (NED) and angiogenesis are two prominent phenotypes of NEPC, whose direct molecular links have been largely unknown.

Methods: In this study, we have employed a number of genetic manipulations (gene overexpression and silencing) and pharmacological perturbations (chemical activators and inhibitors) for genes under investigation to study their connections to each other and their implications in NED and angiogenesis. Several prostate cancer cell and xenograft models in culture and in mice were utilized. ADT was achieved by treating androgen-responsive cells in culture with MDV3100 and by growing them in hormone-deprived media, as well as by surgical castration of male mice for xenograft tumor growth. Gene expression data from TCGA and other public sources, as well as tissue microarrays of prostate normal and tumor tissues from patient were used to evaluate the clinical relevance of this work.

Results: NED and angiogenesis are molecularly connected through EZH2 (enhancer of zeste homolog 2). NED and angiogenesis are both regulated by ADT-activated CREB (cAMP response element-binding protein) that in turn enhances EZH2 activity. We also uncover anti-angiogenic factor TSP1 (thrombospondin-1, THBS1) as a direct target of EZH2 epigenetic repression. TSP1 is downregulated in advanced prostate cancer patient samples and negatively correlates with NE markers and EZH2. Furthermore, castration activates the CREB-EZH2 axis, concordantly affecting TSP1, angiogenesis and NE phenotypes in tumor xenografts. Notably, repressing CREB inhibits the CREB-EZH2 axis, tumor growth, NED and angiogenesis *in vivo*.

Conclusions: We have elucidated a new critical pathway, consisting of CREB-EZH2-TSP1, underlying ADT-enhanced NED and angiogenesis during prostate cancer progression. The investigation of molecular mechanisms underlying EZH2 activation by CREB signaling and the induction of NED by EZH2 is underway.

Effect of radiation cystitis on urinary bladder mechanics Marissa Grobbel, Ph.D. Student in Mechanical Engineering at Michigan State University

Bernadette M.M. Zwaans, Elijah P. Ward, Laura E. Lamb, Department of Urology, Beaumont Health and Sara Roccabianca, Mechanical Engineering Professor at Michigan State University

Effect of radiation cystitis on urinary bladder mechanics

Authors: Marissa Grobbel, Bernadette M.M. Zwaans, Elijah P. Ward, Laura E. Lamb, Sara Roccabianca

Background

Radiation cystitis is a type of urinary bladder dysfunction resulting from irradiation treatment for pelvic cancer. This is a debilitating disease that limits a cancer survivor's ability to recover and significantly affects their quality of life. To our knowledge, no previous studies have examined the effect of irradiation on urinary bladder (UB) remodeling from a mechanical perspective. Therefore, this study aims to analyze the effect of irradiation on UB mechanics.

Methods

UBs of 8 week old, C57BL female mice were subjected to irradiation treatment (n=5) at 3 x 20 Gy, along with untreated controls (n=5). Following treatment, micturition data and weight of the mice were recorded every week from both groups. At 11 weeks post-treatment, the mice were euthanized and their UBs isolated. Four UBs (2 irradiated, 2 control) were sectioned and stained with Masson's trichrome for measurement of collagen content. The extracellular matrices of the remaining UBs were isolated and subjected to a circumferential stress-stretch test.

Results

Irradiation of the mice was well-tolerated as the body weights of the mice were not affected by the treatment, nor was the macroscopic size or shape of the UB at this timepoint. Micturition data showed no significant difference between the irradiated and control groups. However, stress-stretch testing of the isolated extracellular matrix showed a significant decrease in UB distensibility (Figure 1). Histological analyses suggest fibrosis of the UB wall—specifically, an increase from 12.45% to 34.39% collagen (n=1 each group—measurements are ongoing).

Conclusions

Though the micturition data showed no change with irradiation, there is evidence of remodeling that had an effect on the mechanical behavior of the UB. Specifically, the irradiation treatment resulted in fibrosis of the UB wall, leading to decreased distensibility. A decrease in UB distensibility as a result of irradiation can not only cause symptoms that interfere with cancer recovery, but also pose a threat to proper kidney function, outlining the importance of studying radiation cystitis from a mechanical perspective.

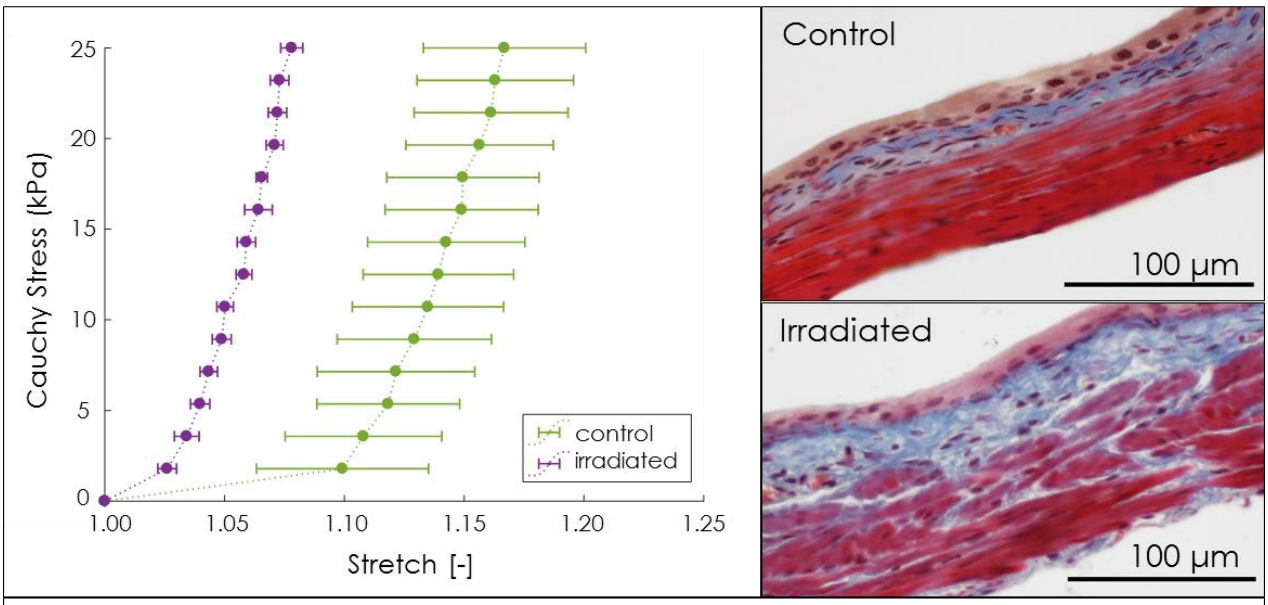

Figure 1. Left: stress-stretch data showing stiffening of irradiated bladders (average +/- standard error). Right: Masson's trichrome showing fibrosis of irradiated bladder (blue = collagen, red = cells).

Pharmacogenetic inhibition of afferent excitability alleviates VEGF-induced visceral allodynia and hyperalgesia in a mouse model of urological chronic pelvic pain syndrome (UCPPS)

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Randall B. Meacham, M.D., Professor, Chair of the Division of Urology, Department of Surgery, UC Denver; Anna P. Malykhina, Ph. D. Associate Professor, Division of Urology, Department of Surgery, UC Denver

Author: Alison X. Xie*, Randall B. Meacham, Anna P. Malykhina

Title: Pharmacogenetic inhibition of afferent excitability alleviates VEGF-induced visceral allodynia and hyperalgesia in a mouse model of urological chronic pelvic pain syndrome (UCPPS)

Abstract:

a) Background:

Patients with urological chronic pelvic pain syndrome (UCPPS) experience chronic pelvic pain (CPP) and lower urinary tract symptoms (LUTS). The UCPPS symptoms are closely associated with nociceptive sensitization in the nervous system, which underlies visceral allodynia and hyperalgesia. Previous studies suggested that afferent hypersensitivity in bladder-projecting sensory neurons plays an important role in the generation and the maintenance of UCPPS symptoms, especially bladder pain and urinary frequency. In this study, we tested the hypothesis that visceral hypersensitivity and the symptoms of UCPPS could alleviated by pharmacogenetic inhibition of sensory neuronal excitability in a mouse model of UCPPS.

b) Methods:

Vascular endothelial growth factor (VEGF) level is correlated with CPP in UCPPS patients, and in animal models of bladder overactivity. Intravesical instillation of $VEGF_{165}$ induced nociceptive sensitization and bladder nerve remodeling in both male and female C57BL6/J mice. Bladder overactivity and pain were evaluated by *in vivo* cystometry and Von Frey assay, respectively. To directly manipulate afferent sensitivity, *Designer Receptors Exclusively Activated by Designer Drugs* (DREADDs) were expressed in bladder-projecting sensory neurons via targeted adeno-associated viral vector (AAV) injections. Transgenic mice expressing Cre-recombinase and fluorescence reporters in bladder sensory neurons and afferent nerves were used to guide cellular expression of DREADDs as well as to reveal the potential nerve remodeling via neuroimaging.

c) Results:

We found that $VEGF_{165}$ induced sensory nerve remodeling in the urinary bladder, bladder overactivity, and visceral mechanical allodynia and hyperalgesia. The $VEGF_{165}$-induced symptoms were likely due to VEGF receptor signaling-mediated upregulation of nociceptors in bladder sensory nerves. Pharmacogenetic inhibition of bladder-projecting sensory neurons using DREADDs significantly attenuated $VEGF_{165}$-induced visceral mechanical allodynia and hyperalgesia and alleviated the symptoms of bladder overactivity.

d) Conclusion:

Our data suggests a functional role for VEGF signaling in peripheral sensory neurons in a murine model of UCPPS. Further investigation of the molecular targets in VEGF signaling pathways in bladder sensory neurons will shed lights on alternative treatments for UCPPS.

Identification of multipotent prostate basal stem cells from single-cell RNA sequencing

Helen He ZHU, Professor, Shanghai Jiao Tong University *Xue Wang, Ph.D. candidate; Wei-Qiang Gao, Professor*

Identification of multipotent prostate basal stem cells from single-cell RNA sequencing

Xue Wang[1,2], Wei-Qiang Gao[1,2] and Helen He Zhu[1]

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- **Background:** The prostate basal cell compartment is postulated to contain stem/progenitor cells. However, the true prostate stem cell subpopulation within prostate basal cells is undetermined.

- **Methods:** We performed single-cell RNA sequencing of over 9000 mouse prostate basal cells. Additional functional assays including organoid forming and xenograft experiments, as well as lineage tracing and genetic ablation experiments were conducted to test the putative stem cell population.

- **Results:** Utilizing unbiased single cell transcriptomic analysis of over 9000 mouse prostate basal cells, we find that a Zeb1^{+} basal cell subset shares gene expression signatures with both epithelial and mesenchymal cells and stands out uniquely among all the basal cell clusters. Zeb is exclusively expressed in a prostate basal cell subpopulation based on both immunocytochemical and cell lineage tracing analysis. The Zeb1^{+} prostate epithelial cells are multipotent prostate basal stem cells (PBSCs) that can self-renew and generate functional prostatic glandular structures with all three epithelial cell types at the single-cell level. Genetic ablation studies further reveal an indispensable role for Zeb1 in prostate basal cell development. Moreover, Zeb1^{+} epithelial cells can be detected in mouse and clinical samples of prostate tumors.

- **Conclusions:** Zeb1 marks a multipotent prostate basal stem cells and Zeb1 itself is required for prostate basal cell development. Identification of the PBSC and its transcriptome profile is crucial to advance our understanding of prostate development and tumorigenesis.

FOXA2 Promotes Prostate Cancer Growth in Bone Zachary M. Connelly, LSUHSC Shreveport

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Background: Bone metastases frequently occur in advanced-stage prostate cancer (PCa) patients. Understanding the mechanisms that support the establishment and growth of PCa cells in bone may lead to the identification of therapeutic targets for the lethal disease. We found that the pioneer transcription factor, forkhead box A2 (FOXA2) expressed in embryonic prostate development and late-stage PCa is expressed in a subset of PCa bone metastasis specimens.

Methods: Bioinformatic analysis of human PCa databases, Patient Derived-Xenografts, and cancer cell lines provided evidence of FOXA2 expression in a subset of PCa bone metastases. To determine the functional role of FOXA2 in PCa metastasis, we stably knocked down the expression of FOXA2 in aggressive PCa PC3 cells, which are well-established to grow in bones and elicit an osteolytic reaction.

Results: We found that FOXA2 knockdown caused a significant decrease in PCa PC3-mediated bone destruction *in vivo* following intra-tibial injection. To explore the mechanisms by which FOXA2 promotes the cancer-mediated bone lesions, we conducted RNA-Seq analyses and found that the mRNA levels of Parathyroid Hormone-related Protein (PTHrP) decreased following FOXA2 knockdown. ChIP assays indicate that FOXA2 directly regulates the expression of PTHLH (PTHrP gene). PTHrP is a well-established factor that regulates the turnover of bone tissue in both normal physiology and cancer metastases. FOXA2's regulation of PTHrP could provide a mechanism to promote PCa cells' interaction with the bone microenvironment and facilitate PCa bone colonization.

Conclusion: These results indicate that FOXA2 is involved in PCa growth in bone.

PRMT5 as a novel target for the treatment of castration-resistant prostate cancer

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2019 Travel Award Winner
Jake Owens, BA, Xuehong DengChang-Deng Hu, PhD

Background:

Emergence of castration-resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT) is one of the biggest challenges in prostate cancer therapy. Androgen receptor (AR) reactivation via various mechanisms is the driver of the ADT resistance. Current CRPC therapies that target AR signaling are not curative and only prolong survival by 4-5 months. Thus, the development of novel approaches for CRPC treatment is in urgent need.

Recently it was shown that protein arginine methyltransferase 5 (PRMT5), an emerging epigenetic enzyme and putative splicing regulator, is required for the hormone-naïve prostate cancer (HNPC) growth. Mechanistically, it was demonstrated that in HNPC PRMT5 epigenetically activates AR transcription. Considering the role of AR in CRPC and that PRMT5 regulates AR in HNPC, we aimed to determine whether PRMT5 regulates AR expression in CRPC.

Methods:

shRNA against PRMT5 and inhibitor BLL3.3 were used to target PRMT5 in CRPC cells C4-2 (AR overexpression), 22Rv1 (AR-V7 expression) and VCaP (AR gene amplification). Transcriptome-wide gene expression was measured via RNA-seq. AR and AR target genes expression were analyzed using Western Blot and RT-qPCR. Cell proliferation was measured using MTT assay. Chromatin immunoprecipitation was used to analyze presence of PRMT5 and associated histone methylation marks at the AR promoter. 22Rv1 lines with shRNA inducible expression were established for use in xenograft studies.

Results:

PRMT5 targeting reduced cell proliferation and decreased the protein and mRNA levels of both AR full length and V7 in all CRPC cell lines tested. Consistently, expression of full length AR or AR-V7 target genes was decreased. PRMT5 and H4R3me2s were present at the AR promoter. To further explore the role of PRMT5 in CRPC, we performed RNA-seq analysis in 22Rv1 upon PRMT5 knockdown. Interestingly, 293 genes were down- and 329 genes were upregulated upon PRMT5 knockdown contrary to the common perception of PRMT5 as an epigenetic suppressor. Additionally, exon mapping revealed differential up- and down-regulation of AR isoforms in PRMT5 knockdown samples suggesting that PRMT5 regulates AR splicing. PRMT5 knockdown significantly reduced the growth of 22Rv1 xenografts in castrated NRG male mice.

Conclusions

Our results suggest that PRMT5 acts as a regulator of AR expression in CRPC cells via both epigenetic regulation of transcription and mRNA splicing. Based on these findings, we propose that targeting PRMT5 may present a novel treatment approach for CRPC via eliminating AR and its splice variants expression.

Transcription factor PROX1 drives neuroendocrine differentiation and cellular plasticity in prostate cancer

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Transcription factor PROX1 drives neuroendocrine differentiation and cellular plasticity in prostate cancer

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Introduction and Objectives:

With the application of the next-generation AR pathway inhibitor (ARPIs), such as enzalutamide, patients with metastatic castration resistant prostate cancer (mCRPC) acquire a significant survival benefit. However, a proportion of patients undergo neuroendocrine differentiation (NED) and progress to neuroendocrine prostate cancer (NEPC), and finally show resistance to ARPIs. The underlying mechanisms remain largely unknown. In our previous study, we have demonstrated that Prospero-related homeobox 1 (PROX1) as a critical downstream factor of DAB2IP induced HIF-1alpha protein accumulation and an epithelial-mesenchymal transition (EMT) response, leading to a unique non-skeletal metastasis of PCa. In this study, we will further explore the role of PROX1 in the regulation of NED and cellular plasticity (i.e., EMT and cancer stemness) of PCa.

Methods:

Immunohistochemistry (IHC) was performed to evaluate the expression of PROX1 in prostate adenocarcinoma and NEPC tissues. The expression of PROX1 and gene copy number were analyzed in different NEPC models (clinical tissues, patient-derived xenograft models, transgenic mouse models and cell lines) from public databases or published literatures. *In vitro*, qPCR and western blot were used to detect the expression of PROX1 in different NEPC cell models (LNCaP with TP53/RB1 loss, PC3, hypoxia-treated 22Rv1, and NCI-H660 cell lines). qPCR was used to evaluate the expression of PROX1 in LNCaP cells which were treated with DHT or charcoal stripped medium, in order to explore the association between PROX1 and AR signalling pathway. The changes of NED and cellular plasticity in C4-2B/MDVR cells with PROX1 knockdown were measured by Boyden chamber assay, qPCR and western blot analysis. *In vivo*, subcutaneous xenografts in nude mice were used to detect tumorigenicity, and the expression of PROX1, NSE, E-cadherin and CD44 was detected by IHC.

Results:

PROX1 was uniquely upregulated in NEPC clinical specimens, PDX models, transgenic mouse and cell models due to TP53/RB1 loss, *PROX1* gene amplification and hypoxia. Also, activation of AR signalling pathway decreased the expression of PROX1 gene. Furthermore, PROX1 was upregulated in C4-2B/MDVR cell model with NEPC phenotype, and knockdown of PROX1 could reverse NED and cellular plasticity of C4-2B/MDVR cells and restore the sensitivity to enzalutamide treatment *in vitro* and *in vivo*.

Conclusions:

PROX1 was significantly upregulated in NEPC, which could drive PCa cell NED and cellular plasticity for the development of enzalutamide resistance.

Source of Funding:

National Natural Science Foundation of China (NSFC 81202014 to KW), the "New-Star" Young Scientists Program of Shaanxi Province (to KW) and Fundamental Research Funds for the Central Universities in China (to KW).

Keywords:

PROX1; prostate cancer; NED; cellular plasticity; EMT; enzalutamide resistance

Site Specific DNA Methylation Silences Forkhead Box A1 Expression in Advanced Bladder Cancer

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Site Specific DNA Methylation Silences *Forkhead Box A1* Expression in Advanced Bladder Cancer

Jenna M. Buckwalter Ph.D.*, Lauren M. Shuman M.S., Thomas C. Wildermuth, Vonn Walter Ph.D., Joshua Warrick M.D., Xue-Ru Wu M.D., Jay Raman M.D., David J. DeGraff Ph.D.

Introduction and Objective: Bladder cancer (BC) has significant molecular and morphologic heterogeneity. Transcription factor forkhead box A1 (FOXA1) is required for maintenance of urothelial differentiation, and decreased *FOXA1* expression is associated with basal-squamous BC. As basal-squamous BC responds differentially to therapeutic intervention, manipulation of *FOXA1* expression to control subtype specification is an attractive concept. The aim of this study was to determine the mechanism(s) responsible for loss of *FOXA1* expression in BC.

Methods: Computational analysis of the TCGA BC study was used to examine the relationship between *FOXA1* mutational, copy number and methylation status with gene expression. The UCSC genome browser was used to identify CpG islands in the *FOXA1* gene as potential sites of methylation. A PCR-based system for detecting methylated CpG islands was used to determine the methylation status of *FOXA1*-associated CpG islands in a panel of human BC cell lines. In addition, human basal BC cell lines that fail to express *FOXA1* were treated with DNA methyltransferase (DNMT) inhibitors in an effort to influence methylation status and *FOXA1* expression.

Results: Decreased *FOXA1* expression is not correlated with mutational status and/or copy number alterations in BC which suggested a role for epigenetic silencing of *FOXA1* in basal-squamous BC. Three CpG islands were identified in the *FOXA1* which include islands 99, 123, and 143. Analysis of TCGA DNA methylation data identified significant methylation at CpG island 99 ($p<0.0009$; Wilcoxon rank sum; Bonferonni) relative to normal adjacent control tissue. Methylation analysis for CpG islands in human BC cell lines determined CpG island 99 was methylated specifically in basal-squamous BC cell lines which fail to express *FOXA1*, while CpG island 143 was unmethylated. CpG island 123 was methylated in all ten cell lines. Treatment of SCaBER and HT1376 basal-squamous BC cell lines with the DNMT inhibitors 5-Aza- 2'deoxycytidine and Zebularine individually and in combination increased *FOXA1* expression and decreased DNA methylation at CpG island 99.

Conclusions: Our data indicates site-specific methylation of CpG island 99 is implicated in the repression of *FOXA1* expression in human BC and preclinical models. Importantly, *FOXA1* methylation is reversed by DNMT inhibitor treatment, thus confirming methylation as an epigenetic regulatory mechanism controlling *FOXA1* expression in BC. Our study additionally shows the importance of examining promoter methylation in association with gene expression to determine the functional consequences of epigenetic alterations.

A novel non-canonical EZH2 function as a chaperone to mediate box C/D snoRNP assembly Yang Yi, Post-Doctoral Fellow, Northwestern University

Qingshu Meng, Post-doctoral fellow; Qiaqia Li, Graduate Student; Kaifu Chen, Associate Professor; Wei Zhao, Professor; Qi Cao, Associate Professor

A novel non-canonical EZH2 function as a chaperone to mediate box C/D snoRNP assembly

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Role: Presenting Author

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Disclosure:

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Role: Author

Disclosure:

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Role: Author

Disclosure:

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Disclosure:

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Disclosure: Nothing to Disclose

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Role: Author

Disclosure:

Disclosure Status: Complete

Disclosure: Nothing to Disclose

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No financial relationships or conflicts of interest.

Basic Mechanisms in Urologic Disease

Introduction & Objective

Overactivated ribosome biogenesis is a common feature of prostate cancer. However, the underlying molecular mechanism remains elusive. EZH2 is a histone methyltransferase and a well-known oncogene of prostate cancer. Deciphering the link between EZH2 and ribosome biogenesis in prostate cancer may provide critical means to overcome this devastating disease.

Methods

We use co-immunoprecipitation, GST pulldown and immunostaining to investigate the interacting partners of EZH2. ChIP-qPCR, RIP-qPCR and Northern Blot assays were performed to discover the role of EZH2 in rRNA transcription and processing. Immunohistochemistry and Western Blot were utilized to examine the function of EZH2 in cancer-related translation control.

Results

We identified a direct interaction between EZH2 and FBL in the nucleolus. Suppression of EZH2 affected ribosome biogenesis in three aspects: increased transcription of pre-rRNA, disorder of rRNA processing and alteration of rRNA methylation levels. Whereas EZH2 regulates pre-rRNA transcription in a PRC2-dependent way, the other two consequences were found to be SET domain-independent and closely related to the impaired functions of FBL. We further demonstrated that EZH2 strengthens the FBL-NOP56 interaction and thus facilitates the assembly of snoRNP complex. Additionally, by targeting FBL, EZH2 exerts its function in translational control and activates internal ribosome entry site (IRES)-dependent translation of key oncogenes including XIAP in

prostate cancer.

Conclusions

Our study uncovers a novel non-canonical role of EZH2 to orchestrate epigenetic regulation and translational control in prostate cancer.

Funding and Keywords

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Repression of Transcription Factor AP-2 Alpha by PPAR gamma Reveals a Novel Transcriptional Circuit in Basal- squamous Bladder Cancer

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Repression of Transcription Factor AP-2 Alpha by PPARγ Reveals a Novel Transcriptional Circuit in Basal-squamous Bladder Cancer

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Background: The discovery of bladder cancer transcriptional subtypes provides an opportunity to identify high risk patients, and tailor disease management. Recent studies suggest tumor heterogeneity contributes to regional differences in molecular subtype within the tumor, as well as during progression and following treatment. Nonetheless, the transcriptional drivers of the aggressive basal-squamous subtype remain unidentified. As PPARχ has been repeatedly implicated in the luminal subtype of bladder cancer, we hypothesized inactivation of this transcriptional master regulator during progression results in increased expression of basal-squamous specific transcription factors (TFs) which act to drive aggressive behavior. In this study, our purpose is to identify basal TFs repressed by luminal driver PPARγ.

Methods: We initiated a pharmacologic and RNA-seq-based screen to identify PPARχ-repressed, basal-squamous specific TFs.

Results: Hierarchical clustering of RNA-seq data following treatment of 3 human bladder cancer cells with a PPARχ agonist identified a number of TFs regulated by PPARχ activation, several of which are implicated in urothelial and squamous differentiation. Among these TFs, we show TFAP2A and its paralog TFAP2C are overexpressed in basal-squamous bladder cancer and in squamous areas of cystectomy samples, and that these overexpression is associated with increased lymph node metastasis and distant recurrence, respectively. Biochemical analysis confirmed the ability of PPARɣ activation to repress TFAP2A, while knockdown by siPPARγ and PPARχ antagonist studies indicate the requirement of a functional receptor. *In vivo* tissue recombination studies show TFAP2A and TFAP2C promote tumor growth in line with the aggressive nature of basal-squamous bladder cancer.

Conclusions: Activation of PPARγ (Luminal subtype driver) represses TFAP2A expression overexpressed in basal subtype of human bladder cancer. TFAP2A and its palalog TFAP2C are associated with basal-squamous bladder cancer and both TFs play a role to promote cell proliferation, migration, invasion as well as tumorigenesis. This study identified basal subtype TFAP2A repressed by luminal subtype driver PPARγ in human bladder cancer. This finding suggests the potential mechanism of the transcriptional interplay between molecular subtypes of human bladder cancer.

N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer

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N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer

Background: Despite the development of highly effective androgen receptor (AR)-directed therapies, nearly 37% of prostate cancer patients develop resistance. A further third of these men develop aggressive neuroendocrine prostate cancer (NEPC) for which no effective therapies exist. Lineage plasticity, a process by which differentiated cells lose their identity and acquire an alternative lineage phenotype, has been proposed as a mechanism of resistance to targeted therapies, however, the molecular programs underlying this transformation are poorly understood. We observed that the majority of NEPC and 20% of castration-resistant prostate cancer (CRPC) aberrantly overexpress the transcription factor *MYCN* (N-Myc). Despite this frequent occurrence, the role of N- Myc in driving lineage plasticity and the epigenetic mechanisms which regulate disease progression remain to be elucidated.

Methods: We analyzed overall survival and whole transcriptome data from a cohort of over 200 prostate cancer patients. We also assessed epigenetic modifications along with the N-Myc transcriptome, cistrome and chromatin-bound interactome by performing ChIP-seq, RNA-seq and RIME in a combination of mouse models, human prostate cancer cell lines, and NEPC patient-derived organoids following acute and chronic androgen withdrawal. Finally, we used CRISPR-based approaches to modulate the expression of N-Myc-interacting proteins to assess changes in chromatin accessibility by ATAC-seq.

Results: Expression of N-Myc is correlated with reduced overall survival and NEPC tumors are significantly enriched for stem cell and neural lineage-defining genes. The N-Myc cistrome is androgen-dependent and drives a transcriptional program leading to epithelial plasticity and the acquisition of clinically relevant neural lineage markers. N-Myc interacts with the known AR co-factors HOXB13 and FOXA1 at neural lineage genes. Interestingly, histone marks at these N-Myc-bound, neural lineage genes are epigenetically reprogrammed by EZH2 and can accurately classify prostate cancer patients in our cohort. Finally, chromatin accessibility is altered by N-Myc in a chromobox family-dependent manner, leading to deregulation of gene expression.

Conclusions: We describe a functional role for N-Myc in driving NEPC, characterized by changes in the N-Myc cistrome and interacting co-factors, as well as reprogramming of the epigenome in an androgen context-dependent manner. Ongoing studies are addressing tumor heterogeneity during lineage plasticity using single cell-based RNA-seq and ATAC-seq approaches and will identify novel actionable targets for future therapeutic interventions.

Fibroblast growth factor receptor 1 in reprograming cell metabolism in prostate cancer cells

Fen Wang, Ph. D., Texas A&M University
Ms. Yuepeng Ke, Mr. Ziying Liu, Dr. Sheng Pan

Fibroblast growth factor receptor 1 in reprograming cell metabolism in prostate cancer cells

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Background:

Metabolic reprograming from oxidative phosphorylation to aerobic glycolysis is a common event in cancer progression. Although glycolysis is less efficient than oxidative phosphorylation for providing energy with respect to the number of ATP per glucose, it meets the demand of rapidly growing cancer cells for building blocks. In addition, increased glycolysis results in glucose deprivation and lactate accumulation in the tumor microenvironment, which suppresses lymphocyte infiltration and compromises anti-immunotherapies. In line with those effects, the glycolytic phenotype is associated with prostate cancer (PCa) progression and aggressiveness. Understanding of how PCa cells reprogram their metabolism may provide a novel approach to selectively inhibit aerobic glycolysis in the cells. Fibroblast growth factor (FGF) signaling in which FGF and FGF receptor (FGFR) are partitioned between the epithelial and stromal compartments. That precisely balanced communication is critical for preserving the tissue homeostasis and function of the prostate. There is extensive evidence that ectopic activation of the FGF/FGFR signaling axis are associated with PCa development and progression. Among them, loss of resident FGFR2 and acquire of ectopic FGFR1 are the most frequently found associated with PCa progression although the detailed molecular mechanism remains not well known

Methods:

The FGFR1 alleles in DU145 cells were ablated. The glycolysis, oxidative phosphorylation, and ATP production profiles were measured with a Seahorse Analyzer, production of lactate was determined with a lactate detection kit, the phosphorylated peptide profiles were characterized with quantitative mass spectrometry.

Results:

Ablation of FGFR1 in DU145 cells significantly reduced ATP production via glycolysis and increase ATP production via oxidative phosphorylation. It also reduced lactate production of the cells. Unbiased mass-spectrometry based proteomic assay also showed that ablation of FGFR1 in PCa cells changed the profile of protein expression and posttranslational modifications.

Conclusions:

Our results indicate that ectopic FGFR1 expression reprograms the energy metabolism of PCa cells, representing a hallmark change in PCa progression.

Identification of cognate proximal cell types of the mouse and human prostate and their enrichment in human Benign Prostatic Hyperplasia

Diya Binoy Joseph, UT Southwestern Medical Center

2019 Travel Award Winner

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Identification of cognate proximal cell types of the mouse and human prostate and their enrichment in human Benign Prostatic Hyperplasia

Background: Benign prostatic hyperplasia (BPH) is highly prevalent in aging men and poses a significant healthcare burden associated with the treatment of Lower Urinary Tract Symptoms (LUTS). BPH/LUTS remain difficult to treat because of phenotypic heterogeneity, resulting in the need for surgical intervention. The cell types that originate BPH growth are not known. Anatomical studies have shown that the transition zone located near the urethra is the site of BPH growth. We previously identified two novel cell types, club and hillock, that are enriched in the prostatic urethra and proximal ducts. Here, we identify a new proximal fibroblast in the human as well as cognate hillock epithelia and proximal fibroblasts in the mouse. We assess the contribution of each proximal cell type in human BPH, establishing a new paradigm for urethral epithelia and proximal fibroblasts in discrete BPH phenotypes.

Methods: We used an unbiased approach by single cell RNA-sequencing (scRNA-seq) to identify cognate cell types of the mouse and human prostate and develop new flow cytometry and IHC antibody panels to purify and locate each cell type. The frequency of proximal fibroblasts and urethral epithelia in BPH vs. normal human prostate was assessed using scRNA-seq, flow cytometry, and immunofluorescence.

Results: Hillock and club cell identity is established early and these cells extend into the proximal ducts of the adult prostate transition zone. Proximal fibroblasts surround the urethra and proximal ducts. BPH patients have increased club and hillock cells within glandular nodules compared to normal prostate tissue and proximal fibroblasts are increased in areas of peri-urethral fibrosis. Mouse scRNA-seq and IHC data confirmed the existence of cognate proximal fibroblasts and hillock epithelia, but club cells were not found.

Conclusions: Our results show that club and hillock epithelia of the urethra and proximal ducts are established before prostate budding and are enriched in BPH glandular nodules, suggesting a potential cellular origin for new prostate growth. We also identify the proximal fibroblast of the human as the cellular source of collagen deposition in prostatic fibrosis, setting up a new phenotype to target in patients with LUTS. We created a cellular atlas of the mouse urethra and proximal prostate, which will allow for the generation of specialized mouse models to trace urethral and proximal fibroblast lineages. The cell types of the proximal ducts could become novel targets for the treatment of BPH/LUTS.

PBRM1 mutation develops a tumor-favoring microenvironment in renal cell carcinoma

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Katie Wu, ph.D, M.D, Lei Li, Ph.D, M.D

PBRM1 mutation develops a tumor-favoring microenvironment in renal cell carcinoma

Shan Xu, Kaijie Wu, Lei Li

Background

Tumor microenvironment plays crucial roles in tumor development, and may lead to the failure of immunotherapy. Polybromo-1 (*PBRM1*) is the second most frequently mutated gene after Von Hippel-Lindau (*VHL)* in renal cell carcinoma (RCC), and loss of *PBRM1* was correlated with advanced tumor stage, high grade, and poor overall survival in the previous reported studies. However, the effect of *PBRM1* mutation on tumor microenvironmental characterization is poorly known.

Methods

In this study, the tumor microenvironmental infiltration patterns of RCC patients in the cancer genome atlas (TCGA) database were assessed by the estimation of stromal and immune cells in malignant tumors using expression data (ESTIMATE). Meanwhile, bioinformatics tools, such as, gene set enrichment analysis (GSEA), single sample gene set enrichment analysis (ssGSEA), and complex heatmap were used to analyze the characteristics and clinicopathologic features of RCC. Mast cell recruitment assay, clone and clonogenic assay, cell cycle analysis, ELISA assay, tube formation assay, and western blot assay were all applied to test the function in vitro.

Results

We discovered that RCC patients with *PBRM1* mutations were associated with inhibiting T-cell infiltration, infiltrating more resting mast cells and macrophage M2, speciously recruitment of more resting mast cells in tumor microenvironment, but

not with the *VHL* mutation status. Meanwhile, silenced *PBRM1* improved the capacity of mast cell recruitment, promoted angiogenesis, and enhanced RCC cell proliferation in 786-O and ACHN cells. Furthermore, the molecular mechanisms underlying mast cells infiltration in *PBRM1*-silenced cells were CCL5 and CCL5 evoked immune related pathways, including increased interferon-inflammatory signaling, interferon-gamma signaling, interferon-alpha signaling, and IL-6-JAK-STAT3. CCL5 amount, CCL5 secretion in condition mediums, and the recruitment capability of mast cells in 786-O and ACHN cells. The upregulated hypoxia and hypoxia inducible factor related processes were responsive for tumor angiogenesis and cell proliferation in RCC patients with *PBRM1* mutations.

Conclusion

PBRM1 mutation facilitates a tumor-favoring microenvironment and results in an aggressive tumor behavior. *PBRM1* mutation-CCL5-activated interferon-gamma signaling and IL-6-JAK-STAT3 pathways could explain why *PBRM1* status affects responsiveness to immune therapy efficacy. Therefore, our results help to interpret the responses of RCC to immunotherapies and provide new strategies for the treatment of cancers.

FKHD-Mutant FOXA1 Promotes Androgen Independence and Prostate Cancer Progression

Xiaodong Lu, PhD, Northwestern University

Bohan Xu, PhD; Bing Song, PhD; Jung Kim, PhD; Ming Hu, PhD; Jonathan C. Zhao, PhD; Jindan Yu, PhD

TITLE: FKHD-MUTANT FOXA1 PROMOTES ANDROGEN INDEPENDENCE AND PROSTATE CANCER PROGRESSION

Background: FOXA1, a member of the forkhead (FKHD) family transcription factor, is required for the proper differentiation of prostate epithelial cells during development and for the maintenance of the differentiated status in the adult prostate. Transgenic knockout of FOXA1 in adult prostate epithelial cells led to altered cell morphology, increased proliferation, and elevated expression of basal cell markers. Concordantly, FOXA1 is down-regulated in metastatic, castration-resistant prostate cancer (CRPC). We and others have previously reported that loss of FOXA1 not only leads to oncogenic AR reprogramming, but promotes epithelial-to-mesenchymal transition (EMT) of prostate cancer (PCa) cells. Recent genomic profiling of patient tumors have identified FOXA1 as a most frequently mutated genes in PCa, with a hotspot at its FKHD domain, which is required for its interaction with both DNA and the androgen receptor (AR). How these mutations contribute to PCa progression, however, remains unclear.

Methods: We performed RNA-seq and ChIP-seq of HA (FOXA1) and AR in prostate cancer cells with endogenous FOXA1 knockdown followed by rescue of WT FOXA1 or its cancer-associated mutations. Bioinformatics analyses were utilized to interrogate the genomic data. FOXA1 interactions with AR and DNA were futher determined by Co-IP and electrophoretic mobility shift assays (EMSA), respectively.

Results: We found that FKHD-mutant (FKHDm) FOXA1 have deficiency in DNA binding and thus greatly reduced cistrome. However, FKHDm FOXA1 exhibited increased ability to interact with AR protein, thereby preventing AR binding to DNA and reducing AR cistrome. Concordantly, these mutants caused a decrease in AR signaling and cell growth in the presence of androgen. However, under hormone-deprived conditions, FKHDm enhanced AR activities and induced PCa cell growth, likely due to their failure to bind DNA and dilute residual AR. Interestingly, we found that FKHDm binds to some new genomic sites that are not shared with the WT FOXA1 and regulate the expression of many pro-metastatic genes. The clinical relevance of our findings was validated in the TCGA dataset wherein we observed decreased AR signaling but enhanced EMT in PCa with FKHDm FOXA1.

Conclusion: Our studies revealed the mechanistic details by which FKHDm FOXA1 promotes PCa progression, characterized by changes in FOXA1 and AR chromatin binding. These altered cistromes conferred a unique transcriptome that favors androgen-independent growth and promotes PCa aggressiveness.

Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) is upregulated in PCa and promotes resistance to androgen deprivation therapy

Praveen Kumar Jaiswal, Postdoctoral Fellow, LSUHSC-Shreveport

Sweaty Koul, Kashyap Koul, Runhua Shi, Hari K Koul

Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) is upregulated in PCa and promotes resistance to androgen deprivation therapy

Praveen Kumar Jaiswal[1,4], Sweaty Koul[2,4], Kashyap Koul[3,4], Runhua Shi[4], Hari K Koul[1,4]

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Background: Cap-dependent translation, is essential to maintain high protein synthesis and translation of specific mRNAs responsible for various tumorigenic properties in cancer cells and as such offers a novel therapeutic target. Loss of PTEN, stimulation of PI3K/AKT signaling and therapy dependent decrease in 4EBP1 play important role in upregulation of cap-dependent translational reprograming in castration resistant prostate cancer (CRPC), suggesting that this pathway may offer new and novel treatment approach for the patients with CRPC and therapy resistance. An interaction between EIF4G1-EIF4E is critical for the formation of the EIF4F complex and initiation of cap-dependent translation. In the present study, we tested the effects of modulating EIF4G1 in CRPC/ENZ resistant prostate cancer.

Methods: EIF4G1 mRNA expression was analyzed in multiple clinical cohorts. EIF4G1 protein expression was evaluated using Human Prostate Cancer tissue microarray (TMA) by immunohistochemistry. Enzalutamide (ENZ) sensitive C4-2B cells and ENZ resistant C4-2B (C4-2B ENZR) cells were used in the current study. EIF4G1 knockdown by shRNA and EIF4G-EIF4E complex inhibitor (4EGI-1) were used to test the function of EIF4G1. *In-vitro* functional assays such as clonogenic, cell proliferation, cell viability, trans-well migration/invasion, and 3D prostasphere formation; were used to test the role of EIF4G1.

Results: We observed that 11% of the localized PCa patients, 16% of metastatic PCa patients and 59% of CRPC/Neuroendocrine PCa patients have genetic alteration in EIF4G1 such as amplification and mRNA up-regulation. Our analysis of EIF4G1 expression in TMA showed a graded increase in EIF4G1 protein levels as the disease advances. We found an elevated level of EIF4G1 protein in C4-2B ENZR cells as compared to parental C4-2B cells. Treatment with 4EGI-1 or knocking down of EIF4G1 impaired colony formation, 3D-prostasphere formation, cell migration/invasion, cell viability/proliferation suggesting a critical role of EIF4G1 in promotion of aggressive phenotype and therapy resistance. Treatment with 4EGI-1 inhibitor also resulted in decreased EIF4G1 protein levels in C4-2B ENZR cells. Moreover, knocking down EIF4G1 as well as treatment with 4EGI-1 inhibitor sensitized CRPC cells to ENZ treatment.

Conclusions: EIF4G1 plays a critical role in CRPC and ENZ resistance and may be a novel target for overcoming therapy resistance in prostate cancer.

NOX4 is upregulated during PCa progression and plays a key role in hypoxic survival of PCa cells Saikolappan Sankaralingam, LSUHSC-Shreveport

Binod Kumar, Sweaty Koul, Praveen Kumar Jaiswal, Hari K Koul

NOX4 is upregulated during PCa progression and plays a key role in hypoxic survival of PCa cells.

Saikolappan Sankaralingam[1], Binod Kumar[2], Sweaty Koul[3, 4], Praveen Kumar Jaiswal[1, 4], Hari K Koul[1, 4]

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Background: Hypoxic tumor microenvironment plays an important role in prostate cancer progression. ROS are generated during hypoxia and ROS have been shown to promote tumor progression. In this study, we evaluate the role of NOX4 isoform in PCa growth and progression during hypoxia.

Methods: We analyzed TCGA datasets for NOX4 expression in clinical samples of PCa. LNCaP, PC3 and RWPE1 cells were obtained from ATCC. HRLNCaP were derived from LNCaP cells by subjecting them to multiple cycles of H/R over six months. Where indicated, cells were subjected to 12h hypoxia. ROS were measured by CM-H_2DCFDA (H_2O_2) and dihydroethidium (superoxide) fluorescence. NOX4 knock out LNCaP cells were generated using CRISPR/Cas9. Protein and mRNA expression was measured by Western blot/Immunofluorescence, and real time-PCR respectively.

Results: Analysis of TCGA data revealed that NOX4 mRNA levels are significantly ($p < 1E-12$) elevated in PCa tissues as compared to normal prostate tissues, and there is a positive correlation with increasing Gleason grade. We observed that NOX4 protein as well as mRNA levels were increased in PCa cells in response to hypoxia. NOX4 knockout reduced the expression of HIF1α, VEGFA and decreased p38 mitogen activated protein kinase (p38 MAPK) activity. Furthermore, pharmacological inhibition of NOX4 as well as knockout of NOX4 gene impaired cell survival under hypoxia and decreased clonogenic activity.

Conclusions: Our results suggest that NOX4 plays a key role in survival of PCa during hypoxia and highlight a critical role for NOX4 in the pathogenesis of PCa.

Androgen-sensitive differential expression of cytokines and growth factors in primary BPH stromal cells and normal adjacent stromal cells

Wei Chen, Ph.D, University of Pittsburgh

Laura E. Pascal, Ph.D, Zhou Wang, Ph.D , Rajiv Dhir, MD, Uma Chandran, Ph.D, Zhou Wang, Ph.D, Alex Chang

Title: Androgen-sensitive differential expression of cytokines and growth factors in primary BPH stromal cells and normal adjacent stromal cells

Authors: Wei Chen, Laura E. Pascal, Rajiv Dhir, Uma Chandran, Alex Chang and Zhou Wang

Background: The prostate is an androgen-sensitive organ composed of glandular cells embedded in a surrounding stroma, and stromal AR and androgens play critical roles in prostatic development and adult homeostasis. Our preliminary data showed the stromal cells derived from BPH, but not stromal cells from normal adjacent prostate, are able to enhance prostatic epithelial cell proliferation in 3-demensional co-culture. Furthermore, androgens significantly increased the proliferation of prostatic epithelial cells in the presence of primary BPH stroma cells but not the paired normal stromal cells. However, the underlying mechanisms of this phenomenon are still unknown.

Method: Primary stromal cells were derived from human BPH specimens obtained through transurethral resection of the prostate (TURP). Cytokines arrays and RNA sequencing were utilized to characterize the androgen-responsive genes and cytokines in BPH primary stromal cells and paired normal adjacent stromal cells. Cytokines and androgen-responsive genes that are differentially expression between paired BPH stromal cells and normal prostatic stromal cells were validated in clinical BPH specimens via IHC and qPCR.

Results: Stromal cells derived from the BPH tissues secreted higher level of CCL family proteins (CCL8, CCL7, CCL11, CCL13 and CCL28), CXCL proteins (CXCL6, CXCL12), interleukins (IL6, IL7 and IL32), and growth factors than those from the paired normal adjacent tissues. RNA sequencing data showed that several cytokines and growth factors were up-regulated in BPH stromal cells after androgens stimulations but not in normal adjacent stromal cells, which are confirmed by qPCR.

Conclusions: These findings suggest that the primary stromal cells derived from BPH tissues expressed different cytokines and growth factors than normal adjacent stromal cells. Androgens have been shown to influence the expression of several genes including CXC and interleukins in BPH stromal cells, but not normal adjacent stromal cells. Our results suggest that androgen signaling in BPH stromal cells is dysregulated and could contribute to prostatic epithelial growth and provide a strong foundation to elucidate the mechanisms of androgen-dependent stromal regulation of epithelial cell growth in BPH.

Effect of new AR-V7 inhibitor in enzalutamide resistant prostate cancer

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Effect of new AR-V7 inhibitor in enzalutamide resistant prostate cancer

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ABSTRACT

Background: Castration resistance is the universal feature of lethal prostate cancer (CaP), secondary anti-androgen/hormone therapy (SAT) has emerged as the most widely used therapy for CRPC due to its ease of administration as well as low toxicity profile. However, SAT-resistance emerges relatively quickly and the clinical benefit is limited. For example, the anti-androgen enzalutamide has been reported to improve median overall survival by only 4.8 months in men with the approved indication of post-docetaxel CRPC. AR-V7 is an AR splice variant and has a truncated c-terminal region that includes the ligand binding domain. Due to this truncation, AR-V7 is constitutively active as a transcription factor even under androgen-deprived condition. Previous studies reported that AR-V7 expression is induced by ADT and correlates with resistance to both enzalutamide and abiraterone in CaP. Therefore, in this study, we studied the effect of new AR-V7 inhibitor in enzalutamide resistant prostate cancer.

Methods: We are attempting to discover PROteolysis TArgeting Chimeras (PROTACs) for Advanced Prostate Cancer targeted towards AR-V7, the most predominant variant of the Androgen Receptor (AR), which has been reported as a possible cause of resistance to Enzalutamide and Abiraterone. Furthermore, in the enzalutamide resistant prostate cancer cell (22Rv1-Enz^R) xenograft mice model, tumor direct injection of AR23 was performed.

Results: Treatment of AR-V7 inhibitor, AR23, decreased AR-V7 as well as full length AR (AR-FL). AR23 treatment also decreased cell growth of enzalutamide resistant prostate cancer cell line, 22Rv1-Enz^R. Tumor direct injection of AR23 attenuated 22Rv1-Enz^R growth in the xenograft mice model.

Conclusions: These findings suggest that AR-V7 inhibitor treatment can be effective therapeutic target when currently available AR antagonist fail.

Osteopontin exacerbates the inflammatory environment in the prostate

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Osteopontin exacerbates the inflammatory environment in the prostate

Background: Chronic inflammatory processes are thought to contribute to the development of lower urinary tract symptoms (LUTS) in elderly men either via stimulating proliferation and triggering benign prostatic hyperplasia (BPH) or by triggering fibrosis in the periurethral region. Approximately 30% of patients with LUTS are resistant to existing therapies and identifying the inflammatory processes provoking tissue remodeling are essential to develop more efficient treatments. Our study aims to identify the role of osteopontin (OPN), a cytokine and fibrosis-associated protein elevated in experimental prostatitis, in inflammation and tissue remodeling in the prostate. **Methods:** OPN expression was detected by immunohistochemistry in prostates from patients without medical therapy or treated with α-blockers, 5-α reductase inhibitors or both and undergone surgery to relieve LUTS (sBPH, n=30). Incidental BPH (iBPH) is from the transitional zone of prostates from radical prostatectomy for low volume, low grade prostate cancer (n=8). Slides were captured with a Nuance multispectral camera and segmented and scored with inForm software. Immortalized stromal (BHPrS-1) and epithelial (NHPrE-1 and BHPrE-1) cell lines were used to determine the secretion of OPN in response to recombinant IL-1β and TGF-β1 by ELISA and genes activated by OPN were identified by qPCR. **Results:** OPN expression was detected in both glandular and stromal cells, although, it was not significantly elevated in sBPH patients compared to iBPH. However, OPN positivity was significantly higher in sBPH prostates when highly fibrotic samples with atrophic glands were eliminated from the iBPH group (p=0.0099). Multiple splice variants of OPN are expressed in the cell lines and its secretion is stimulated by TGF-β1 in NHPrE-1

and both Il-1β and TGF-β1 in BHPrS-1 cells. Most interestingly, we observed an increase in the expression of inflammatory genes in response to OPN including *CXCL1*, *CXCL2*, *CXCL8*, *PTGS2* and *IL6* in BHPrS-1, but this effect was not replicated in epithelial cells. **Conclusions:** Elevated levels of OPN exacerbates inflammation by stimulating stromal cytokine production. Pharmacological inhibition of OPN may have multiple beneficial effects to relieve LUTS by repressing the inflammatory environment in the prostate.

Identification of genes that drive resistance to enzalutamide in castration-resistant prostate cancer cells Wisam Awadallah, Case Western Reserve University

Sarah Kohrt, Robert A. Philips, Renjie Jin, Xiuping Yu, Jianghong Zhang, Tom C. Case, Peter E. Clark, Robert J. Matsusik, Yajun Yi Philip D. Anderson, Magdalena M. Grabowska

Abstract category: Big Data for Discovery

Identification of genes that drive resistance to enzalutamide in castration-resistant prostate cancer cells

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Background: As the majority of prostate cancers are adenocarcinomas that express androgen receptor (AR), androgen deprivation is a mainstay of treatment for prostate cancer patients. Tumors initially respond to androgen deprivation therapy, but progress to castration-resistant prostate cancer (CRPC). The next line of treatment for CRPC patients are next-generation androgen deprivation therapies, like the potent anti-androgen enzalutamide. However, tumors eventually progress to enzalutamide-resistant CRPC. The goal of this study was to define novel targets for re-sensitizing CRPC to enzalutamide.
Methods: One third of a commercially available bar-coded lentiviral short hairpin (sh) RNA library (DECIPHER from Cellecta) was transduced into castration-resistant C4-2B cells, targeting approximately 5,000 genes. Cells were split into three groups: cells before treatment (representing the starting population of shRNAs), cells treated with vehicle control (DMSO), and cells treated with enzalutamide for six days. DNA was isolated and shRNA was quantified by sequencing. The ratio of the abundance of each shRNA in the enzalutamide group versus both control groups was calculated. A given shRNA was considered a ‘‘hit’’ if it showed at a least 2-fold abundance decrease relative to both vehicle control and initial sample. Candidate genes were then verified in C4-2B and 22RV1 CRPC cells by using duplicate short interfering (si) RNA constructs to knock down our genes of interest. Quantitative PCR (qPCR) and crystal violet cell survival assays were used to validate knockdown, changes in *AR,* and cell survival in response to enzalutamide.
Results: We identified 11 genes (*ACAT1, AR, CAD, CDC37, COL5A2, EIF6 GABRG2, MAP3K11, PSMD12, SPDEF, TFAP2C*) that when knocked out, increased sensitivity of C4-2B cells to enzalutamide. Of the genes identified, four validated *in vitro* via crystal violet cell survival assays: *ACAT1, PSMD12, MAP3K11*, and *AR*. Importantly, the knockdown construct targeting both full length AR and a constitutively active AR splice variant (AR-V7) was more efficient at inducing enzalutamide sensitivity. Knockdown of these four genes also showed changes in expression of *AR* and others of our 11 gene list.
Conclusions: Our studies have identified 11 genes that drive resistance to enzalutamide in prostate cancer cells *in vitro*. Four of these genes show promise after further validation. Ongoing studies are working to elucidate how these genes are driving resistance and if these genes drive resistance *in vivo*.

Role of Estrogens in Fibrosis and Myofibroblast Phenoconversion of Prostate Stromal Cells

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Role of Estrogens in Fibrosis and Myofibroblast Phenoconversion of Prostate Stromal Cells

Background:
Benign prostatic hyperplasia (BPH) is a non-malignant progressive disease characterized by the enlargement of the prostate. Approximately 90% of men over 80 years old have BPH. Men often develop lower urinary tract symptoms (LUTS) secondary to BPH, including urinary retention, increased frequency and nocturia. The causes of LUTS in BPH can be multifactorial, which include prostate proliferation, changes in smooth muscle contractility and increase in prostate fibrosis. Aging associated fibrotic changes in the tissue microenvironment can lead to disease in multiple organ systems. Of interest, CXCL12, a CXC-type chemokine commonly expressed with aging, has been shown to increase prostatic fibrosis. This may occur through the phenoconversion of fibroblasts to myofibroblasts, a high collagen secreting cell type that can accumulate in tissue. It is also known that sex steroid hormones, such as estrogens, are involved in prostate development and disease. However, the molecular link between estrogens and fibrosis is still elusive.

Methods:
The goal of this study was to assess whether estrogens affected CXCL12 expression and induced fibroblast to myofibroblast differentiation in prostate stromal cells *in vitro*. We treated prostate stromal cell lines (i.e. BHPrS1, WMPY1 and N1) with 17-β estradiol (E2). We performed RT-qPCR for common fibrosis-associated markers, such as collagen and smooth muscle actin. We also monitored fibroblast to myofibroblast phenoconversion by evaluating prostate stromal cells morphology and co-expression of smooth muscle actin and collagen by immunocytochemistry.

Results:
BHPrS1 cells showed an increase in COL1A1 and ACTA2 expression after E2 treatment. We anticipate observing an increase in those markers, as well as an increase in CXCL12 expression in our prostate stromal cells treated with E2. We also expect to observe morphological changes consistent with a myofibroblast phenotype in prostate stromal cells treated with E2.

Conclusions:
As men age, testosterone levels decrease while estrogen levels increase or remain constant. This suggests that estrogens may play a role upon aging in BPH/LUTS. These results would support the concept that the aging associated changes in steroid hormones can influence prostate fibrosis. A better understanding of these mechanisms may provide a more targeted approach for personalized treatment of BPH/LUTS.

Novel role of ketone body metabolism in acquired gemcitabine resistance

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Novel role of ketone body metabolism in acquired gemcitabine resistance.

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Background: Urinary bladder cancer accounts for 81,000 new cases per year and 4.7% of the new cancer diagnosis in the US. Even though most of the patients suffer from non-invasive lesions, around 70% will recur following surgical intervention. Chemotherapy with gemcitabine in combination with cisplatin is a common treatment option for recurrent disease, however 60% of the patients will relapse due to resistance. Gemcitabine is a synthetic pyrimidine nucleoside prodrug, without a known mechanism for resistance. In examining mechanisms of resistance, we identified ketone body metabolism to be important. We found that OXCT1, a rate-limiting enzyme in ketone metabolism, was highly associated with bladder cancer death Hoglund et al. $P = 2.3x10^{-3}$ and TCGA, $P = 6.5x10^{-4}$. **Methods:** We measured the expression of OXCT1 in RT4, UMUC3, T24 and 5637 bladder cancer cell lines and observed that expression correlated with gemcitabine resistance. We generated gemcitabine resistant 5637 cells (5637GR) with continuous exposure to the drug. This caused the elevation of OXCT1 expression, as well as PGC1a and SIRT1 involved in mitochondrial biogenesis. Using CRISPR/Cas9 we were able to knockout OXCT1 to further study the role of this gene. **Results:** Removal of OXCT1 was able to sensitize inherently resistant UMUC3 and 5637GR lines. Given that OXCT1 is a rate limiting enzyme in ketone metabolism, we evaluated oxygen consumption rate among parental and resistant cells with Seahorse. Surprisingly we found that resistant cells have higher oxygen consumption rate when exposed to the drug. Additionally, we evaluated the role OXCT1 has on cancer stemness through sphere forming assays (hanging drop and forced floating methods) to reveal that the OXCT1-KO lines had reduced spheres compared to their parental lines. We identified bone morphogenic protein (BMP) signaling to be induced in promoting SOX9 expression, in OXTC1-dependent manner. Gene expression analysis further corroborated the novel role of ketone metabolism in stemness and therapeutic resistance. **Conclusions:** OXCT1 expression is important in response to Gemcitabine and it may have an undiscovered non-metabolic role affecting stemness. Importantly, OXCT1 is an enzyme and a potential “druggable” target which may provide better outcomes to Gem + Cisplatin combination therapy.

Tumor Microenvironment Characterization in Bladder Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures

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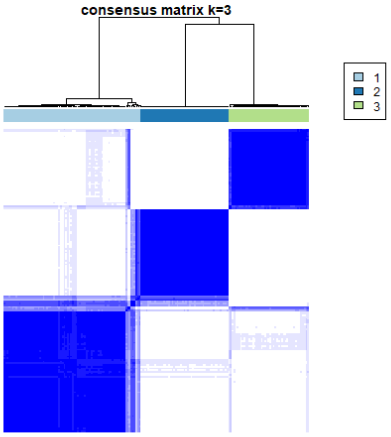
Jin Zeng, Kaijie Wu, Yule Chen, Shan Xu

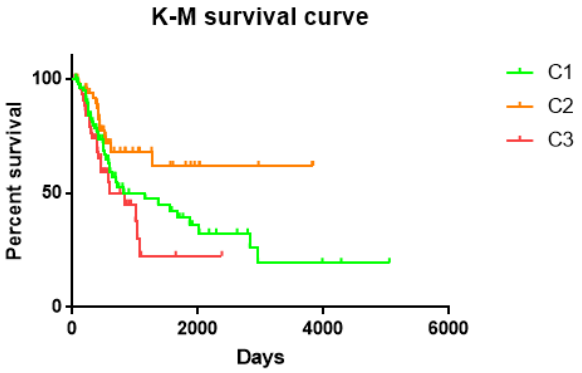
Background: The tumor microenvironment of bladder cancer plays an important role in tumor growth, invasion and metastasis. The purpose of this study is to identify the immune molecular subtypes of bladder cancer, so as to establish a new method for the immunotherapy and prognosis prediction of bladder cancer.

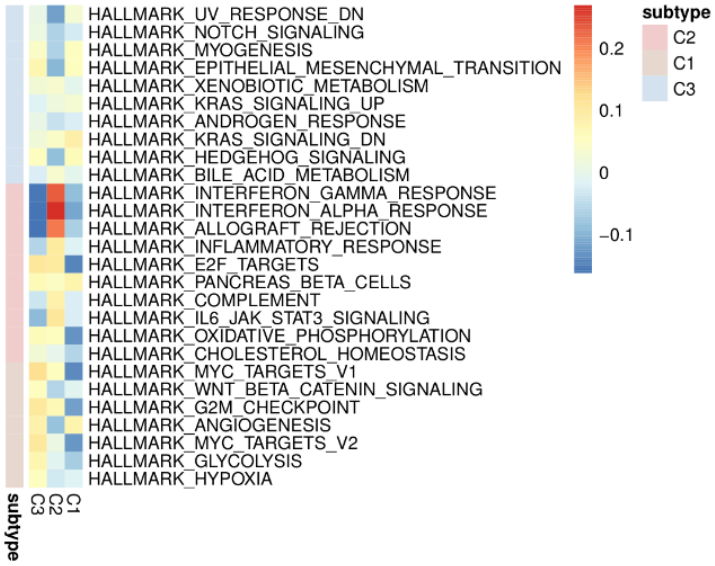
Methods: We used RNA-seq data of bladder cancer in TCGA database to estimate the relative proportion of 22 immune cells in tumor by CIBERSORT algorithm and LM22 gene set, and then carried out unsupervised k-means clustering, k-m survival curve analysis, muti-edgeR differentially expressed genes analysis and muti-GSEA analysis to find tumor immune molecular subtypes and characteristic genes.

Results: We identified three immune subtypes and a number of characteristic genes that can determine the immune characteristics and immunotherapy of bladder cancer.

Conclusions: By using RNA-seq data and CIBERSORT algorithm, we calculated the relative proportion of tumor cell invasion, and identified three immune subtypes and some characteristic genes through clustering and difference analysis, which can predict the prognosis of patients and the effect of immunotherapy, providing a new direction for the treatment of bladder cancer.







SPINK1 is associated with androgen independence in prostate cancer cells

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BACKGROUND

Prostate cancer (PCa) remains the second leading cause of cancer-related mortality in males in the US. Preliminary RNA-seq data from our laboratory discovered SPINK1 to be one of the most differentially expressed genes enriched in PCa stem cells compared to non-diseased progenitor population. We sought to investigate its role in disease progression in a prostate cancer stem-like cell line, HuSLC.

METHODS

Human RNA-seq data sets were queried for differential expression of SPINK1 in metastatic and localized PCa. Stable knockdown of *SPINK1* in HusLC cells using lentivirus was utilized to study its effects on proliferation (MTT assay), migration (scratch assay), stemness (prostasphere assay, RT-PCR), and subcutaneous xenograft formation and serial propagation in the presence or absence of androgens.

RESULTS

Analysis of RNA-seq data set of annotated human PCa metastasis from Stand Up to Cancer and Johns Hopkins University found an increase in SPINK1 mRNA expression compared with localized tumors suggesting disease stage-specific functions for SPINK1. SPINK1 mRNA levels were increased in HuSLC prostaspheres compared to HuSLC cells cultured in monolayer suggesting a role in maintenance of a progenitor population. Knockdown of SPINK1 (SPINK1-KD) however did not change the sphere-forming capacity by size or number but did decrease stemness genes Nanog, SOX2, and OCT4 again suggesting a role in affecting stemness. Knockdown of SPINK1 decreased migration and proliferation of HuSLC in monolayer suggesting a decrease in SPINK1 is associated with a less aggressive PCa phenotype. Subcutaneous xenografts developed in non-castrate nude mice and with supplemental exogenous testosterone did not reveal a difference in tumor weight or with serial transplantation. SPINK1-KD xenografts however were grossly more secretory (PSA-positive by Western blot) compared to control HuSLC xenografts suggesting an increased responsiveness to androgens. Indeed, when the knock-down and control xenografts were cultured in castrate conditions, SPINK1-KD xenografts were drastically smaller than control HuSLC suggesting that SPINK1 expression is associated with conference of androgen independence in this PCa stem-like cell line.

CONCLUSIONS

The clinical significance of SPINK1 in PCa remains controversial. However, taken together, our results present supporting evidence to suggest a role for SPINK1 in conferring androgen independence in the progression of PCa disease with implications for targeted treatment in PCa stem or stem-like cells.

Ferroptosis induction as a novel therapeutic approach for advanced prostate cancer

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Title: Ferroptosis induction as a novel therapeutic approach for advanced prostate cancer

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Background

For most advanced prostate cancers (PC) treated with hormonal therapy, response is transient and the majority of patients will progress towards castration-resistant prostate cancer (CRPC), which is largely responsible for PC associated death. *Ferroptosis* is a type of programmed cell death characterized by the accumulation of iron dependent lipid reactive oxygen species (ROS) in cells, a mechanism distinct from traditional apoptosis and necrosis. Ferroptosis is induced by inhibiting two mediators, glutathione peroxidase 4 (GPX4) or SLC7A11 which results in accumulation of intracellular ROS. In this study, we evaluated the therapeutic potential of ferroptosis induction as a novel therapeutic strategy for advanced PC.

Methods

Erastin (SLC7A11 inhibitor) and RSL3 (GPX4 inhibitor) were used to induce ferroptosis in the PC cell lines DU145, PC3, ARCaP, C4-2, LNCaP, 22Rv1, and neuroendocrine prostate cancer cells (NEPC) H660. We determined the vulnerability of PC cell lines to ferroptosis induction by measuring viability, colony formation, and migration ability in vitro. In vivo, the therapeutic potential of ferroptosis induction was evaluated using xenograft tumor models treated with erastin. To assess the synergic therapeutic potential of second-generation anti-androgens (enzalutamide and abiraterone) with ferroptosis inducers on PC cells (C4-2 and LNCaP cell lines) we performed in vitro colony formation and migration, and in vivo xenografts.

Results

We analyzed the expression of SLC7A11 and GPX4 using The Cancer Genome Atlas Prostate Cancer dataset and demonstrated that SLC7A11 and GPX4 have significantly higher expression in PC. Moreover, patient-derived xenograft models derived from metastatic PC showed high levels of SLC7A11 and GPX4 expression at protein level. PC cells are vulnerable to the ferroptosis inducers erastin and RSL3 in vitro and in vivo. Both ferroptosis inducers significantly reduced PC cell viability, colony formation, and migration in vitro. Ferroptosis induction by erastin treatment delayed PC growth using DU145, PC3, ARCaP, and H660 xenograft models. Ferroptosis inducers also strongly synergize with second-generation anti-androgens in inhibiting PC cell growth and migration in vitro and in vivo xenografts.

Conclusions

Our results suggest that induction of ferroptosis could represent a new therapeutic strategy for CRPC and NEPC as a monotherapy or in combination with second-generation anti-androgens.

ONECUT2 and its Extremely Long 3‚Äô-Non-coding Region Cooperate To Drive Aggressive Prostate Cancer

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ONECUT2 and its Extremely Long 3'-Non-coding Region Cooperate To Drive Aggressive Prostate Cancer

Background: We recently reported that the transcription factor ONECUT2 (OC2) is a master regulator operating in mCRPC that suppresses AR activity and promotes neural differentiation and tumor cell survival (Rotinen et al. *Nat Med* 2018). OC2 mRNA possesses an unusually long (14,574 nt), evolutionarily conserved 3'-untranslated region (3'-UTR) with many microRNA binding sites, including up to 26 miR-9 sites. This is notable because mir-9 targets many of the same neurogenic genes as the OC2 protein. Paradoxically, OC2 expression is highest in tissues with high miR-9 expression. The length and complex secondary structure of the OC2 mRNA suggests it may serve as a potent master competing endogenous RNA (ceRNA) by sequestering miRNAs. Here we describe a novel role for the OC2 3'-UTR in lethal prostate cancer consistent with a function as a ceRNA.

Methods: Bioinformatics strategies, enforced expression, CRISPR, microarray, 3'-RACE and bead capture of mRNA were used. OC2-driven phenotypes were assessed using luciferase activity, proliferation, invasion, migration, colony forming and anoikis assays.

Results: A plausible ceRNA network in OC2-driven tumors was constructed computationally using prostate, neuroblastoma and non-small cell lung cancers. This network suggests that the OC2 3'-UTR activates neurogenic genes regulated by miR-9 and miR-124. 3'-RACE and RNA-seq data confirmed that the full-length OC2 mRNA is expressed in prostate cancer. Bead capture of the 3'-UTR demonstrated miR-9 binding to the OC2 mRNA, and PCR confirmed OC2 mRNA is highly insensitive to miR-9-targeted degradation. Overexpression of OC2 3'-UTR absent the protein coding region drove strong expression of mRNAs targeted by miR-9 and miR-124. Likewise, expression of OC2 3'-UTR alone (without protein) increased metastatic potential. Genes regulated by the OC2 3'-UTR exhibited more than 40% overlap with genes driven by overexpression of the OC2 protein in the absence of the 3'-UTR, indicating a cooperative functional relationship between the OC2 protein and its 3'-UTR. Both gene sets are enriched for neuronal differentiation and show increased Polycomb Repressive Complex activity.

Conclusion: In addition to the transcriptional role played by the *ONECUT2* gene, the 3'-UTR appears to function independently as a ceRNA. The overlapping induced networks suggest an evolutionarily conserved mechanism to reinforce OC2 transcription by protection of OC2-regulated mRNAs from miRNA suppression. These coordinate mechanisms appear to operate in ~ 1/3 of mCRPC.

Notch3 promotes Prostate Cancer-Induced Bone Lesion Development by Modulating the Bone Microenvironment via MMP-3.

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Notch3 promotes Prostate Cancer-Induced Bone Lesion Development by Modulating the Bone Microenvironment via MMP-3.

Background:

Prostate cancer metastases primarily localize in the bone where they induce a unique osteoblastic response. Notch signaling is known to be dysregulated in PCa, but its role in PCa-induced bone metastasis is not known. Better understanding of the host/tumor interactions that trigger and drive metastatic processes could provide additional avenues for therapeutic intervention.

Methods:

PCa cells, in which Notch3 expression was manipulated, were injected into the tibiae of SCID mice. Development of bone lesions was monitored by x-ray and measured using Metamorph software. The tibiae were harvested at end time points for histological analyses and qRT-PCR. Cultured bone marrow from naïve mice was used for *in vitro* differentiation of osteoblasts or osteoclasts in the presence or absence of conditioned medium from cancer cells in which Notch3 expression was manipulated. Proliferation of osteoblasts or osteoclasts was read out by MTT assay and differentiation was read out by ALP or TRAP staining, respectively. TMA containing patients visceral and bone metastases was analyzed by IHC.

Results:

PCa cell lines that induce mixed/osteoblastic lesions in bone expressed 5-6 times more Notch3, than tumor cells that produce osteolytic lesions. Over-expression of active Notch3 (NICD3) in osteolytic tumors reduced lytic lesion area and enhanced expression of osteoblastogenic markers. Loss of Notch3 in osteoblastic tumors enhanced osteolytic lesion area and decreased osteoblastogenic markers. This was accompanied by a respective decrease and increase in the number of osteoclasts and osteoblasts at the tumor-bone interface, without any effect on tumor proliferation.

Conditioned medium from NICD3-expressing cells enhanced osteoblast differentiation and proliferation in vitro, while simultaneously inhibiting osteoclastogenesis. MMP-3 was specifically elevated in NICD3-expressing tumors, and inhibition of MMP-3 rescued the NICD3-induced block in osteolytic lesions, suppressed osteoblast proliferation in vitro, and stimulated osteoclastogenesis. Clinical osteoblastic bone metastasis samples had higher levels of Notch3 and MMP-3 compared to patient matched visceral metastases or osteolytic metastasis samples. Furthermore, PC3-NICD3 conditioned media induced the expression of Jagged-1 ligand in mouse bone marrow derived-osteoblasts cells and Jagged-1 induced osteoblastogenesis.

Conclusions:

These studies define a new role for Notch3 in manipulating the tumor microenvironment in bone metastases.

CDK4/6 Pathway as Therapeutic Target for Bladder Cancer Ai-Hong Ma, Ph.D., University of California Davis

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CDK4/6 Pathway as Therapeutic Target for Bladder Cancer

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Abstract

Background: Perturbation of the CDK4/6 pathway is frequently observed in advanced bladder cancer. We investigated the potential of targeting this pathway alone or in combination with chemotherapy as a therapeutic approach for the treatment of bladder cancer

Methods: The genetic alterations of the CDK4/6 pathway in bladder cancer was first analyzed with The Cancer Genome Atlas (TCGA) database and validated in our bladder cancer patient-

derived tumor xenografts (PDXs). Bladder cancer cell lines and mice carrying PDXs with the CDK4/6 pathway perturbations were treated with a CDK4/6 inhibitor palbociclib to determine its anti-cancer activity and the underlying mechanisms. The combination index method was performed to assess palbociclib and gemcitabine drug-drug interactions.

Results: Through analyzing the TCGA database, we found that 79.2% of bladder cancers harbor alterations along the cell cycle regulation pathway. Palbociclib induced G0/G1cell cycle arrest but with minimal apoptosis in vitro. In mice carrying PDXs, palbociclib treatment reduced tumor growth and prolonged survival from 14 days to 32 days compared to vehicle only controls ($p=0.0001$). Palbociclib treatment was associated with decrease in Rb phosphorylation in both cell lines and PDXs. Palbociclib and gemcitabine exhibited antagonistic cytotoxicity *in vitro* (CI >3) and in vivo.

Conclusions: The CDK4/6 pathway is feasible as a potential target for the treatment of bladder cancer. A CDK4/6 inhibitor should not be combined with gemcitabine.

PBM nano-formulation inhibits hedgehog signaling in docetaxel-resistant prostate cancer

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PBM nano-formulation inhibits hedgehog signaling in docetaxel-resistant prostate cancer

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Background: Docetaxel (DTX) remains the preferred choice of treatment for advanced prostate cancer (PCa), but modest drug responses, systemic toxicities, and drug resistance limit their efficacy. Among several complex signaling pathways studied, an aberrant change in Hedgehog signaling (Hh) is found to be involved in progression, invasion, and metastasis and even therapy-resistance of PCa. Therefore, there is an increasing demand for alternatives for effective chemotherapeutic treatment of multi-drug resistant PCa cells. In our study, we determined the utility of natural compound thymoquinone (TQ)-loaded biodegradable PBM- nanoparticles coated with polycaprolactone-polyethylene glycol copolymers conjugated to prostate-specific membrane antigen (PSMA) aptamer to target drug-resistant cells.

Methods: We generated a novel planetary ball milled nanoparticles (PBM-NPs) that use a natural polysaccharide (starch; FDA approved) to create a drug-polysaccharide nanoparticle as a core, subsequently coated with PSMA Aptamer (A10)-conjugated poly (ε-caprolactone) / poly (ethylene glycol) copolymer, encapsulated with TQ. For the study, DTX resistant cells (C4-2B-R and LNCaP-R) were generated from the parental C4-2B and LNCaP cells. Cell proliferation was estimated by MTT assay treated with PBM-NP (A10-TQ-NP). Immunohistochemistry, western blots were used to investigate the expression level of Shh signaling markers.

Results: Treatment of PCa cells (PC3-R and C4-2B-R) with A10-TQ-PBM-NPs have shown its cytotoxic effect and was found effectual as confirmed through cell viability tests such as MTT assay. Also, decreased expression of P-gp efflux protein and increased apoptosis confirmed the effective cytotoxicity of A10-TQ-PBM-NPs in both PCa cell lines. Further, tissue microarray analysis showed an elevated expression of Shh in PCa patients. The qRT-PCR and western blot assays showed a significant decline in the expression levels of Sonic hedgehog (Shh), Patched 1(PTCH1), and Glioma-associated oncogene homolog 1 (Gli1) in A10-TQ-PBM-NPs treated PCa cells, while upregulated in control cells.

Conclusions: Our findings unveil a novel and effective treatment strategy for the use of PBM-NPs to inhibit the Hh signaling pathway and suppress invasion and metastasis in PCa, thereby improve the quality of life of patients.

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The Role of SOX2 in Promoting Enzalutamide Resistance in Castration-Resistant Prostate Cancer

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The Role of SOX2 in Promoting Enzalutamide Resistance in Castration-Resistant Prostate Cancer

Background

Despite potent inhibition of AR pathway activation, many patients develop castration-resistant prostate cancer (CRPC). Second-line therapies such as enzalutamide have increased overall survival in CRPC. However, resistance to these therapies inevitably emerges, suggesting other pathways apart from AR signalling are contributing to the failure of treatments. We and others have previously demonstrated that *SOX2* [SRY (sex determining region Y)-box 2] is an AR-repressed gene that is found in a large percentage of high Gleason grade prostate tumours, as well as in most metastases. Additionally, expression of SOX2 in a castration sensitive cell line is sufficient to enable tumour formation *in* vivo.

Methods

We utilized chromatin immunoprecipitation (ChIP) to identify genes bound by SOX2 in prostate cancer and embryonic stem cells. Further, we also performed transcriptome sequencing (RNA-seq) on SOX2 expressing CWR-R1 cells and CRISPR-Cas9 edited SOX2 knockout cells in both vehicle and enzalutamide treated conditions. Phenotypic analysis includes cell cycle distribution, BrdU incorporation, Seahorse Metabolic assays and growth assays.

Results

Our results indicate that SOX2 binds distinct sets of in prostate cancer compared to embryonic stem cells, suggesting SOX2 in prostate cancer cells regulates different genes and potentially functions in a different manner. Phenotypic characterization of SOX2 reveals that SOX2 knockout leads to decreased growth over time and this phenotype is enhanced with enzalutamide treatment. Further, enzalutamide treatment leads to a G_1 arrest in the SOX2 knockout cells. SOX2 knockout also leads to decreased migration of cells. Metabolic assays reveal SOX2 expressing cells have an increased basal rate of glycolysis, and these cells are more capable of responding to increased energetic demand during mitochondrial respiration. Following enzalutamide treatment, gene set enrichment analysis indicates an enrichment of genes involved with cell cycle transition and DNA damage. Protein analysis indicates a decrease in the level of key cell cycle progression and DNA repair proteins, while showing increased levels of DNA damage response proteins.

Conclusions

SOX2 appears to have a different function in castration-resistant prostate cancer, and drives proliferation and migration through metabolic, cell cycle, and DNA damage response pathways. A potential implication of this research could suggest using SOX2 as a biomarker for determining which patients might respond better to radiation therapy.

The effects of autoimmune inflammation on androgen receptor signaling in adult prostate stem cells

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Title: The effects of autoimmune inflammation on androgen receptor signaling in adult prostate stem cells

Background: Inflammation and benign prostate hyperplasia (BPH) are strongly correlated, with up to 70% of BPH cases exhibiting inflammation. Adult prostate stem cells (PSC) have also been implicated in hyperplastic disease; however, the impact of prostatitis on PSC is not fully understood. We previously identified an inflammation-driven increase in *in vivo* PSC proliferation and *ex vivo* differentiation of the resulting organoids. Since the androgen receptor (AR) is a major driver of prostate development and differentiation, we hypothesized that inflammation affects AR signaling in the PSC.

Methods: To induce inflammation, ovalbumin-targeting T-cells from Rag1-/- Thy1.1+ OT-I are activated *ex vivo* and adoptively transferred to POET-3 mice. The resulting epithelial and stromal hyperplasia closely resemble human autoimmune diease. Prostates from inflamed and naïve, castrate and intact POET-3 mice are harvested, digested, and separated by FACS to isolate an enriched PSC population defined as lineage$^-$ (CD45$^-$ CD31$^-$), stem cell antigen-1$^+$, CD49f$^+$ (LSC). These are then cultured under 3D androgen-free conditions for further analysis.

Results: Data presented herein demonstrate a novel finding that inflammation increases AR and its target genes in the LSC and resulting organoids in a manner independent of exogenous androgens. Notably, organoids grown from PSC derived from inflamed POET-3 mice maintain increased AR and AR target genes and AR-dependent proliferation, and differentiation despite the absence of androgen in culture.

Conclusions: These results establish a novel link between inflammation and persistent androgen-independent AR signaling, which may contribute to treatment-resistant hyperplastic disease.

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Keywords: Prostate, stem cells, inflammation, androgen receptor

Age-related Increased Incidence of Prostate Cancer was Revealed by a Spatially and Temporally Controlled Prostate-Specific Pten Knockout Mouse Model Generated through Adenovirus-Assisted In vivo Approach

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Age-related Increased Incidence of Prostate Cancer was Revealed by a Spatially and Temporally Controlled Prostate-Specific Pten Knockout Mouse Model Generated through Adenovirus-Assisted *In vivo* Approach

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Background

Prostate cancer (PCa) is associated with advanced age. To investigate the role of age in PCa, it would be advantageous to develop a spatially and temporally controlled PCa mouse model at different ages. PTEN is one of the most frequently mutated tumor suppressor genes in human cancers. *Pten* conditional knockout (KO) mouse model has become one established preclinical model for PCa. However, there is a limitation to use this model to study the effects of aging on PCa. As *Pten* deletion in this model is triggered in the 2-week-old prostate, when comparing the onset of PCa between aged and non-aged mice, it is difficult to distinguish whether the onset of PCa is due to the acceleration of the normal aging process or due to the manifestation of the PCa pathologies.

Methods

We present a protocol to intraductally injecting an adenovirus expressing Cre-recombinase with the luciferin tag into the prostate of $Pten^{L/L}$ mice at different ages. *In vivo* imaging of the luciferin signals following viral injection to confirm expression and activity of Cre-recombinase. Immunohistochemical staining was performed on prostate tissue sections harvested from mice after viral injection to confirm *Pten* loss and phospho-*Akt* activation. Mice genitourinary (GU)-bloc weights and histopathology was compared between aged and non-aged mice at different time points after viral injection.

Results

The Adenovirus were infected to most epithelial cells but not in the stromal cells or other organs. Aged mice had significantly increased PCa compared to non-aged mice after viral injection at different time points.

Conclusions

Prostate-specific *Pten* KO mouse model can be induced at different ages by the adenovirus-assisted *in vivo* conditional KO approach. These models allow comparing tumor growth in the same time interval post-*Pten* excision between the aged and non-aged mice, and better understanding the effects of aging on prostate carcinogenesis.

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Targeting GAPDH-related glycolysis in castration-resistant prostate cancers
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Targeting GAPDH-related glycolysis in castration-resistant prostate cancers

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Background: Although it was widely used as internal control for gene expression study, increased Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) GAPDH expression was reported in multiple cancers with poor prognosis[1]. Recent studies showed that GAPDH activity is a critical rate-limiting factor in cancer cells with high levels of aerobic glycolysis[2]. Alternol is a natural compound that disrupts metabolic flux preferentially in cancer cells and interacts with 4 Krebs cycle enzymes and GAPDH in prostate cancer cells[3].

Methods: GAPDH expression was analyzed using the GEO, OncoMine and cBioportal databases. GAPDH activities were analyzed in prostate cancer cell lines. Alternol interaction was assessed using in silicon modeling and in cell culture-based CETSA assay. Glycolytic activity was analyzed using Seahorse analyzer. Metabolic alteration was examined using GC-MS approach.

Results: GAPDH genetic alteration is significantly associated with disease progression and poor overall survival (Fig 1-2). GAPDH expression is significantly higher (2-5 folds) in primary and metastatic prostate cancer tissues compared to the benign compartments (Fig 3). Castration resulted in a significant increase of GAPDH expression in mouse prostate and in LuCaP365 xenograft tissues grown in NOD/SCID mice (Fig 4). GAPDH enzymatic activity is about 2-fold higher in CRPC cells compared to benign prostate cells and Alternol treatment reduced its activity to the levels close to the benign cells. Computer modeling revealed that Alternol interacts with GAPDH protein on one of the NAD^+ binding sites (Fig 5). As expected, Alternol directly inhibited GAPDH dehydrogenase activity in an *in vitro* assay with purified enzyme (IC_{50} = 5.593 nM). These inhibitory effects were associated with reduced glycolytic flux in cancer cells as assessed by extracellular acidification rate (ECAR) and metabolomic analysis.

Conclusions: These results suggest that aberrant GAPDH expression is critical in CRPC progression and that Alternol might be used to block CRPC progression by suppressing GAPDH activity.

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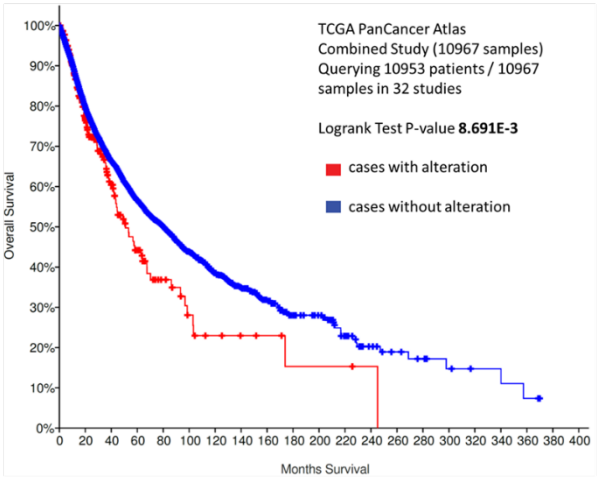

Fig 1. GAPDH gene alteration with overall survival in cancer patients.

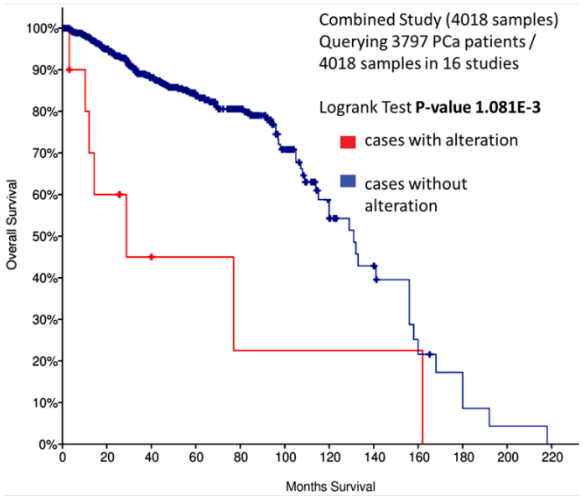

Fig 2. GAPDH gene alteration with overall survival in prostate cancer patients.

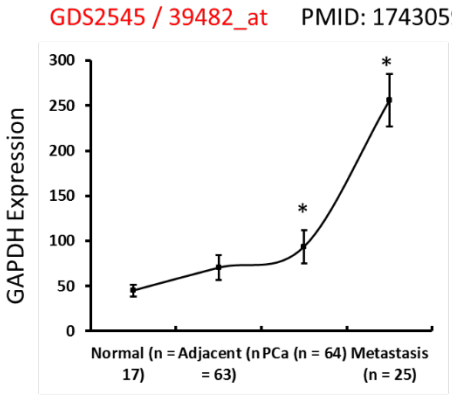

Fig 3. Analysis of metastatic prostate tumors and primary prostate tumors. Normal tissue adjacent to the tumor and normal donor tissue also examined.

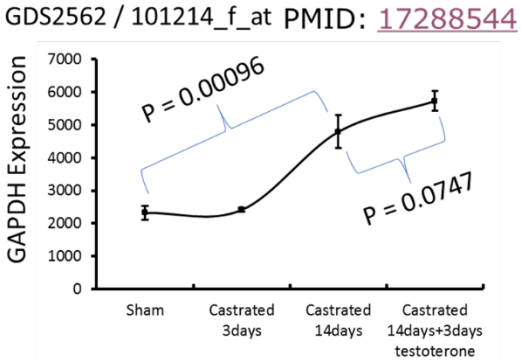

Fig 4. Analysis of prostate of animals following castration and subsequent hormone replacement with testosterone.

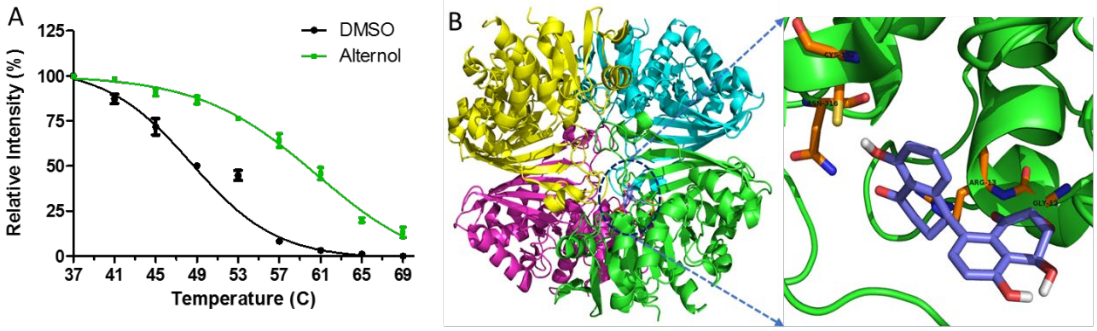

Fig 5. Alternol interacts with GAPDH by CETSA assay in prostate cancer cells (A) and by computer modeling (B).