1

Targeting CBP/p300 and its downstream transcriptional machinery in advanced PCa

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Abstract

Background: Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in the US. The androgen receptor (AR), a hormone-activated transcription factor, plays vital roles in the development and progression of PCa. Thus, androgen-deprivation therapy (ADT) is a standard-of-care first-line therapy for metastatic PCa. Resistance to ADT leads almost uniformly to lethal disease, termed castration-resistant prostate cancer (CRPC). As such, there is a largely unmet clinical need to identify and develop novel strategies, that work either alone or in concert with AR-directed therapeutics, to combat CRPC. The highly conserved histone acetyltransferases CBP/p300 are potent co-activators for AR, and high p300 expression is associated with locally advanced disease and castration-resistant AR function. This study shows that CBP and p300 are highly expressed and correlate closely with AR gene expression and AR activity score in primary PCa and CRPC. Thus, it will be critical to determine the role of CBP/p300 in PCa in order to potentially develop novel therapeutic targets for precision medicine to enhance patient outcome.

Methods: By employing clinically relevant PCa models, the clinical significance of CBP/p300 expression in PCa patients as well as mechanistic evaluation of CBP/p300 transcriptional reprogramming and DNA damage response pathways have been undertaken. Lastly, the molecular response to CBP/p300 inhibition will be assessed to discern novel metrics for precision medicine for PCa patients to improve therapeutic efficacy.

Results: Previous studies have relied on non-specific compounds and genetic silencing to target CBP/p300 and its associated transcriptional machinery. CCS1477 is a first-in-class bromodomain inhibitor designed by Cell Centric and targeted to inhibit CBP/p300 mediated bromodomain activity, and thus regulate cell survival. The IC50 values of CCS1477 in hormone therapy-sensitive and CRPC cell lines were determined to demonstrate effective inhibition in growth and clonogenicity assays. Inhibition of the CBP/p300 bromodomain with CCS1477 resulted in significant downregulation of AR-FL, AR-V7, and its targets' mRNA expression, as well as inhibition of associated factors such c-MYC and its downstream targets, in PCa cell lines as well as PDX models. Transcriptomic analysis indicated that both CBP and p300 expression correlate with expression of genes involved in double strand break (DSB) DNA repair process including non-homologous end joining (NHEJ) and homologous recombination (HR) in both primary PCa and CRPC models. CCS1477 directly impacted DNA damage response and repair dynamics, as shown via RAD51 and gH2AX foci. Inhibition of CBP/p300 activity decreased tumor cell proliferation, blocked CRPC xenograft growth in vivo, and decreased proliferation ex vivo in patient-derived prostate tumor explants. Importantly, CBP/p300 expression correlated with HR genes in human prostate tissue samples. Moreover, inhibition of CBP/p300 activity also decreased HR gene expression in patients further supporting the essential role CBP/p300 plays in DNA repair. In sum, CBP/p300 inhibition mediates HR repair and impacts patient outcome.

Conclusion: Thus, these studies identify CBP/p300 as a driver of PCa tumorigenesis through coordinated control of critical transcriptional events and lay the groundwork to optimize therapeutic strategies for advanced PCa via CBP/p300 inhibition, potentially in combination with AR-directed therapies. Combined, these studies have the capacity for significant near-term impact in the prevention and/or management of metastatic disease.

2

Heterotypic cell-cell communication is substantially changed in the absence of SRD5A2

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Abstract

Heterotypic cell-cell communication is substantially changed in the absence of SRD5A2

Background: Steroid 5α reductase 2 (SRD5A2) is the predominant enzyme responsible for prostatic development and growth. Despite the introduction of steroid 5α-reductase inhibitors (5ARI) for benign prostatic hyperplasia (BPH), the progression of LUTS is only slowed by 34% with 5ARI-response. Little is known why patients with BPH do not uniformly respond to 5ARI therapy. We previously generated a SRD5A2 null (Srd5a2-/-) mouse model with ~50% prostate involution of the original size. We demonstrated that luminal cells with lineage signature and progenitor signature diminished, whereas luminal cells with estrogen response genetic signature stably survived when SRD5A2 was absent. Here we demonstrate changes in the cell-cell interactions between stromal cells and luminal cells with WNT5A as a critical modulator in the absence of SRD5A2.

Material and Methods: Single-cell RNA sequencing (scRNA seq) and bulk RNA seq were performed with prostate tissues from Srd5a2-/- and Srd5a2+/- littermate control mice. Unbiased scRNA seq with 10x genomics platform, followed by unsupervised clustering, was utilized to generate cell clusters based on differentially expressed (DE) gene profiles. The heterotypic cell-cell communication was analyzed using CellphoneDB2. Identified ligand-receptor pairs were validated by qPCR in BHPrS1 human prostatic stromal cell line and human prostatic tissues with BPH. RNAscope was performed to identify the transcription expression of target genes.

Results: The expression of SRD5A2 was exclusively identified in fibroblasts and myofibroblasts. The absence of SRD5A2 induced the increase of stromal cells (11.3% vs. 18.0%) but a decrease in luminal cells (53.2% vs. 31.8%). Analysis of ligand-receptor pairs indicated that stromal cells were the most prolific interactors, and myofibroblasts were the most "outbound" cells. Meanwhile, the fibroblasts in Srd5a2-/- mice had the most significantly increased weighted value (366 vs. 493), and LE1, one subset of luminal cells, had the most significantly decreased weighted value (205 vs. 96). Myofibroblast and muscle system processes were most significantly upregulated in Srd5a2-/- mice as assessed by bulk RNA-seq analysis. Nine highly significant interactions were identified which regulate stromal–luminal cell communication, including WNT5A/PTPRK, TGFB2/TGFBR1, and (NRP1, NRP2, FLT1)/VEGFA. RNAscope data showed a significant upregulation of WNT5A in the anterior prostate of Srd5a2-/- mice ($p<0.0001$). In human BPH specimens, WNT5A was positively correlated with SRD5A2 ($R=0.54$, $p=0.02$), although in SRD5A2-overexpressed BHPrS1 cells, WNT5A was not significantly changed.

Conclusion: Our data suggest that the heterotypic cell-cell communication is substantially changed in the absence of SRD5A2. The stroma-niched signaling, particularly WNT5A and WNT signaling pathway, might serve as a therapeutic target for managing BPH patients who lack SRD5A2 expression and may be unresponsive to 5ARI therapies.

3

KLF5 Drives Basal Cell Identity to Promote Prostate Cancer Lineage Plasticity

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Abstract

Background:

Most prostate cancers have a luminal epithelial cell identity driven by the androgen receptor (AR) transcription factor. As a result, locally advanced or metastatic prostate cancer can be treated with androgen deprivation therapy (ADT) alone or in combination with high affinity AR antagonists like enzalutamide. Unfortunately, these therapies are not curative, and men will eventually develop castration-resistant prostate cancer (CRPC). About 70-75% of CRPC restores AR signaling, while the remaining 25-30% display lineage plasticity evidenced by AR negative subtypes like neuroendocrine CRPC (NEPC) or “double negative” CRPC (DNPC) lacking both AR and neuroendocrine markers. These AR-independent manifestations of CRPC represent a clinical challenge because no effective therapies are available and attractive therapeutic targets are largely unknown. The goal of this study was to elucidate mechanisms that mediate lineage plasticity and resistance to AR-targeted therapies.

Methods:

We utilized prostate cancer cell lines, patient derived xenografts (PDX), and publicly available data from clinical CRPC specimens to identify and investigate factors promoting lineage plasticity and progression.

Results:

We identified the stem cell transcription factor KLF5 as a CRPC oncogene that regulates prostate cancer lineage plasticity. KLF5 opposes AR transcriptional activity to regulate the balance between luminal and basal cell identities. We found that KLF5 levels are highest in DNPC patient tumors, which corresponds to this AR-independent form of CRPC having a basal cell identity. Using the LTL331 PDX model, which progresses from hormone-sensitive adenocarcinoma to NEPC after castration, we found that KLF5 levels are upregulated after castration and coincide with erosion of luminal identity and emergence of basal cell identity. These luminal-to-basal transitions preceded development of NEPC. Using a DNPC-like cell line model derived from LTL331, we found that knockdown of KLF5 decreased expression of markers that define basal cell identity.

Conclusions:

These Results: suggest a mechanism by which tumors upregulate KLF5 under the selective stress of ADT to promote a lineage plastic state of high basal cell identity and reduced luminal cell identity that can ultimately progress to NEPC. These studies nominate KLF5 as a potential therapeutic target to block early events in prostate cancer lineage plasticity and thereby increase durability of AR-targeted therapies.

4

Interactions between Siglec-7/9 and their ligands promote T cell immune evasion in prostate cancer

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Abstract

Background: Cancer immunotherapy has emerged as a potent strategy for cancer treatment to improve patient's survival. However, currently available immune checkpoint inhibitors are ineffective for prostate cancer. The interactions between Sialic acid-binding immunoglobulin-type lectin proteins (Siglec) and their ligands, sialylated glycoproteins, have been implicated as an immune checkpoint pathway. In several cancer types including leukemia, melanoma, and lung cancer, the Siglec-7/9-sialic acid axis has been identified as a potential immune checkpoint. We tested whether this pathway might be involved in prostate cancer.

Methods: Siglec-7/9 Fc binding protein conjugated with FITC was used to evaluate Siglec-7/9 ligand expression levels by flow cytometry. Siglec-7/9 expression on immune cells was evaluated by flow cytometry. T cell-mediated cytotoxicity was used to evaluate the interactions of Siglec-7/9 and their ligands on prostate cancer cells in a co-culture system. FXYD5 and CD59 knockout cells were generated by the CRISPR/cas9 system and validated by western blot.

Results: We demonstrated that Siglec-7/9 expression was correlated with cytotoxic T cells in tumor tissue, and Siglec-7/9 ligands were expressed in prostate cancer cell lines and tumors. Blocking Siglec-7/9 and interaction with their ligands sensitized prostate cancer cells to T cell-mediated killing. CRISPRi screening in prostate cancer cells identified FXYD5 and CD59 as potential Siglec-7 and Siglec-9 ligands, respectively. FYD5 and D59 knockout in prostate cancer cells reduced Siglec-7 and Siglec-9 binding capacity and enhanced T cell-mediated killing.

Conclusions: Interactions between Siglec-7/9 and their ligands on prostate cancer cells are involved in T cell immune evasion, and blocking these interactions promoted T cell-mediated cancer cell death. We provide a strong rationale for targeting Siglec-7/FXYD5 and Siglec-9/CD59 immune checkpoints as potential therapeutic approaches for prostate cancer.

5

NUSAP1 binds to ILF2 and DHX9 and promotes R-loop-dependent DNA damage

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Abstract

Background: Increased expression of nucleolar and spindle-associated protein 1 (NUSAP1) has been identified as a robust prognostic biomarker in prostate cancer and many other malignancies. We have previously shown that NUSAP1 promotes prostate cancer invasion and metastasis, and NUSAP1 expression is regulated by E2F1. To further expose the biological function of NUSAP1, we used affinity purification and mass spectrometry (AP-MS) proteomic analysis to identify NUSAP1 interactors. We hypothesized that cancer progression could be driven through direct interactions of NUSAP1 with protein binding partners.

Methods: AP-MS was performed on 293 T cells to characterize the NUSAP1 interactome. Selected NUSAP1 interacting proteins were verified by co-immunoprecipitation (co-IP) and immunofluorescence (IF) in prostate cancer cells. Deletion constructs of protein interacting domains of flag-NUSAP1 with ILF2 and DHX9 were tested by co-IP and IF. R-loops were detected using the S9.6 antibody and Dot-blots. Knockdown of NUSAP1, ILF2, and DHX9 in the absence and presence of camptothecin (CPT) was used to characterize their effects on R-loop formation and DNA damage.

Results: We characterized 85 unique proteins in the NUSAP1 interactome, including DHX9 and ILF2. Using proteomic approaches, we uncovered a novel R-loop-dependent DNA damage response function for NUSAP1 and ILF2 through their interactions. Pull-down experiments and co-IF confirmed the direct interaction of NUSAP1 with ILF2 and DHX9 in prostate cancer cells and revealed the microtubule and charged helical domains of NUSAP1 were important for the interactions. Depletion of NUSAP1 reduced CPT-induced R-loop accumulation and DNA damage, while both were increased by depletion of DHX9 and ILF2. Depletion of NUSAP1 significantly reduced the CPT-induced R-loop accumulation and DNA damage induced by depletion of ILF2 alone. In agreement with the proteomic findings, we found in prostate adenocarcinoma that NUSAP1 and ILF2 mRNA expression levels are correlated and are elevated in tumor tissues and associated with poor clinical outcomes.

Conclusions: These Results: identified NUSAP1 interactions with ILF2 and DHX9 and reveal novel roles for NUSAP1 and ILF2 in R-loop accumulation and DNA damage.

6

Novel HSP70 inhibitors improve enzalutamide treatment in prostate cancer

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Abstract

Background: Ubiquitin proteasome system is suppressed in enzalutamide resistant prostate cancer cells and that the HSP70/STUB1 machinery is involved in AR and AR variant protein stabilization. Targeting HSP70 could be a valuable strategy to overcome the resistance of androgen receptor signaling inhibitors (ARSI) in advanced prostate cancer. In this study, we tested novel HSP70 inhibitors in enzalutamide resistant cells and patient-derived prostate cancer cell models.

Methods: Expression of AR-V7, HSP70 and STUB1 were determined by qRT-PCR and western blot. Expression of HSP70 and STUB1 was downregulated using specific siRNA. HSP70/STUB1 and AR-V7 interaction was determined by co-immunoprecipitation and dual immunofluorescence. Tumor organoids were established from the patient derived xenografts (PDX) and the viability was stained by LIVE/DEAD™ Cell Imaging Kit. The effects of HSP70 inhibitors on enzalutamide sensitivity were examined in cell line model and patient-derived cell models.

Results: A novel HSP70 inhibitor JG98 significantly suppressed drug resistant C4-2B MDVR and CWR22Rv1 cell growth and enhanced enzalutamide treatment. JG98 also suppressed the PDX derived organoid growth and induced organoid death in a dose dependent manner. Mechanistically, JG98 suppressed AR/AR-V7 expression in resistant cells and promoted STUB1 entering into the nucleus and bound to AR-V7. Knockdown of STUB1 significantly diminished JG98 anti-cancer effect. A more potent HSP70 inhibitor JG231 was developed from JG98. JG231 significantly improved the drug solubility and showed better pharmacokinetic characteristics than JG98. Moreover, JG231 effectively suppressed the cell growth and enhanced enzalutamide treatment in resistant cells and PDX derived prostate cancer cells.

Conclusions: The HSP70/STUB1 machinery involved in AR/AR-V7 regulation and enzalutamide resistance. Targeting HSP70 by novel HSP70 inhibitors overcome the ARSI resistance and improve their therapy.

7

LSD1 inhibition disrupts super-enhancer-driven oncogenic transcription programs in castration-resistant prostate cancer

Mr. Muqing Li Master

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Abstract

Title: LSD1 inhibition disrupts super-enhancer-driven oncogenic transcription programs in castration-resistant prostate cancer

Authors: Muqing Li, Mingyu Liu, Wanting Han, Zifeng Wang, Dong Han, Susan Patalano, Jill A. Macoska, Eva Corey, Shuai Gao, and Changmeng Cai

Background: As a histone demethylase, LSD1 (lysine-specific demethylase 1, also called KDM1A) functions as a repressor by demethylating mono- and di-methylated histone 3 lysine 4 (H3K4me1/2). It can also function as an activator by demethylating H3K9me1/2 and other non-histone proteins, such as FOXA1. LSD1 plays important oncogenic roles in various malignancies including prostate cancer (PCa), and multiple clinical trials are ongoing to investigate the therapeutic potential of LSD1 inhibitors. While we previously demonstrated that LSD1 inhibition (LSD1-i) can suppress tumor growth of castration-resistant PCa (CRPC) via disrupting the androgen receptor (AR) transcription program, it remains elusive whether LSD1 inhibition targets other oncogenic pathways in CRPC. Elucidating the molecular basis of LSD1 inhibition in CRPC could provide important insights for developing combination therapies for CRPC patients.

Methods: Bulk RNA-Seq was performed in an array of CRPC xenograft models (derived from cell lines and PDXs) that have received LSD1-i treatment to identify LSD1-i targeted oncogenic pathways. We also conducted single-cell RNA-Seq in a PDX model to determine the cellular responses to LSD-i. Chromatin immunoprecipitation assay together with integrative computational analysis was performed to understand the chromatin-associated regulatory network in which LSD1 is involved. Combination therapy of LSD1-i and inhibitors targeting LSD1-regulated oncogenic pathways was assessed in multiple CRPC xenograft models.

Results: Our transcriptomic profiling studies in the CRPC xenograft models uncovered that LSD1-i consistently decreased MYC signaling through suppression of MYC expression. This finding was further corroborated by a single-cell RNA sequencing analysis performed in a heterogenous CRPC PDX model. As MYC is a well-known direct target of BRD4 and is driven by super-enhancers (SEs), we interrogated the association of LSD1, FOXA1 and BRD4 at the MYC-associated super-enhancer site and found that LSD1-mediated FOXA1 chromatin binding could regulate BRD4 recruitment. Moreover, we found that LSD1, similar to BRD4, was highly enriched at SEs and formed phase separation. Therefore, we tested the combination treatment by co-targeting LSD1 and BRD4 in a series of CRPC xenograft models and found great synergy between LSD1-i and BET-i in decreasing tumor growth and various key transcriptional programs, including newly identified CRPC-specific SEs.

Conclusion: Our study demonstrates that LSD1-i can decrease MYC signaling and disrupt SE activities in CRPC and that co-targeting LSD1 and BRD4 can achieve great synergy in suppressing CRPC tumor growth. Our finding provides novel insights into the molecular action of LSD1-i in CRPC and suggests a novel combination therapy by co-targeting two key epigenetic factors. The study will lead to rapid translation in the clinic for CRPC patients.

8

Exploring the role of ASCL1 in neuroendocrine prostate cancer

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Abstract

Most patients with prostate adenocarcinoma (PAd), an androgen receptor (AR) driven cancer, develop resistance to therapies targeting AR. Consequently, a portion of these patients develop neuroendocrine prostate cancer (NEPC), a rapidly progressing cancer with limited therapies and poor survival outcomes. Current research to understand the transition of PAd to NEPC suggests a model of lineage plasticity, where AR-dependent luminal tumors progress towards an AR-independent neuroendocrine lineage. Several groups have shown human NEPC tumors have lost RB1 and TP53, and in experimental models, loss of both genes mediates the transition to a neuroendocrine lineage. Notably, NEPC histology and gene expression resemble another neuroendocrine cancer, small cell lung carcinoma (SCLC), also characterized by loss of RB1 and TP53. In SCLC, transcription factor ASCL1 is required for tumor cell growth in vitro and for SCLC formation in vivo. ASCL1 is also present in NEPC tumors and in NEPC cell line models. In fact, ASCL1 was shown to regulate neuronal stem cell-like lineage programming in NEPC in vitro. This project is aimed at determining the requirement and function of ASCL1 in the transition of PAd to NEPC in vivo. To model this transition, we established genetically engineered mouse models (GEMMs) harboring loss of Rb1 and Tp53 with Myc overexpression (RPM) by administering adenovirus expressing Cre recombinase directly to the prostate. These animals display prostate tumors with small cell neuroendocrine histology, and we are currently investigating what the concomitant loss of ASCL1 does in this model. Using these same GEMMs to establish prostate organoids, we show their capacity to generate subcutaneous allograft tumors displaying mixed adenocarcinoma, squamous, and NE small cell features. ASCL1, along with other NE markers such as SYP, INSM1, and UCHL1, are detected in regions with small cell histology as well as some regions histologically resembling adenocarcinoma. Subcutaneous allografts from prostate organoids lacking ASCL1 exhibit a dramatic reduction in tumor formation efficiency and often result in the development of fluid-cysts. When tumors do form, they have retained the ASCL1 gene. These Results: demonstrate a key function for ASCL1 in NE lineage programming in vivo and suggest additional stem cell-like functions in supporting prostate tumor formation with mixed histology.

9

A targeted CRISPR/Cas9 screen identifies FOXA1 as a key transcription factor involved in MYC-driven and diet-dependent prostate cancer outcomes

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Abstract

Background: Accumulating evidence points to a role for high-fat diets and obesity in driving prostate cancer progression. Chromatin remodeling and epigenetic reprogramming are emerging mechanisms of prostate cancer progression, representing dynamic avenues through which a cell may respond to a high-fat diet or obese environment. However, the link between diet and chromatin-related processes in driving aggressive disease thus far has been uncharacterized.

Methods: Our experimental designs are articulated around a murine model of MYC-driven prostate cancer that recapitulates several molecular features of human prostate adenocarcinoma. To identify candidate chromatin- and epigenomic-related factors critical for prostate tumorigenesis, we performed CRISPR/Cas9 knock-out screens *in vitro* using custom CRISPR/Cas9 libraries targeting epigenetic-related genes. To validate the candidate genes, we generated stable knock-out cells using CRISPR/Cas9 and characterized their oncogenic phenotypes *in vitro* using a battery of cellular assays, *in vivo* under conditions of either a control diet (CTD; 10% kCal fat) or diet-induced obesity (DIO; 60% kCal fat), and through a series of next-generation sequencing-based experiments and analyses (*e.g.*, RNA-seq, ATAC-seq, ChIP-seq).

Results: Our *in vitro* CRISPR/Cas9 screen identified the pioneer transcription factor forkhead box A1 (FOXA1) as a top candidate in promoting MYC-driven prostate cancer cellular proliferation. Indeed, genetic knock-out of FOXA1 confirmed that FOXA1 contributes to prostate cancer cellular proliferation, as well as cell-cycle progression and the maintenance of mitochondrial respiration *in vitro*. Transcriptomic analysis further implicates FOXA1 in regulating metabolic processes such as glycolysis and fatty acid metabolism. Along this line, FOXA1 supports tumor initiation and growth under CTD *in vivo*. Critically, loss of FOXA1 abrogates DIO-dependent tumor growth.

Conclusions: Our studies suggest that FOXA1 supports diet-dependent MYC-driven tumorigenesis of the prostate. We expect the Results: of our molecular profiling experiments to further elucidate the ways in which diet dictates FOXA1 function, thereby influencing prostate cancer outcomes. By doing so, we would be setting the stage for the use of targeted therapies that exploit aggressive prostate cancer's dependency on FOXA1 and other diet-dependent epigenetic factors.

10

Fatty acid biomarkers in urine for prostate cancer diagnosis

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Abstract

Prostate cancer (PCa) diagnosis relies primarily on the prostate specific antigen (PSA) results, however this test lacks accuracy and often causes men to undergo unnecessary and invasive prostate biopsies. There is a clinical need for a more accurate diagnostic tool for PCa diagnosis and alternative biomarkers have been explored to fulfill this purpose. Among the candidates explored as PCa biomarkers are fatty acids (FA) for their role as cell structure components, being essential for normal cell proliferation and PCa rapid proliferation. We aimed to investigate the use of FAs in urine to produce a model that can differentiate between positive and negative PCa patients with a higher accuracy than PSA. To construct a FA model for PCa diagnosis 280 urine samples (142 PCa positive and 138 PCa negative) were analyzed by stir bar sorptive extraction (SBSE) and thermal desorption-gas chromatography and mass spectrometry (TD-GCMS). For comparison, a PSA model for PCa diagnosis was constructed using the same pool of samples. The FA model (AUC 0.709 95% CI) was determined to perform better than the PSA model (AUC 0.606 95% CI) for PCa diagnosis. These Results: support the potential of FA as more accurate PCa biomarkers, and the use of our method provide a solventless and non-invasive alternative for PCa diagnosis.

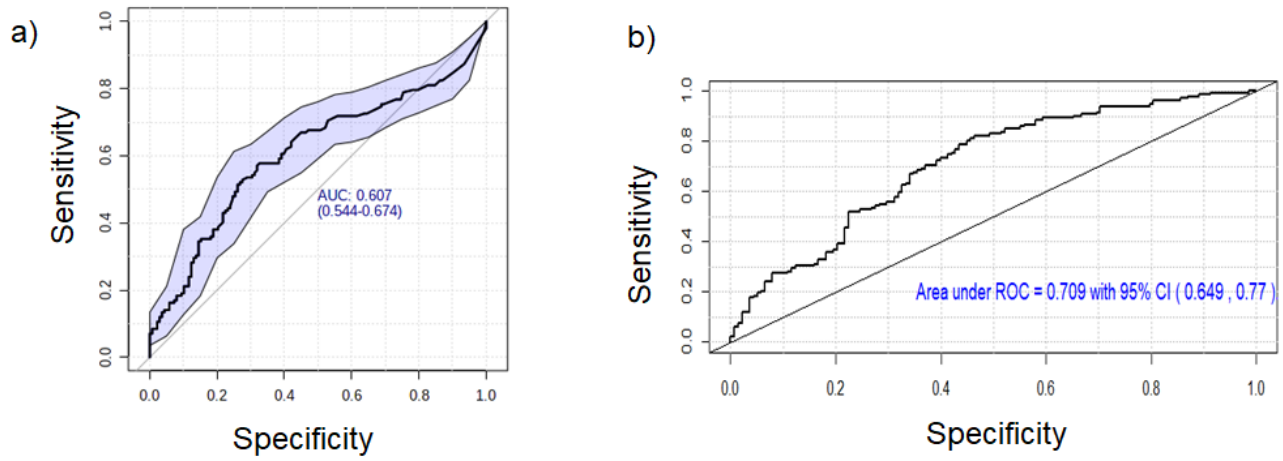

Figure 1. Receiver operating characteristic curves for prostate cancer diagnosis. a) ROC plot for PSA model (AUC 0.606, blue band represents 95% CI). b) ROC plot for fatty acid model (AUC 0.709 95% CI).

11

Epigenetic Reprogramming following inactivation of FOXA1 Results: in Interferon-Independent Bladder Cancer

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Abstract

Background: Forkhead Box A1 (FOXA1) is a pioneer transcription factor (TF) critical in epigenetic regulation of chromatin states and cell fate determination. We previously identified that reduced expression of FOXA1 is independently associated with poor overall survival in bladder cancer (BC) patients. In this study, we determined the impact of FOXA1 inactivation on chromatin epigenetic modification and associated gene expression, and also identified a functional FOXA1 binding site within the human CD274 promoter.

Methods: We used CRISPR/Cas9 to delete FOXA1 in human luminal BC cells (FOXA1KO). We performed RNA-seq and Chip-seq for H3K27ac, an epigenetic mark of active enhancers/promoters. Motif and Gene Set Enrichment Analysis (GSEA) analysis of the Chip/RNA-seq data identified FOXA1 KO-induced increases in accessibility to TF binding sites and affected pathways, respectively. Western blotting (WB) and qPCR confirmed FOXA1-regulated CD274/PD-L1 expression. Additionally, we completed DNA affinity purification assays using biotinylated wild-type and mutant human CD274 promoter fragments.

Results: Following CRISPR/Cas9-mediated KO of FOXA1 in UMUC1 BC cells, 8,230 differentially expressed genes (FDR, $q<0.05$) were identified. GSEA identified IFNα/ɣ gene signatures, which include CD274/PD-L1, as significantly upregulated following FOXA1 KO. Significant changes in H3K27ac at specific genomic loci were also identified following FOXA1 KO. Integrated analysis of ChIP-seq and RNA-seq data showed increased H3K27ac was consistently associated with upregulated genes of the IFNα/ɣ expression signatures. Motif analysis of genomic sequences enriched for H3K27ac identified significant enrichment of interferon response factor 1 (IRF1) binding sites in regulatory elements throughout the genome. Interestingly, FOXA1 KO also resulted in significant increases in IRF1 expression, even in the absence of IFNɣ stimulation. Using the CD274 promoter as a model interferon-responsive cis regulatory element, affinity DNA purification assays identified a functional FOXA1 binding site within this promoter. In addition, we show increased IRF1 binding to this region within the CD274 promoter following FOXA1 KO.

Conclusions: Collectively, our Results: indicate FOXA1 inactivation in BC Results: in increased accessibility to IRF1 binding sites throughout the genome. In conjunction with increased IRF1 expression following FOXA1 silencing, these events culminate in the epithelial-intrinsic activation of interferon target genes in an IFNɣ-independent manner. These observations suggest that epithelial-specific genetic changes can result in the autoactivation of interferon signaling and associated gene expression in malignant bladder epithelium.

12

Targeting of ERG Positive Prostate Cancers with ERGi-USU-6 salt derivative

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Abstract

Background: Prostate cancer (PCa) is the second leading cause of cancer deaths among men in the United States. Approximately 50% of PCa patients harbor the oncogenic TMPRRS2-ERG gene fusion in their primary and 35% of patients with metastatic castration resistant prostate cancers. We have identified a potent small molecule inhibitor, ERGi-USU-6 salt derivative 7b, that selectively inhibits the growth of ERG positive tumor cells at concentrations within the range of FDA approved cancer drugs. To gain first insights into the cancer-selective properties of ERGi-USU-6 salt 7b we assessed cell cycle regulation, cell death related activities of the compound.

Methods: The biological activities of salt derivative 7b, were assessed in hormone-refractory metastatic tumor derived ERG positive prostate cancer cell line, VCaP. We monitored the cell cycle by cell sorting and assessed cell cycle-regulator proteins by immunoblot assays in response to 7b treatment. Also, we monitored the levels of the RIOK2 kinase, previously shown to bind the parental ERGi-USU compound. The normal primary endothelium derived HUVEC cells were used as normal control due to the normal endogenous expression of ERG in endothelial cells including HUVEC.

Results: The cell cycle analyses, and pathway mapping by protein assessment assays suggests that salt derivative 7b treatment inhibits the ERG positive prostate cancer by modulating the expression of ATF3 gene that has been implicated in ferroptosis. We have observed activations in proteins involved in the iron-dependent cell death process ferroptosis. Consistent with reports on G0/G1 cell-cycle arrest of prostate cancer cells under induced ferroptosis, we observed cell-cycle arrest G0/G1 phase in 7b-treated prostate cancer cells accompanied by modulation of cell cycle regulating proteins. In contrast, the salt derivative 7b had no effect on ERG positive normal endothelia cells indicating cancer selectivity.

Conclusions: Our Results: showed that the ferroptosis inducer ATF3 gene is involved in the cancer-selective activity of ERGi-USU-6 salt derivative 7b. Further, based on our observations we hypothesize that ferroptosis may be the mechanism of cancer selective activity of salt derivative 7b. In normal ERG positive endothelium-derived cells, toxicity or biological activity of salt derivative 7b was not observed. Indeed, development of ERG inhibitors may benefit prostate cancer patients including delaying cancer progression in patients on active surveillance, as well as, treating patients who have developed ERG positive metastatic castration resistant PCa.

13

Immunosuppressive signaling is activated in enzalutamide resistant prostate cancer

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Abstract

Background: Emerging data suggested that enzalutamide-treated prostate cancer patients with increased programmed death-ligand 1 (PD-L1) expression may benefit from anti-PD-L1 treatment. Unfortunately, the Phase III IMbassador250 trial revealed that the combination of atezolizumab (PD-L1 inhibitor) and enzalutamide failed to extend overall survival in patients with castration-resistant prostate cancer (CRPC). The mechanisms underlying treatment failure remain unknown. In this study, we investigated the regulation of immunosuppressive signaling in enzalutamide resistant prostate cancer and characterized the immune infiltrating cells in mouse prostate tumors.

Methods: The expression of interferon gamma-related genes was determined using qRT-PCR and/or western blotting. Androgen receptor (AR) and AR-V7 levels were downregulated using specific siRNA. Myc-CaP cells were chronically exposed to increasing concentrations of enzalutamide (5-50 μM) for >12 months, and the cells resistant to enzalutamide were referred to as Myc-CaP MDVR. The gene-regulating mechanisms in drug-resistant prostate cancer cells were determined by RNA sequencing analyses. Myc-CaP and Myc-CaP MDVR xenograft tumors were established in FVB mice, and tumor-infiltrating lymphocytes were isolated using Ficoll. The stained immune cells were determined by flow cytometry, and the data were analyzed using Flowjo.

Results: Immune-related signaling pathways (interferon alpha/gamma response, T cell activation, and cell chemotaxis pathways) were suppressed in C4-2B MDVR cells. However, PD-L1 expression was highly upregulated in C4-2B MDVR cells. DHT treatment significantly suppressed the RNA and protein expression of PD-L1 in a dose- and time-dependent manner. Knockdown of full-length AR, but not AR-V7, significantly increased PD-L1 expression. Enzalutamide treatment effectively suppressed Myc-CaP tumor growth. The treatment also increased $CD45^+$ tumor-infiltrating cells and $CD3^+$ T cells but decreased myeloid-derived suppressor cells (MDSC) ($CD11b^+$, $Gr1^+$) in tumors. However, regulatory T cells (Tregs) ($CD4^+$, $FoxP3^+$) and MDSC populations were significantly increased in Myc-CaP MDVR tumors compared to those in Myc-CaP parental tumors. Co-culturing bone marrow-derived macrophages (BMDMs) with Myc-CaP MDVR cells significantly increased the MDSC population and enzalutamide treatment further increased the MDSC percentage.

Conclusions: Immunosuppressive signaling is activated in enzalutamide-resistant prostate cancer and AR signaling negatively regulates immune checkpoint PD-L1 expression. MDSC and Tregs are highly enriched in enzalutamide-resistant mouse prostate tumors.

14

Expression of mutant *Pik3ca* in murine urothelial cells provides a novel model of bladder tumorigenesis

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Abstract

Background:

Despite the fact that approximately 70% of newly diagnosed bladder cancers are non-invasive tumors with high rates of recurrence, non-invasive bladder cancer is significantly understudied. In part, the lack of appropriate models to validate the contribution of specific molecular drivers of bladder tumorigenesis is a significant issue. Activating mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) are a frequent event in early stage bladder cancer, yet an in vivo model for understanding these mutations in bladder cancer is not currently available.

Methods:

To address this gap, we created a novel *Upk2-Cre/Pik3ca*^{H1047R} mouse model which expresses one or two alleles of mutant *Pik3ca*^{H1047R} in a urothelial-specific manner. The functionality of mutant *Pik3ca* in this model was confirmed by quantifying p-Akt expression using immunohistochemistry (IHC). Experimental and genetic control mice were then characterized at 6 and 12 months of age by assessing the thickness of the urothelium, nuclear atypia, and immunohistochemical expression of luminal (Foxa1 and Pparγ) and basal (Krt5/6 and Krt14) urothelial differentiation markers. Additionally, 6 month old mice were treated with 0.01% *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) via their drinking water for 10 weeks to determine the contribution of *Pik3ca* mutation in sensitivity to carcinogen exposure. Urothelial thickness, nuclear atypia, and luminal/basal marker expression were again assessed in these mice.

Results:

At 6 months of age, mice carrying one or two alleles of mutant *Pik3ca* develop increased urothelial thickness and nuclear atypia. At 12 months, mice carrying one allele of *Pik3ca*^{H1047R} fail to progress further, however, activation of two alleles Results: in possible evidence of early papillary tumor formation. IHC analysis shows these mice maintain luminal differentiation characterized by high Foxa1 and Pparγ expression as well as low Krt5/6 and Krt14 expression in the urothelium. In addition, 6 month old mice were exposed to BBN for 10 weeks, however, significant changes in urothelial thickness, nuclear atypia, and luminal/basal marker expression in the mutant mice were surprisingly not observed.

Conclusions

These data describe the establishment and characterization of a novel model of early luminal urothelial tumorigenesis driven by mutant *Pik3ca* expression. Further studies including longer aging periods and higher or lower BBN concentrations could determine if these mice progress to form mature papillary tumors or show increased sensitivity to carcinogen.

15

AR-V7 Accelerates Prostate Cancer Metastasis by Activating a Full-Length AR-Independent Transcription Program

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Abstract

AR-V7 Accelerates Prostate Cancer Metastasis by Activating a Full-Length AR-Independent Transcription Program

Background: Castration-resistant prostate cancer (CRPC) is particularly challenging to treat as it is characterized by the reactivation and reprogramming of Androgen Receptor (AR) signaling following androgen deprivation therapies (ADTs). One critical mechanism that contributes to the resistance is the elevated expression of the ligand-binding domain-truncated AR splice variants, primarily AR-V7, which is constitutively active without ligands. While it is clear now that AR-V7 plays a critical role in restoring AR signaling and allows tumor cells to be resistant to ADTs, it remains undetermined whether AR-V7 may have a functionally distinct activity to promote prostate cancer progression, such as metastasis.

Hypothesis: We hypothesize that AR-V7 can maintain a full-length AR (AR-FL)-independent and functionally distinct transcription program to accelerate prostate cancer progression.

Methods: Lentiviral stable lines that overexpress tetracycline-regulated V5-tagged AR-V7 or AR-FL using AR-V7 negative LNCaP and C4-2 cell lines were generated. The mouse intratibial injection was used to assess the *in vivo* impact of AR-V7 or AR-FL expression on prostate cancer bone metastasis. ChIP-seq analyses of AR-V7, AR-FL, FOXA1, and H3K27ac, ATAC-seq analyses, and RNA-seq analyses were conducted in these stable lines to characterize the global transcription activity of AR-V7 in comparison with AR-FL.

Results: Our *in vivo* analyses demonstrated a strong promoting effect of AR-V7 on bone metastasis with a dramatic induction of SOX9 expression, which is a well-studied metastasis marker. On the contrary, overexpressing AR-FL failed to induce metastasis in these models. Through integrated cistromic and transcriptomic analyses, we found that AR-V7 can transcriptionally activate a unique metastasis program, including SOX9, through binding to cryptic AREs (androgen-responsive elements) that are generally not accessible to AR-FL under castrated conditions. Moreover, we also demonstrated that this metastasis-promoting AR-V7 activity was dependent on its Ser81 phosphorylation, which is potentially mediated by CDK9.

Conclusions: AR-V7 has a distinctive function of AR-FL in promoting prostate cancer metastasis through transcriptionally activating a metastasis program. This activity is dependent on CDK9-mediated AR-V7 Ser81 phosphorylation. Therefore, CRPC patients with high levels of AR-V7 may therapeutically benefit from CDK9 inhibitor treatment.

16

Development of a novel pre-clinical animal model to assess the immature rat kidney and bladder in response to urinary tract obstruction

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Abstract

Development of a novel pre-clinical animal model to assess the immature rat kidney and bladder in response to urinary tract obstruction

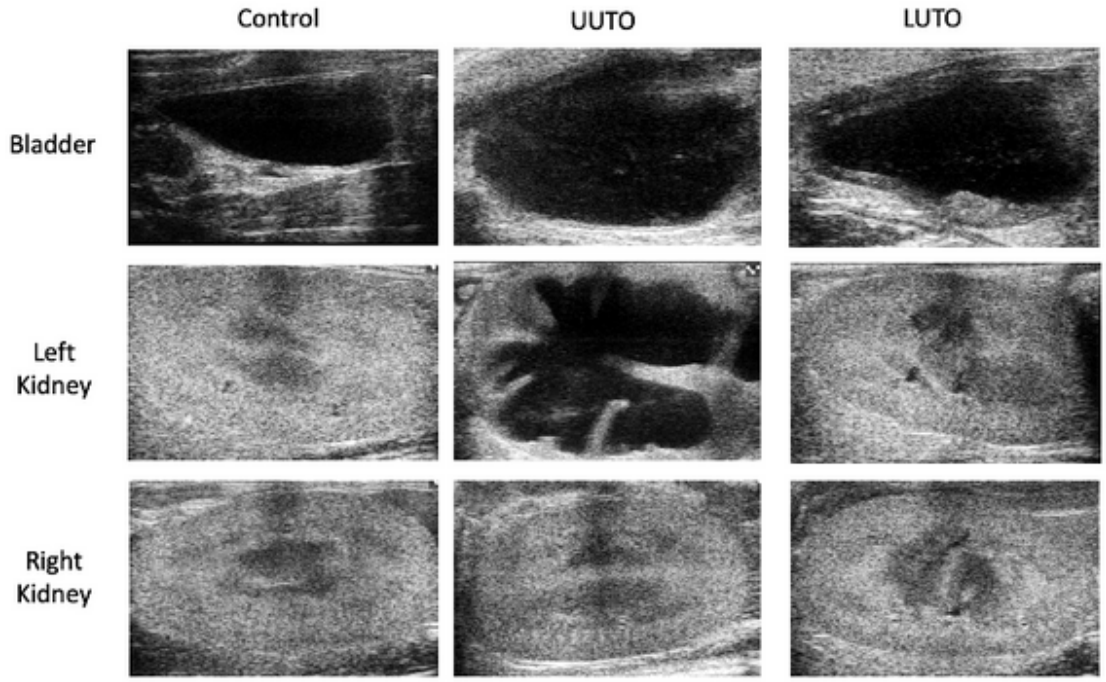
Background: Congenital urinary obstruction is a common cause of ESRD in pediatrics. Despite an in-utero insult, obstruction is not identified until irreversible renal and bladder damage has occurred. There has been no record of an animal model of lower urinary tract obstruction (LUTO) without operating on fetus within a gravid uterus, limiting studies to large and costly animals. The purpose of this study was to develop an economical model of LUTO in rat neonates with immature kidneys, as nephrogenesis is not complete at birth, and compare to age-matched healthy controls as well as neonates with upper ureteral tract obstruction (UUTO).

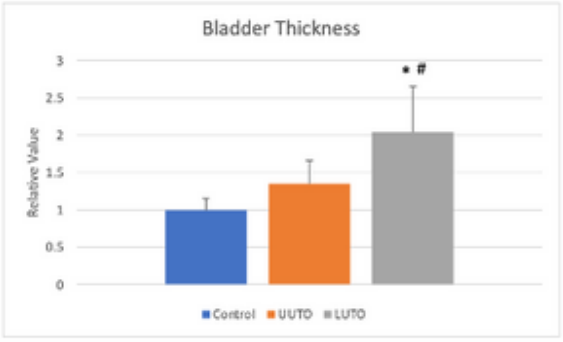
Methods: Male rats (N=3 per group) were operated on d3 of life. For control (C), the kidney and bladder were identified only. For UUTO, partial left (L) ureteral obstruction was performed via placement of a 0.4 mm wire next to the ureter and ligation with an 6-0 nonabsorbable suture, followed by removal of the wire. For LUTO, partial urethral obstruction was created via modified clip applier which left an opening of 0.13 mm below the prostate. Endpoint analysis included ultrasound, creatinine, and histology. Tukey-Kramer post-hoc test was used for all pair-wise comparisons using JMP software (SAS Institute, Cary, NC) ($p<0.05$).

Results: At 21d post-operatively, there were no differences in creatinine (C 0.25+/-0.08; UUTO 0.16+/-0.04; LUTO 0.23+/-0.06 mg/dL). Ultrasound confirmed L UUTO hydronephrosis via area of renal pelvis ($p<0.001$) (R: C 1+/-0.14, UUTO 1.42+/- 0.44, LUTO 0.98 +/-0.25; L: C 1+/-0.25, UUTO 7.5+/-1.43, LUTO 0.95+/-0.22 relative units) and bladder wall thickening in LUTO ($p<0.001$) (C 1+/-0.15; UUTO 1.34+/-0.31; 2.04+/-0.62 relative units) (**Figure 1**).

Trichrome demonstrated a significantly increased smooth muscle cell/collagen ratio in LUTO bladders compared to C ($p=0.012$) and UUTO ($p=0.014$) (C 0.15+/-0.04; UUTO 0.20+/-0.06; LUTO 1.99+/-0.92 relative units) consistent with smooth muscle hypertrophy (**Figure 2**). In the kidneys, there was a higher percentage of fibrosis in the L UUTO group compared to the R ($p<0.001$) and compared to all other L kidneys (C $p<0.001$; LUTO $p<0.001$) (**Figure 3**).

Figure 1: (A) Representative images of renal-bladder ultrasound, (B) corresponding bladder thickness, and (C) area of renal pelvis





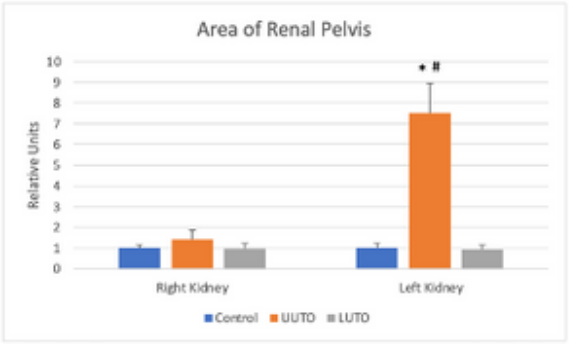
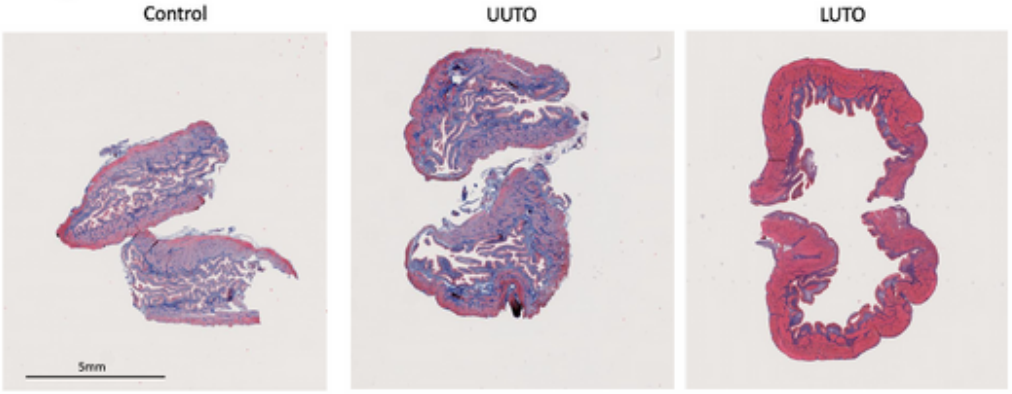


Figure 2: (A) Representative histologic analysis of bladder pathology (Trichrome) and (B) SMC/Collagen ratio within whole mount bladder tissue. (C) Representative histologic analysis of renal pathology (Trichrome) and (D) percentage of fibrotic area on whole mount trichrome staining of renal tissue.



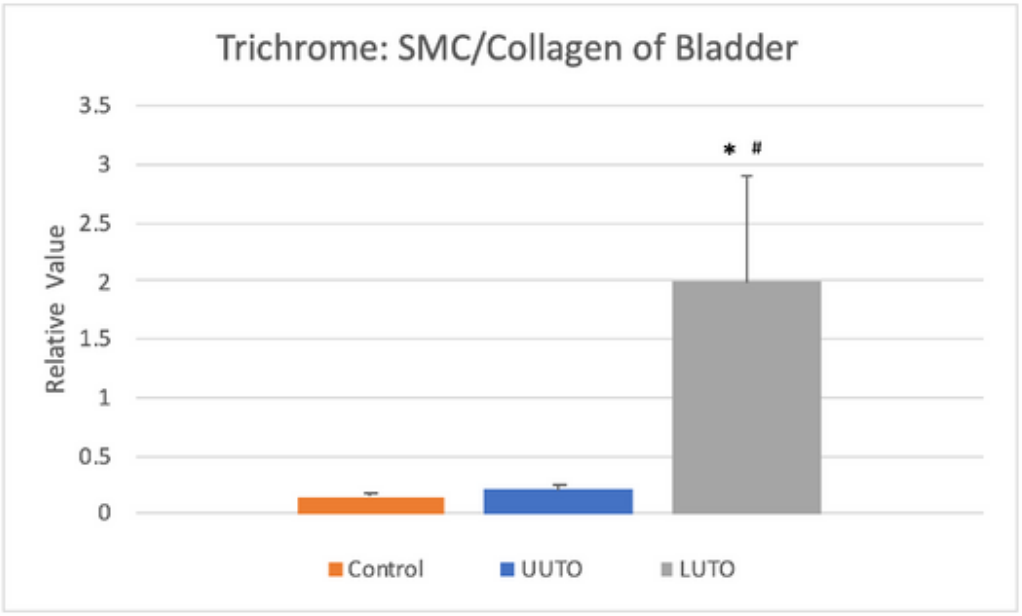
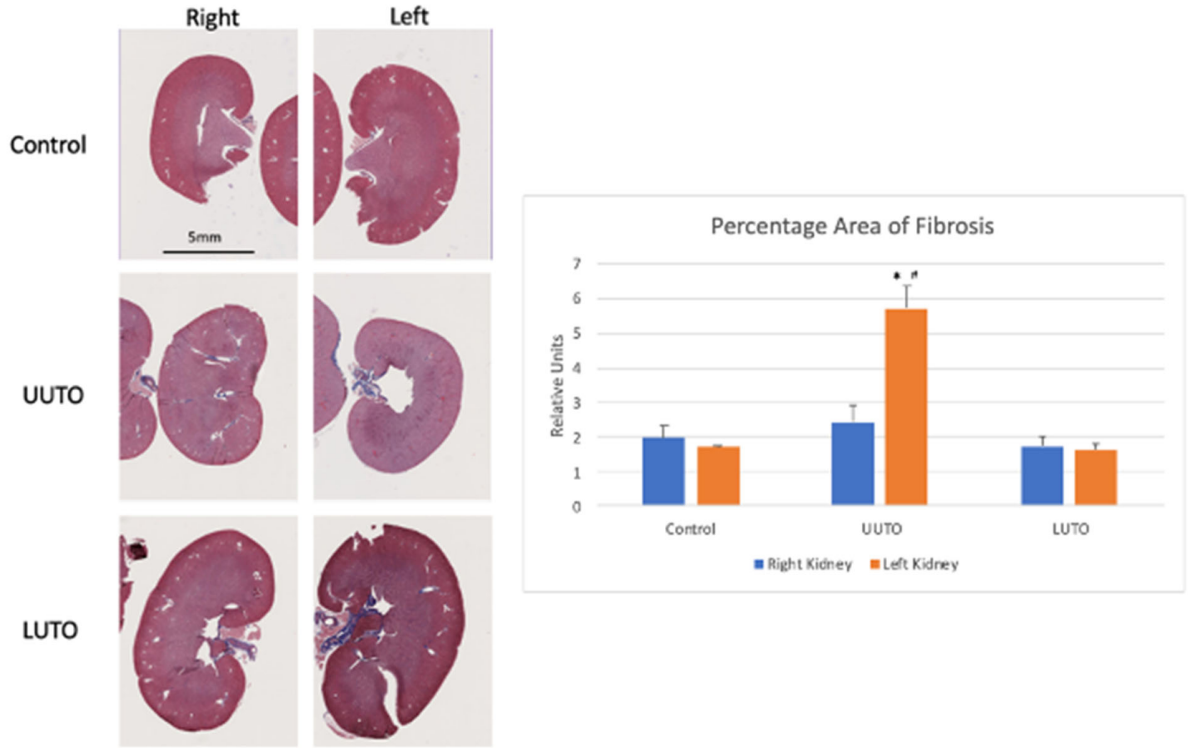


Figure 3: (A) Representative histologic analysis of renal pathology (Trichrome) and (B) percentage of fibrotic area on whole mount trichrome staining of renal tissue.



Conclusions: This is the first study of its kind to develop an economically feasible model for congenital LUTO in a rat animal model of the immature kidney. Minimal changes in creatinine with radiologic and pathologic changes in the kidney and bladder 21d after obstruction indicate it is a representative model of the clinically silent progression seen in urinary obstruction. Future work will evaluate further time points, prognostic markers between groups, and interventions to mitigate irreversible injury.

17

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

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Abstract

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

Methods: Protein levels of Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) in benign prostate, localized PC, castration-resistant PC (CRPC), and NEPC patients were analyzed via immunohistochemistry. Stable cell lines were generated by knockdown/knockout of UCHL1 in TROP2-driven NEPC (TD-NEPC) cell line or overexpression of UCHL1 in 22Rv1 and C4-2 cell lines. Colony formation and 3D-matrigel dot invasion assay were used to test cell growth and invasion in vitro. The functional role of UCHL1 on NEPC tumor growth and metastatic colonization in vivo was evaluated by subcutaneous xenograft implantation and intracardiac injection. Mass spectrometry was performed on TD-NEPC UCHL1 knockdown cells to define UCHL1 targets. LDN-57444, a UCHL1 inhibitor, was tested on NEPC tumor growth and metastasis in vivo.

Results: We demonstrate that UCHL1 is highly expressed in NEPC patients when compared to localized PC and CRPC. Overexpression of UCHL1 increases NEPC tumor growth and NE phenotype, while loss of UCHL1 significantly decreases cell growth, tumor growth, and metastasis in NEPC. Proteomics analysis demonstrates that pathways involved in regulation of NE phenotype acquisition such as E2F targets, MYC targets, and mTOR signaling, are altered upon downregulation of UCHL1. Most importantly, treatment with LDN-57444 significantly suppresses NEPC xenografts and NEPC patient-derived xenografts (PDXs) tumor growth in vivo. Further, inhibition of UCHL1 decreases the expression of NE markers and significantly decreases the metastatic burden of NEPC in vivo.

Conclusions: UCHL1 represents as a potent therapeutic target for NEPC, and is a novel molecular indicator for this malignancy.

18

Engineering Large Chromosomal Deletions in Mice to Advance Precision Oncology for Prostate Cancer

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Abstract

Background: Human prostate cancer is notable for its high number of somatic copy number alterations (CNAs), which include both focal and broad arm-level amplifications and deletions. Up to 90% of prostate tumors harbor aberrant CNAs, and genomic deletions affect about 40% of these tumors. Many chromosomal deletions are highly recurrent and occur in more than 10% of prostate tumors, including 8p (40~50%), 6q14–16, 10q23, 13q14, and 16q22–24 (20~30% each). Most of these deletions are large, often including hundreds of genes. Notably, several large deletions, including 6q14–16 and 16q22–24, are significantly associated with poor patient outcomes, suggesting that these large chromosomal deletions play a functional role in prostate tumorigenesis, and highlighting the critical need for elucidation of the mechanisms underlying large-deletion driven tumorigenesis. However, due to the limited cloning capacity of targeting vectors (~300 Kb) and the rarity of on-target homologous recombination events in traditional gene-targeting technology, modelling large chromosomal deletions in mice has proven highly challenging, and the in vivo role of such lesions in tumorigenesis has therefore been significantly understudied.

Methods: Here, we developed an innovative approach to chromosomal engineering over large genetic distances through CRISPR/Cas9 technology in mouse embryonic stem (ES) cells. The resulting ES clones carrying the desired genome edits make possible the creation of conditional knockout mice that accurately mimic prostate cancer-associated large deletions.

Results:/Conclusions: We will utilize our innovative approach to generate and fully characterize two novel genetically engineered mouse (GEM) models that harbor prostate cancer-associated 10q23– and 6q14–16– deletions, respectively. The development and characterization of these GEM models will contribute greatly to the implementation of precision oncology research, open new opportunities for modelling human diseases associated with large chromosomal deletions, and lead to novel insights into the biology of prostate cancer-associated large deletions, which will facilitate the identification of deletion-specific vulnerabilities that can be exploited for prostate cancer treatment.

19

The expression of prostatic androgen receptor shows lobe-specific changes during aging in mouse

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Abstract

BACKGROUND:：The androgen receptor (AR) is a nuclear receptor transcription factor that can be activated by the androgens, testosterone (T) and dihydrotestosterone (DHT). As men age, the serum levels of T decreases, while the level of estrogen (E_2) increases. Studies suggest that the prostate diseases, such as benign prostate hyperplasia (BPH) and prostate cancer, are closely linked to hormones/receptor changes and aging. However, these detailed cellular and molecular mechanisms remain unclear. Since both men and mice show similar changes in hormones (T and E_2) as they age, the study of hormones/receptor changes may show insight into disease development and progression. Here, we evaluated the expression of nuclear AR in the aging male mouse prostate.

METHODS: we examined the anterior prostate (AP), ventral prostate (VP), and dorsal lateral prostate (DLP) from 2-month-old and 24-month-old C57Bl/6 mice. AR expression was examined via immunohistological Methods:. The percentage of AR positivity was determined using 2-bin (AP) and 4-bin (VP and DLP) scoring by Inform software. Through comparison of the prostatic AR intensities between different age groups, we ascertained a basic relationship between AR and aging.

RESULTS: In the AP, more prostatic AR was expressed in 2-month-old (young) mice (92.21% positivity, n=9), while 24-month-old (old) mice showed reduced AR expression (54.09% positivity, n=7). Similarly, young mice (n=19) showed a higher positivity score in the VP, compared with old mice (n=14). However, no significant difference in AR expression was observed between young and old mice in the DLP.

CONCLUSIONS: Aging, the single greatest risk factor for BPH, is associated with decreased prostatic AR expression in mouse AP and VP.

20

Ferroptosis is a Novel Therapeutic Target for RB-Deficient Lethal Prostate Cancer

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Abstract

Background:

Although the development of next-generation antiandrogens has significantly extended the survival time of patients with metastatic castration-resistant prostate cancer (mCRPC), drug resistance inevitably developed over time. Therefore, the identification of novel therapeutic strategies targeting mCRPC is urgent. RB deficiency is a common genetic event and contributes to the development of therapy resistance and poor prognosis in prostate cancer. However, effective therapies against RB-deficient lethal prostate cancer remain elusive. Here we determined how RB regulates the sensitivity of prostate cancer cells to ferroptosis, a form of regulated cell death driven by iron-dependent lipid peroxidation, and whether we can exploit ferroptosis for the treatment of RB-deficient lethal prostate cancer.

Methods:

To determine how RB regulates ferroptosis in prostate cancer, we generated RB stable knockdown prostate cancer cell lines using lentivirus-delivered shRNAs. We compared cellular sensitivity to ferroptosis and associated lipid peroxidation between control and RB stable knockdown prostate cancer cells. Using western blotting, qPCR, and ChIP analyses, we examined the regulation of ferroptosis-related genes by RB. We also evaluated the therapeutic efficacy of ferroptosis inducers in cell-derived xenograft, patient-derived xenograft, and genetically engineered mouse models.

Results:

Our studies revealed that RB deficiency sensitizes prostate cancer cells to ferroptosis. Mechanistically, we found that the E2F family of transcription factors directly binds and activates the ACSL4 promoter and that RB inhibits ferroptosis by suppressing E2F-mediated ACSL4 transcriptional activation. More importantly, our preclinical studies demonstrated that induction of ferroptosis by JKE-1674, a recently discovered ferroptosis inducer, significantly blocks RB-deficient prostate tumor growth, and metastasis, and improves overall survival of mice in the absence of obvious toxicity.

Conclusions:

Our findings uncover an RB/E2F/ACSL4 molecular axis that governs ferroptosis and also suggest a new approach to the treatment of RB-deficient lethal prostate cancer.

21

Urinary NGAL levels in children with exstrophy bladder: Premonition of renal damage

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Abstract

Background: Urinary NGAL (Neutrophil gelatinase-associated lipocalin) levels have been shown to predict the inception of renal damage in various medical conditions. The present study was conducted to study the role of urinary NGAL levels in children with exstrophy bladder (EB) post single-stage total reconstruction (SSTR) as markers of early renal function derangement.

Methods: Urine samples were collected from children with EB before SSTR (group A, n=11), 5 years post-SSTR (group B, n=40) and controls (group C, n=41) and stored at -20°C. NGAL levels were estimated using double antibody sandwich ELISA.

Results: The mean NGAL level in groups A, B and C was 1.39, 34.24 and 2.58 ng/ml respectively. Amongst group B, the mean NGAL amongst those with GFR ≥80 ml/min/1.73 m2BSA was 29.8 ng/ml and 31.74 ng/ml in those with GFR <80 ml/min. Urine samples were also evaluated at a 6-monthly follow-up post SSTR. Mean NGAL at 6 months was 6.76 ng/ml, at 12-month was 30.3 ng/ml and remained >30 ng/ml at 18 and 24 months. Corresponding DMSA scans did not show the presence of a scar and GFR remained stable.

Conclusions: Urinary NGAL may detect the earliest signs of renal damage even when standard renal function assessment using DMSA and GFR seems normal.

22

Biological plasticity of Renal Pelvis, Pelvic Volumetry & Depleting Renal Reserves During Non-Operative Management Of PUJO-Type Hydronephrosis

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Abstract

Background: The timing and the need for surgical intervention in antenatally diagnosed moderate degree of hydronephrosis due to pelviureteric junction(PUJ) type obstruction still remains a dilemma. This study aims to find the significance of biological differences in the plasticity in renal pelvic tissues, between different individuals viz a viz, additional pelvic dimensions, other than the anterior-posterior diameter in reflecting the severity of hydronephrosis viz a viz across all stages of SFU grading.

Materials and Methods: Data for all patients with PUJ type hydronephrosis with Split renal function(SRF) ≥ 40% with a delayed pattern of clearance, managed from January 2000 till August 2012 under a single surgeon(MB) was retrospectively collected. Serial ultrasound parameters including AP diameter [both renal and pelvis], pelvis lateral diameter, Craniocaudal length, cortical thickness [at upper and lower pole], renal length, and volume of the renal pelvis were measured at both initial and follow-up scans. These were compared to other follow-up parameters like renal dynamic scans, GFR estimation and serum creatinine. For this sub-analysis patients were grouped into those with increasing (group-a) & declining (group-b) pelvic volumes.

Results: Total 87 patients; mean age 24 months; follow-up 13-60 months; 41 required surgery and 46 were managed non-operatively. Increased pelvic volumes (group- a), reflecting an increase in all the 3 dimensions of the renal pelvis, were observed in the non-operatively followed patients. While SRF was slow to decline in this group, it remained above 40%. On the contrary, pelvic volumes showed a significant decline (group- b) from their initial values in patients who developed indications for surgery [p=0.001] during this period.

Conclusions: An increase in renal parenchymal dimensions [A.P.diameter & renal length] reflecting plasticity of renal pelvis & a reduction in upper & lower pole cortical thickness & fall in SRF were consistently observed in both the groups but significantly marked in group-b. In patients with SFU grade II hydronephrosis, progression of disease in the form of an increase in the craniocaudal diameter of the pelvis and an increase in the - AP & Lateral pelvic diameters was observed, although their SRF remained static during the period of this study. The study highlights the significance of measuring additional pelvic dimensions during the non-operative follow-up of infants with PUJO-Type hydronephrosis & underscores the differences in biological factors which determine the difference between individuals who need early surgical intervention.

23

On-tissue imaging of steroid hormones in mouse prostate using MALDI-2

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Abstract

Background:. Benign prostatic hyperplasia (BPH) impacts the majority of the aging male population, with associated lower urinary tract symptoms (LUTS) costing the healthcare industry around $4 billion annually. Steroid hormone imbalances are implicated in the development and progression of BPH/LUTS, with many treatment strategies targeting enzymes that metabolize these hormones. Multiple imaging strategies exist to collect spatiotemporal information within tissues, such as immunohistochemistry (IHC) and mass spectrometry imaging (MSI), yet these techniques are only applicable to proteins. Recently, a novel postionization technique was developed in tandem with matrix-assisted laser desorption ionization MS (MALDI-MS) to allow for increased detection of compounds with poor ionization efficiency, such as lipids and steroid hormones. Here, we employed this postionization technique, known as MALDI-2, for on-tissue detection of steroid hormones in mouse prostate samples.

Methods:. All tissues were flash frozen using liquid nitrogen and embedded in gelatin. Tissue sections of 5µm were cut using a cryostat and set onto ITO-coated slides. Slide images for future reference of tissue position were obtained prior to 2,5-dihydroxybenzoic acid (DHB) matrix application using a robotic TM sprayer. MALDI-2 spectra were acquired using a Bruker timsTOF FleX with MALDI-2 capabilities.

Results:. Initial experiments using 1µg of steroid hormone standards (b-estradiol, androsterone, and 5a-androstan-3a,17b-diol) show drastic improvements in steroid detection, as the protonated peak for b-estradiol (273.18 m/z) was detected using MALDI-2 yet undetected with MALDI alone. Additional hormone adducts were detected for b-estradiol, indicating good overall coverage and detection of steroid hormones using postionization. Future experiments will utilize these standards to determine limits of detection for each hormone listed as well as in tandem with on-tissue imaging for on-tissue quantification.

Conclusions. MALDI-2 imaging provides a novel approach for obtaining spatial quantitative information of steroid hormones in prostate tissue, informing researchers of how these dynamic levels directly influence disease progression and development.

24

Characterizing oxidative phosphorylation defects associated with aging and benign prostatic hyperplasia (BPH)

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Abstract

Background: Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. A major risk factor for BPH is age, with 90% of men in their eighties impacted, and many developing bothersome lower urinary tract symptoms (LUTS). Mitochondrial dysfunction is a hallmark of aging, but its contribution to age-dependent BPH/LUTS is largely unknown. This study is focused on detailed characterization of oxidative phosphorylation (OXPHOS) defects associated with aging in a mouse model of lower urinary tract dysfunction (LUTD), as well as examining the ability of cultured human prostate cell lines to recapitulate these changes.

Methods: The Oxygraph-2 was used to measure respiration in prostate tissue from young (2 months) and old (>24 months) mice. Additional experiments were performed in human prostate stromal cells (BHPrS1) treated with different doses of rotenone, a complex I inhibitor. Additionally, the Seahorse Mito Stress Test was performed on BHPrS1 cells with a transient knockdown of NADH:ubiquinone oxidoreductase core subunit S3 (NDUFS3), a critical complex I protein.

Results: Preliminary studies indicate that aged prostate has decreased respiration in the OXPHOS pathway, consistent with previous data showing a decrease in expression of NDUFS3. Furthermore, cultured human prostate cell lines subjected to multiple Methods: of OXPHOS disruption appear to recapitulate the OXPHOS dysfunction seen in the aged mouse model of LUTD.

Discussion: This study is an important first step in better understanding the bioenergetic alterations associated with LUTD. Future studies aim to identify mechanisms responsible for OXPHOS dysfunction in the aging prostate in both mouse models and human specimens. U54DK104310 (WAR), DK131175 (WAR, DBD), K01AG059899 (TL), T32GM141013 (AEA).

25

CHARACTERIZATION OF THE H1-H3 LOOP AS A COMMON FKBP52 REGULATORY SURFACE FOR THE SIMULTANEOUS TARGETING OF AR, GR, AND PR IN PROSTATE CANCER

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Abstract

CHARACTERIZATION OF THE H1-H3 LOOP AS A COMMON FKBP52 REGULATORY SURFACE FOR THE SIMULTANEOUS TARGETING OF AR, GR, AND PR IN PROSTATE CANCER

IA RODRIGUEZ-PALOMARES; SM Sakells; MB Cox

BACKGROUND: Prostate Cancer (PCa) is one of the most common life-threatening malignancies diagnosed among American men. Initiation and progression of PCa are dependent upon androgen receptor (AR) regulated genes. Functional receptor conformation is influenced by the cooperation of chaperone and cochaperone proteins including the 52 kDa FK506 binding protein (FKBP52). FKBP52 is known for being a positive regulator of AR, PR (progesterone receptor), and GR (glucocorticoid receptor) activity and is a potential therapeutic target for prostate cancer treatment. Previous studies have identified the BF3 surface as an AR-specific regulatory site for FKBP52, and it is likely that there is another common FKBP52 regulatory surface among all the regulated receptors. GR is known to be more hypersensitive to FKBP52 regulation, and mutations within the GR H1-H3 loop affect FKBP-mediated receptor activities. Thus, we hypothesize that the H1-H3 loop is a common FKBP52 regulatory surface for AR, GR, and PR, and that differences within this surface confer FKBP52 hypersensitivity to GR.

METHODS: We conducted site-directed mutagenesis to identify the residues within the human AR H1-H3 loop that are critical for FKBP52 co-chaperone regulation. Taking advantage of the distinct GR hypersensitivity to FKBP52, two classes of functional mutants were generated to make the human AR H1-H3 loop more like human GR or guinea pig GR. In addition, yeast-based reporter assays were performed to assess the role and relevance of those mutations in receptor activity. Similarly, mammalian reporter assays are currently underway to corroborate our findings in a higher vertebrate model system.

RESULTS: We have identified functional mutants within the AR H1-H3 loop that show hypersensitivity to FKBP52 for normal function.

CONCLUSION: Our current data shows that, mechanistically, the H1-H3 loop acts as an FKBP52 regulatory surface for the steroid hormone receptors AR and GR, and suggests that the H1-H3 loop may represent a novel druggable surface for the simultaneous inhibition of AR, GR, and PR.

26

Inhibition of the Wnt/β-Catenin Pathway Overcomes Resistance to Abiraterone in Castration-Resistant Prostate Cancer

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Abstract

Background:. Abiraterone acetate, an androgen suppression agent, reduces testosterone production by blocking the enzyme, cytochrome P450 17 alpha-hydroxylase and has demonstrated improved survival outcomes in patients with advanced-stage prostate cancer. Despite the reduction in testosterone synthesis, almost all patients with metastatic disease relapse, progressing to a more aggressive and lethal castration-resistant prostate cancer (CRPC). Although the androgen receptor (AR) has been associated with therapy failure, the mechanisms underlying this failure have not been identified. Our bioinformatics analysis predicted that activation of the Wnt/β-catenin pathway and its interaction with AR play a major role in attaining abiraterone resistance. We hypothesize that dual targeting of Wnt/β-Catenin and AR could be highly effective in CRPC treatment. **Methods:** Human CRPC cells, C4-2B-Abi (C4-2B abiraterone resistant) were generated by growing C4-2B cells in the progressive concentration of abiraterone 5-20μM to develop resistance and maintained in 5μM abiraterone in the culture medium for >20 generations. These cells were subjected to RNA-Seq analysis and treated with abiraterone and β-catenin inhibitor ICG001 individually and in combination, followed by assessment of cell viability, crystal violet assay, cell cycle analysis, migration, invasion, and expression of various target proteins by Western blotting. **Results:** Next-gen RNA sequencing data revealed a subset of top-ranked genes regulating the Wnt/β-catenin pathway were associated with abiraterone treatment resistance. Treatment of C4-2B and C4-2B-Abi cells individually with abiraterone (20μM) and ICG001 (2μM) for 72 h exhibited a partial suppressive effect in cell growth compared to C4-2B cells. A combination of abiraterone and ICG001 (20μM + 2μM) exhibited significant cell growth inhibition with a marked increase in G1-phase cell cycle arrest, inhibition of migration, and invasion in C4-2B-Abi cells, compared to their parental counterpart. Furthermore, combination treatment led to a significant reduction in cell proliferation as assessed by PCNA along with a reduction in the protein expression of AR, AR-v7, CDC20, PSA, β-catenin and its downstream targets, cyclinD1, and c-Myc, which were more prominent in C4-2B-Abi cells, compared to parental C4-2B cells. Co-targeting AR and β-catenin potentiated PARP cleavage in C4-2B-Abi cells. **Conclusion:** Combined treatment with abiraterone and ICG001 appears to be an effective regimen for the treatment of Abi-resistant prostate cancer cells. This opens a new therapeutic modality for castration-resistant prostate cancer patients. **Grant Support:** Department of Defense grants W81XWH-18-1-0618 and W81XWH-19-1-0720 to SG. Ibrahim M. Atawia is thankful to the Egyptian Ministry of Higher Education for scholarship support.

27

Transcriptional and epigenetic alterations associated with alternative promoter usage during prostate cancer progression

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Abstract

Background: Despite effective treatments for localized prostate cancer (PCa), some tumors recur and progress to lethal metastatic castration resistant prostate cancer (mCRPC) during androgen deprivation therapy (ADT). This progression is accompanied by changes in how transcription factors (TFs) bind to regulatory elements in DNA. Directly assaying TFs in mCRPC biopsies is technically challenging. We hypothesized that transcriptional studies of gene promoter usage could circumvent this limitation and directly assess how TFs impact gene regulation by binding at different promoters of the same gene. In this study, we generated ultra-deep RNA-seq for a large cohort of mCRPC tumors and conducted integrative analysis with matched DNA and methylation data to study how the use of alternative promoters (APs) change during tumor progression and contribute to the development of mCRPC.

Methods: We sequenced the transcriptomes of 104 mCRPC biopsies to >450M reads/sample (10x the typical depth) and combined with published data in normal prostate and localized PCa. Promoter activities were estimated in all 274 biopsies using proActiv after modifications that increased candidate promoters by 14%. We inferred TF enrichment at APs by examining motifs, integrating with public ChIP-seq, and previously published DNA and whole genome bisulfite sequencing in matched mCRPC samples.

Results: We identified 459 and 3,217 APs with differential activity in localized PCa and mCRPC compared to normal prostate, respectively. The activation of APs and the corresponding predicted TF binding was associated with increased expression of genes involved in PCa. Elevated androgen signaling in both localized and mCRPC were correlated with increased AP frequency and use of APs with FOXA1 binding, which was absent in canonical promoters. In mCRPC, APs were further enriched for binding of MYC, HIF1A and E2F1. Furthermore, loss of the tumor suppressor RB1 was associated with increased activity of APs harboring E2F binding sites. APs in treatment emergent neuroendocrine prostate cancer (NEPC) were bound by known relevant TFs such as ASCL1. DNA methylation at APs was highly variable, in contrast to canonical promoters which were less variable, and negatively correlated with AP activation, as exemplified by hypomethylation at APs activated in NEPC. This suggests that the effect of promoter DNA methylation on regulation of gene expression is primarily happening at non-canonical promoters.

Conclusions: We conclude that TF binding at APs not active in normal cells drives gene expression during PCa progression and in response to ADT. Promoter use is strongly correlated with androgen signaling and driver genetic alterations in PCa. DNA methylation changes are associated with AP activity and integrative analysis is important to understanding this regulatory process. A better understanding of how epigenetic reprogramming of TF networks drive lethal PCa will help guide efforts to affect this process as a therapeutic strategy.

28

Activation of neural lineage networks and ARHGEF2 gene in enzalutamide resistant and neuroendocrine prostate cancer cells and associated with patient outcomes

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Abstract

Background:

Treatment-emergent neuroendocrine prostate cancer (NEPC) after androgen receptor-targeted therapies has been an aggressive variant of prostate cancer with unfavorable prognosis. Tumor cells upon the AR-targeted treatment acquire stem-like features through tumor cell plasticity and molecular adaptation, which contribute to neuroendocrine differentiation. Although various histology and biomarkers have been identified to characterize NEPC, the underlying mechanism for early neuroendocrine differentiation is poorly defined.

Methods:

Based on the transcriptomic data of enzalutamide-resistant prostate cancer cell line C4-2B MDVR and two NEPC patient databases, we performed the gene set enrichment analysis to identify neural lineage signature (NLS) genes. For the differentially expressed NLS genes, we studied the correlation with patient clinicopathologic features such as Gleason Score and metastasis status. We also performed Kaplan-Meier survival analyses to determine the association with clinical outcomes. Further, we used specific siRNAs to knock down ARHGEF2 gene expression, cell viability and NED was determined in C4-2B MDVR and H660 cells.

Results:

We identified 95 neural lineage signature genes from C4-2B MDVR and NEPC patients. These genes depict the molecular landscape of neural precursor cell proliferation, embryonic stem cell pluripotency and neural stem cell differentiation, which may represent an early or intermediate stage of neuroendocrine differentiation. These NLS genes positively correlate with conventional NE markers such as chromogranin and synaptophysin, and negatively correlate with PSA, AR, and AR target genes in advanced prostate cancer. Differentially expressed NLS genes stratify small cell neuroendocrine prostate cancer from prostate adenocarcinoma, which is closely associated with clinicopathologic features such as Gleason Score and metastasis status. Higher ARGHEF2, LHX2, and EPHB2 levels among the 95 NLS genes correlate with a shortened survival time in NEPC patients. Furthermore, downregulation of ARHGEF2 gene expression suppresses cell viability and markers of neuroendocrine in enzalutamide resistant and neuroendocrine cells.

Conclusions

The 95 neural lineage gene signature illustrates an early molecular alteration toward neuroendocrine differentiation, which could stratify advanced prostate cancer patients for better clinical treatment and serve as potential therapeutic targets in advanced prostate cancer.

29

Bioengineered BERA-Wnt5a siRNA targeting Wnt5a/FZD2 signaling suppresses advanced prostate cancer tumor growth and enhances enzalutamide treatment

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Abstract

Background:

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

Methods:

Wnt5a and FZD2 expression was examined in enzalutamide resistant C4-2B MDVR cells. Wnt5a and FZD2 expression were modulated using specific siRNAs. Cell growth, colony formation, and migration were studied in vitro. Transcriptomic analysis was performed on C4-2B MDVR cells treated with FZD2 knocked down; gene program of non-canonical Wnt signaling, hallmark androgen response and AR-V7 associated genes were analyzed. A novel tRNA bioengineered Wnt5a siRNA was developed to target Wnt5a/FZD2 signaling. The effect of tRNA-siWnt5a on tumor growth and sensitivity to enzalutamide treatment was evaluated in vitro and in vivo.

Results:

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

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

30

Development of a Novel Oncometabolic Inhibitor for the Treatment of Prostate Cancer

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Abstract

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

Methods: To increase our understanding of SREBP biology in PC cells, we defined the cistrome of SREBPs across multiple PC cell lines via ChIP-Seq. We compared the transcriptomic profiles of genetic targeting of SREBP via siRNA and pharmacological targeting via the novel SCAP inhibitor, SCAPi. We evaluated the effect of SCAPi on cell viability, cell invasion, and AR-signaling in multiple PC cell lines using immunoblotting, luciferase reporters, flow cytometry, MTT, and invasion assays. Additionally, we will evaluate the anticancer activity and safety profile of our novel SCAPi using CDX mouse models.

Results: We found that inhibition of SCAP via siRNA and via SCAPi both yielded similar gene signatures, particularly the perturbation of cell cycle and lipogenesis genes. SCAPi dramatically reduces cell viability, migration, invasion, and expression of both AR- and SREBP-target genes in multiple PC cell lines. Through flow cytometry (Annexin V) and western blots (cleaved PARP) we found SCAPi induces apoptosis within 48 hours in PC cells. Pilot studies in tumor-bearing mice show SCAPi significantly increases survival without inducing animal weight loss.

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

31

Alterations in estrogen metabolism alleviates the development of steroid hormone induced lower urinary tract dysfunction

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Abstract

Background: Benign prostatic hyperplasia is a multifactorial disease that, with a combination of smooth muscle dysfunction, prostatic proliferation, and fibrosis, can lead to lower urinary tract symptoms. Regardless of the underlying manifestation of the disease, age is the single greatest risk factor for the development of BPH/LUTS. Steroidogenesis in the prostate is not limited to the metabolism of testosterone (T) to dihydrotestosterone (DHT); these androgen ligands can be further metabolized into estrogen receptor alpha and beta (ERα and ERβ) ligands. One function of aging in men is the decrease in circulating levels of T while levels of estrogens (E) remain the same or increases, which contributes not only to a decreased T:E ratio but also alter the ERα:ERβ activation. In this study, we use a genetic knockout of a steroid hormone enzyme, CYP7B1, that is critical to the metabolism of 3β-diol, a high-affinity ligand for ERβ, to examine the initiation and progression of lower urinary tract dysfunction (LUTD). We hypothesize that the loss of CYP7B1 increases the accumulation of ERβ ligands leading to decreased proliferation and resolution of LUTD.

Methods: Using wild-type and CYP7B1 knockout mice, in a C57Bl6/129S mixed Background:, we examined the development of LUTD using the steroid-hormone induced model of LUTD. We measured the induction of LUTD upon steroid hormone implantation weekly using void spot assays (VSA). Mice were euthanized after four weeks, and tissues were collected for immunohistochemistry and LC-MS.

Results: The B6/129S mixed Background: mice responded to T+E2 hormones as previously characterized in C57Bl6 wild-type mice. The CYP7B1 wild-type mice with steroid hormones showed a significant increase in urine spot counts suggesting an increase in LUTD. The knockout mice, under the same conditions, showed a significant decrease in void spot counts, suggesting an alleviation of voiding dysfunction characteristic of this model. Interestingly, upon euthanasia of these animals at four weeks post implantation, the knockout mice still presented with urine retention as measured by bladder volume.

Conclusion: Although the loss of CYP7B1 in this mouse model showed an improvement in voiding function as measured by VSA, these mice still developed bladder and prostate changes similar to that of wild-type mice. These Results: could indicate the importance of ERβ activation on alleviating the voiding dysfunction but not the cellular changes within the prostate and bladder.

32

Human prostate stromal cell responses to metabolic stress

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Abstract

Background: Benign prostatic hyperplasia is an age-related disease that is associated with the development of prostatic fibrosis. The aging-related decline in mitochondrial function has emerged as a major contributor to the development of fibrosis in multiple organs given its impact on a variety of maladaptive cellular metabolic responses. Since cultured cells can adjust their metabolism towards glycolysis to combat mitochondrial dysfunction, we examined whether human prostate stromal cells in culture adapt a pro-fibrotic phenotype when forced to rely on oxidative phosphorylation for adenosine triphosphate (ATP) production. This approach also enabled us to test the sensitivity of glycolysis-deficient cells to selective mitochondrial dysfunction.

Methods: Benign human prostate stromal (BHPrS-1) cells were seeded in glucose-containing media. After 24 hours, media was replaced with glucose-free media containing galactose (GAL) to block glycolysis. Additionally, to inhibit oxidative phosphorylation, cells were treated with 0-25 nM rotenone, a mitochondrial electron transport chain complex I inhibitor for 48 hours, three days post-media switch. Cell proliferation and viability assays were performed. Quantitative reverse transcription polymerase chain reaction was performed to determine the expression of markers for fibrosis and mitochondrial function.

Results: BHPrS-1 cell proliferation was significantly but reversibly inhibited upon culturing in GAL media. The restrictive effect on cell proliferation was exacerbated with rotenone treatment, which could not be reversed upon replacement of GAL with glucose-containing media. Quantification of pro-fibrotic markers revealed that the expression of type I collagen, type III collagen, and vimentin increased in BHPrS-1 cells grown in GAL, while expression of PTEN-induced kinase 1, a marker of mitochondrial turnover (i.e., mitophagy), decreased. Moreover, BHPrS-1 cells in GAL media treated with rotenone lost their adhesive properties, with features characteristic of anoikis (i.e., detachment-induced apoptosis).

Conclusions: Restricting ATP production through glycolysis may promote the development of fibrosis in human prostate stromal cells. Furthermore, limiting ATP production to oxidative phosphorylation appears to uncover an exquisite sensitivity of prostate stromal cells to complex I inhibition and provides a model system to prove the potential contributions of mitochondrial dysfunction to anoikis and mechanisms of anoikis resistance in the stroma that could drive fibrosis.

33

Single-cell RNA sequencing of human prostate primary epithelial cells reveals zone-specific cellular populations and gene expression signatures

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Abstract

Title

Single-cell RNA sequencing of human prostate primary epithelial cells reveals zone-specific cellular populations and gene expression signatures

Background:

The human prostate is anatomically segregated into three glandular zones – central (CZ), peripheral (PZ), and transition (TZ). Each zone is associated with different prostatic disease incidence; however, the contribution of zonal variation in prostatic disease etiology is not well understood. While most prostate cancer tumors arise in the PZ, benign prostatic hyperplasia (BPH) occurs primarily in the TZ, and prostatic disease rarely originates from the CZ. Interestingly, the CZ is reported to be developmentally linked with seminal vesicles (SV), which are another androgen-driven tissue that rarely develop disease. Identifying baseline molecular differences between the prostate zones (CZ vs. PZ vs. TZ) may provide insight into the zone-specific development of prostatic disease.

Methods:

Using the 10X Genomics platform, we performed single-cell RNA sequencing on human Prostatic Epithelial Cell (PrEC) cultures collected from CZ, PZ, TZ and SV (SVEC). Cell partitioning, library preparation, and sequencing was done in duplicate for each samples (CZ, PZ, TZ, SV) and bioinformatics analysis was completed in collaboration with the UIUC HPCBio core using Cell Ranger and Seurat. PrECs from additional patients and mining publicly-available data were used for validation.

Results:

Aggregated analysis of all samples identified ten distinct clusters by UMAP. As expected, SVECs clustered separately from the PrECs. Interestingly, two of the clusters identified were specific to CZ samples, while the TZ and PZ populations clustered together. Populations were identified using prostate cell-type specific markers: basal (KRT14, 15, 5, 6A, SOX2, DKK1, S100A6), stem (SERPINB1, LCN2, KRT13, KRT6B, SIX1), luminal (KRT8, 18) and transit-amplifying (CD24). Pathway analysis of zone-specific gene expression showed differential expression of branching morphogenesis and stromal/epithelial interactions, which were validated in PrECs cultured from three additional patients. Interestingly, meta-analysis showed several TZ-specific genes upregulated in BPH and PZ-specific genes upregulated in prostate cancer.

Conclusions

Single-cell RNA sequencing revealed distinct cell population differences and gene expression changes between prostatic zones (CZ, PZ, TZ) and SVs. A better understanding of the molecular differences

between prostate zones is necessary to understand prostatic disease etiology and may provide a foundation for improved therapeutics for prostate disease.

34

Loss of FGF-5 in an in vitro model of CRPC leads to morphologic changes, impedes clonogenicity

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Abstract

Loss of FGF-5 in an in vitro model of CRPC leads to morphologic changes, impedes clonogenicity

Mary M. Stangis, William A. Ricke

Background:

Castration resistant prostate cancer (CRPC) is a lethal, treatment-resistant prostate cancer subtype that can develop via loss of androgen receptor (AR) expression. Fibroblast growth factor 5 (FGF-5) is overexpressed in multiple human cancers, including prostate cancer. In this study, we established a putative FGF-5 knockout (KO) in PC3, a prostate cancer cell line that models AR- CRPC, to investigate the effect of FGF-5 loss on this prostate cancer subtype.

Methods:

PC3 cells were transduced with lentiviral hCMV-Blast cas9 nuclease, and cas9 expression was confirmed via western blot. These cells were then co-transfected with pre-designed synthetic guide RNA (sgRNAs) targeting FGF-5 and a plasmid for GFP and puromycin resistance. Clonal populations were isolated and screened for FGF-5 knockdown using in-cell Western. Immunofluorescence (IF) and qRT-PCR were used to further determine loss of FGF-5, and Sanger sequencing will be used to confirm our putative KO. IF images were taken using the Mantra quantitative pathology workstation and processed using inform. Cell structure and shape was analyzed using the Echo Revolve microscope. Anchorage independence and clonogenic potential was assessed using the soft agar colony formation assay.

Results:

Using IF, we observed FITC fluorescence in PC3 cells and absence of FITC fluorescence in our clonal cell line. We also found that FGF-5 mRNA expression was significantly decreased in PC3 cells that received FGF-5 sgRNA (PC3 sgFGF-5, $p=0.0265$), suggesting successful FGF-5 KO. Loss of FGF-5 in PC3 resulted in both morphologic changes and impeded anchorage independence. We found that PC3 sgFGF-5 cells were significantly wider ($p<0.0001$) and had larger cell area ($p<0.0001$) than parental PC3 cells. PC3 sgFGF-5 cells also formed significantly fewer colonies in the soft agar colony formation assay than parental cells (103 and 61, respectively, $p=0.0458$).

Conclusions:

In this study, we found that loss of FGF-5 in PC3 cells led to a significant decrease in clonogenic potential and anchorage independence. We also found that putative KO cells developed a significantly different morphology. Our next step is to confirm alteration at the genomic level via Sanger sequencing. We also plan to further investigate the mechanisms by which these morphologic changes arise, and look into the impact of FGF-5 loss on tumor growth and invasion using in vivo models.

35

Serum proteomics of prostate cancer in men of diverse ancestry

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Abstract

Background: Prostate cancer (PC) is the most common cancer in men and the second deadliest. African American (AA) men and Caribbean men of African ancestry face the highest risk of developing aggressive PC, making it one of the worst health disparities in the US. We and others have described changes in the tumor microenvironment and epigenome between African American (AA) and European-American (EA) prostate cancer patients. In this study, we evaluate the hypothesis that a distinct serum proteomic signature exists in men of African ancestry that is associated with prostate cancer.

Methods: In this study, we analyzed 225 serum samples from primary, localized PC patients and measured the levels of 38 circulating proteins/analytes that represent cytokines, adipokines, inflammatory markers, cancer markers, growth factors, and other metabolites. We used R and Prism to perform two-way ANOVAs and incorporated clinical factors such as age, BMI, diabetes status, smoker status, Gleason score, and biopsy status.

Results: We found that AA patients had a higher Gleason score within our cohort. PSA levels were not correlated with biopsy status or race. We also found that levels of 3 analytes in particular—fetuin B, ANGPTL4, and osteoprotegerin—were significantly different in EA versus AA PC patients.

Conclusion: In this study we identified 3 novel biomarker candidates for further preclinical studies that differ in EA vs. AA PC patients. Previous studies on PC biomarkers have failed to include racially diverse patient cohorts. Additionally, PC biomarkers are essential to identify subpopulations of patients that differ in disease progression and how they respond to treatment. The selection of the appropriate biomarker can help overcome the health disparity that exists in PC by guiding physicians towards more personalized treatment and accurate prediction of patient outcome.

36

SPOP Loss Places the Prostate Luminal Epithelial Cell at a Selective Disadvantage

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Abstract

Background: Prostate cancer (PC) is the single most common and second-most lethal cancer in men, with over 268,000 estimated cases and over 34,500 estimated deaths in the US in 2022. The Speckle-Type POZ protein (SPOP) mutant subclass of PC accounts for 10% to 15% of all primary PC cases. SPOP is an adaptor for Cullin3/Ring (CUL3-RING)-type E3 ubiquitin ligase complexes and provides substrate specificity. The Cancer Genome Atlas (TCGA) studies show that SPOP is the most frequently mutated gene in primary prostate cancer (PC). Interestingly, PC-associated SPOP mutations are always missense and occur in a heterozygous fashion. The current gap in knowledge is the lack of understanding of the role of wildtype SPOP in PC.

Methods: By utilizing prostate specific SPOP knockout (KO) mice, we recently reported increased levels of AR and MYC protein and increased cellular turnover (both proliferation and apoptosis) in the prostate luminal epithelium compared to wildtype prostates. We now characterized these mice for the expression of Cre protein and SPOP mRNA at different age using immunohistochemistry and RNA in situ hybridization. Furthermore, we performed RNA-sequencing analysis in the SPOP knockout mice and matched control littermates. Moreover, we performed RNA-seq in LNCaP, LNCaP-Abl, and RWPE1 cells following SPOP inhibition via siRNA targeting SPOP. Finally, we compared our SPOP inhibition signature from in vitro cell lines and prostate specific SPOP knockout murine model to gain insights about the role of wildtype SPOP protein in the prostate epithelium.

Result: Using our Spopfl/fl;PBCre+ model, we observed SPOP floxed cells are rapidly lost and the murine prostate epithelium was repopulated with SPOP wildtype carrying cells. Similarly, knockdown (KD) of SPOP through siRNA treatment in a panel of PC cell lines resulted in a significant reduction in cell viability. These observations suggest that SPOP is important for the normal prostate cell viability. Further transcriptomic profiling of SPOP KO (from transgenic murine model) as well as siSPOP treated in vitro prostate cell lines revealed a significant reduction in the transcriptional activity of the AR.

Conclusion: Our data illustrate for the first time a critical role for SPOP in the growth and survival of the prostate epithelium and prostate cancer cell. Our findings further validates SPOP as a important therapeutic target for the treatment of prostate cancer.

37

The dynamic duo: PRAC1 epigenetic silencing and AR in castration resistant prostate cancer.

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Abstract

Background:

Resistance to androgen receptor (AR) directed therapies is one of the key drivers of prostate cancer mortality. Although several molecular alterations involved in castration resistant prostate cancer (CRPC) have been catalogued, a deep mechanistic understanding of the diverse resistance pathways operative in CRPC is still lacking. Here, we describe the role of a previously uncharacterized gene, PRAC1 in treatment resistance and the mechanism that involves epigenetic silencing of the AR co-regulator PRAC1.

Methods:

We applied a reductionist approach by performing whole genome methylation analyses using MBD-seq on paired androgen dependent and castration resistant cell lines to identify candidate gene loci with differential methylation in CRPC. Results: from this screen were validated in CRPC cohorts. In vitro and in vivo loss and gain of function experiments were used to delineate molecular interactions, phenotypic and transcriptomic alterations.

Results:

We demonstrate that the PRAC1 gene locus, which encodes for a 6 kDa peptide, frequently undergoes CpG methylation mediated epigenetic silencing. Loss of PRAC1 expression was tightly associated with resistance to AR directed therapies and was present in ~30% of advanced CRPC but not in hormone naïve tumors. Furthermore, tumors with PRAC1 loss showed a significantly shorter time to progression on AR signaling inhibitors (ARSI). Mechanistically, PRAC1 loss allowed for prostate cancer cell growth in the absence of androgens or presence of ARSIs, suggesting that PRAC1 is a key mediator of resistance. Importantly, PRAC1 binds to the AR and was necessary for efficient AR transactivation and using biochemical Methods:, we showed that these two molecules interact at the molecular level. Additionally, PRAC1 loss dampened canonical AR signaling but induced an AR dependent pro-proliferative expression program. Restoring PRAC1 expression in ARSI resistant models re-established sensitivity to AR directed therapies.

Conclusion

Our data suggests that PRAC1 is a novel AR co-regulator that undergoes epigenetic silencing in CRPC. Loss of PRAC1confers resistance to AR directed therapies. Restoring PRAC1 function in CRPC could represent a novel therapeutic approach in advanced CRPC.

38

The oncogenic transcription factor ERG has distinct roles and signaling regulation in different prostate cancer cell types

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Abstract

Background: ERG is the most common oncogene in prostate cancer, as it is aberrantly expressed in about 50% of cases due to the TMPRSS2/ERG gene rearrangement. ERG is not normally expressed in the adult prostate. The normal function of ERG is confined to hematopoietic stem cells and blood vessels. Aberrant expression of ERG in prostate epithelial cells is oncogenic when combined with mutations that activate the PI3K/AKT signaling pathway. The RAS/MAPK pathway can also regulate ERG function through direct phosphorylation of ERG by ERK. The function of these regulatory events and how they can be exploited to develop therapy for ERG-positive prostate cancer remains unclear.

Methods: ERG function is tested by genomic mapping, gene expression, and phenotypic assays in cell lines and xenograft models. ERG can activate some target genes and repress others. These functions can be separated by a phosphomimetic mutant that can only activate and a phosphonull mutant that can only repress.

Results: ERG expression in prostate basal epithelial cells drives epithelial to mesenchymal transition and stemness properties. This function requires ERG phosphorylation via a positive feedback loop with the TLR4 signaling pathway, and can be repressed by a TLR4 inhibitor. Activation of the PI3K/AKT pathway rearranges the ERG cistrome and alters ERG function, where ERG now promotes luminal epithelial differentiation. In the presence of activated AKT, new ERG targets include genes involved in vascular development and ERG phosphorylation is regulated through a positive feedback loop with the VEGF signaling pathway.

Conclusions: ERG function in basal and luminal prostate epithelial cells appear to mirror ERG functions in hematopoietic stem cells and blood vessels, respectively. These findings suggest strategies of combinatorial inhibition of ERG function in both cell types.

39

Investigation of Bladder Biomechanics Using Dynamic MRI and Computational Fluid Dynamics

Mr Juan Gonzalez-Pereira BSc., Mr Labib Shahid MSc., Dr Shane Wells MD, Dr Wade Bushman PhD, MD, Professor Alejandro Roldan-Alzate PhD

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Abstract

Background: Voiding physiology is most studied with video-urodynamics. Its limitations include (1) inability to simultaneously measure pressure at in the bladder and at multiple positions along the urethra and (2) lack of high resolution and quantitative imaging data. We describe the use of dynamic MRI and computational fluid dynamics (CFD) to characterize biomechanics of voiding in a young healthy man.

Methods: MRI was performed on a healthy young man using a clinical 3T scanner according to an IRB-approved protocol, acquiring bladder images every 3.7 s during voiding. 3D anatomic models were created with MIMICS (Materialise, Leuven, Belgium) and analyzed using a novel algorithm for CFD. This uses computational software to simulate fluid behavior under specified constraints of geometry and flow and enables calculation of voided volume, residual, flow, pressures and wall shear stress.

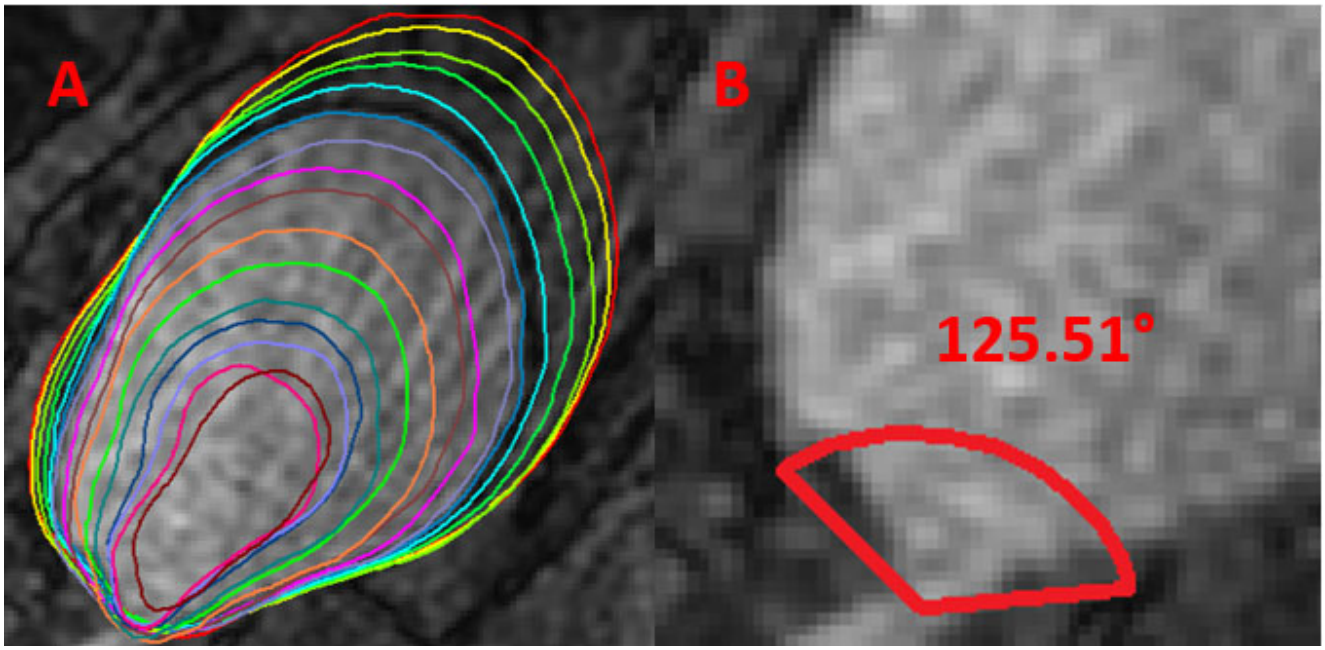
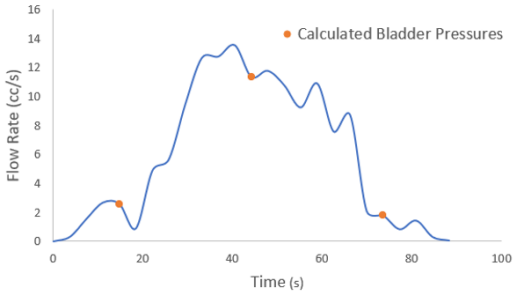
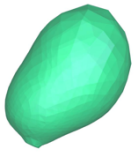


Figure 1: A) Sagittal contour lines of the bladder lumen during voiding. B) Bladder neck angle at maximum flow.

Results: Figure 1 shows a sagittal reconstruction of the change in bladder shape and during the voiding cycle (A) and angle of the open bladder neck at maximum flow. Figure 2 shows the calculated urine flow curve with CFD-determined bladder pressures shown at the indicated time points.



Time from start of voiding (s)	15	44	74
Pressure (Pa) 106000 105400 104800 104200 103600 103000 102400 101800 101200 100600 100000			

TVV (cc)	PVR (cc)	Flow Rate (cc/s)		Bladder Neck Angle (°)		
		Maximum	Average	Pre Void	Maximum Flow	Post Void
521.78	9.63	13.53	5.9	133.9	125.51	175.51

Figure 2: (Left) Urine flow curve determined by time-dependent changes in calculated bladder volume. Timepoints of calculated bladder pressure are indicated by red dots. (Right) Color-coded bladder pressures at three times during voiding. Note: the highest pressure (at 44s) corresponds to near peak flow. Additional calculated metrics shown below.

Conclusion: Dynamic MRI and CFD is a non-invasive alternative to video-urodynamics that provides both superior anatomic detail and a more comprehensive functional assessment of bladder biomechanics during voiding.

40

Dynamic MRI Reveals a Change in Prostatic Urethral Angle During Voiding

Mr Juan Gonzalez-Pereira BSc, Mr Labib Shahid MSc, Dr Shane Wells MD, Dr Wade Bushman PhD, MD, Professor Alejandro Roldan-Alzate PhD

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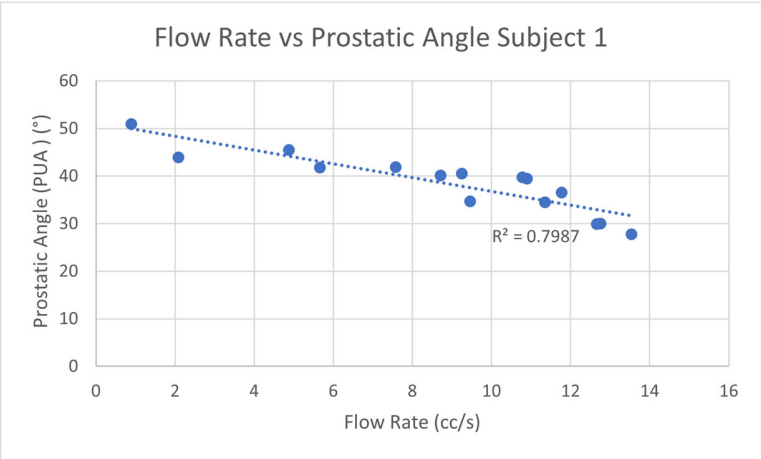
Abstract

Background: Dynamic changes in urethral anatomy during voiding have never been quantitatively assessed. We used dynamic magnetic resonance imaging (MRI) to obtain dynamic, high-fidelity 3D images of the lower urinary tract during voiding. These were then used to create accurate dynamic urethral reconstructions throughput the voiding event coupled with determinations of urine flow, pressure and wall shear stress by computational fluid dynamics (CFD).

Methods: MRI scans were performed on a healthy young man using a clinical 3T scanner according to an IRB-approved protocol to acquire a 3D image every 3.7 s during voiding. 3D models of the urethral lumen were created using MIMICS (Materialise, Leuven, Belgium).

Results: Diameters of the internal and external urethral sphincter (IUS, EUS) and prostatic urethral angle (PUA) were obtained throughout voiding. CFD, using computational software to model and simulate fluid behavior under specified constraints of geometry and flow, was used to determine wall shear stress (WSS), pressures and fluid velocities along the length of the urethra during maximum flow (Figure 1). IUS diameter remains unchanged throughout voiding whereas EUS diameter correlates positively with urine flow rate (data not shown here). Most notably, we noted a striking change in PUA during voiding. PUA becomes less acute as flow increases, being straightest at peak flow rate (Figure 2).

Figure 1: a) Segmentation of the prostatic (purple), membranous (red) and penile (blue) urethras during voiding. b) 3D reconstruction of the urethral lumen. c) Color coded representation of Pressure, WSS and fluid velocity along the course of the urethra during voiding.



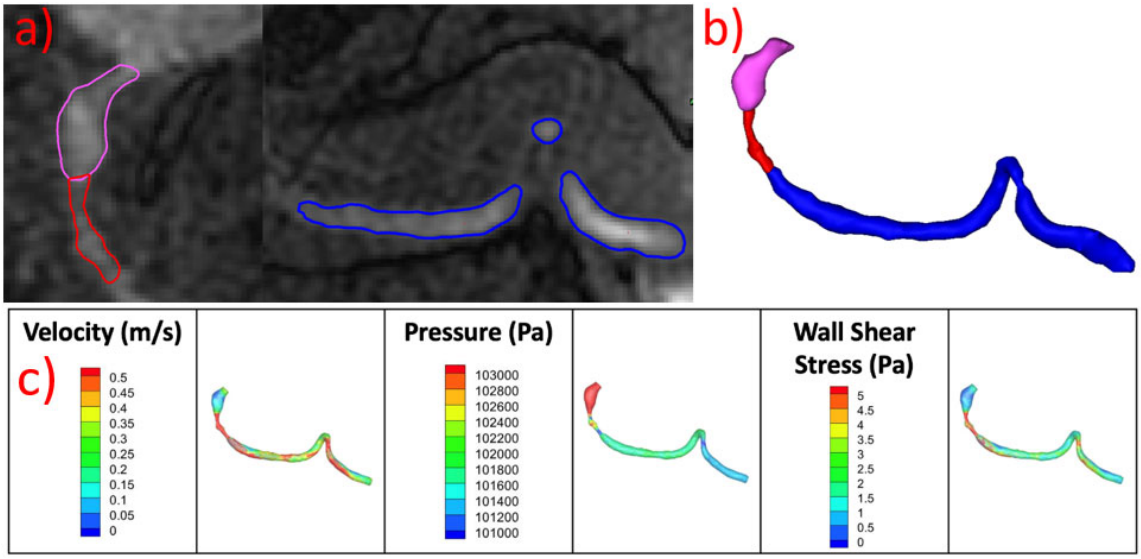


Figure 2: PUA vs flow rate.

Conclusion: Dynamic MRI can be used to define the biomechanics of the urethra during voiding and reveals – for the first time – a change in prostatic urethral angle during voiding that correlates with urinary flow rate.

41

Viral-mediated optogenetic neuromodulation of the rat external urethral sphincter

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Abstract

Background:

Lower urinary tract dysfunction dramatically affects patients' quality of life surviving spinal cord injury. Detrusor-sphincter dyssynergia, where the bladder detrusor muscle and external urethral sphincter muscle are not coordinated, can occur in patients with spinal cord injury. Neuromodulation of the external urethral sphincter motor neurons may be an approach to overcome aberrant sphincter activity. Optogenetics is a powerful and promising method for controlling neural circuits. This study aimed to express opsins into the motor neurons supplying the external urethral sphincter, thereby controlling the external urethral sphincter through light-mediated activation of the opsins.

Methods:

To determine if we can use optogenetics to control the external urethral sphincter, we injected AAV-retro-CAG-ChR2-tdTomato directly into the external urethral sphincter of rats. After five weeks, we evaluated the viral transduction of ChR2-tdTomato into the motor neurons of the external urethral sphincter functionally and histologically.

Results:

We recorded the electromyography (EMG) of the external urethral sphincter while stimulating ChR2 with blue light and evaluated different light intensities and frequencies to replicate filling-induced contractions. We observed that blue light-mediated stimulation of the external urethral sphincter produced EMG activity dependent on light intensity and frequency. We confirmed the retrograde expression of ChR2-tdTomato by histology.

Conclusions:

Our preliminary Results: indicate that using AAV-retro-CAG-ChR2-tdTomato, we could effectively modulate the external urethral sphincter EMG activity. Future studies focus on translating these Results: to rodents with spinal cord injury. We hope these studies will help to improve neuromodulation techniques targeting lower urinary tract function.

42

Renin Angiotensin System activation in Congenital Hydronephrosis and benefits of early surgical intervention

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Abstract

Background:

Controversy surrounds the optimal management of antenatally diagnosed hydronephrosis due to technical limitations in accurately quantifying the degree of obstruction at pelviureteric junction. We have quantified pelvic volumes and plasma renin activity (PRA) as additional measures to evaluate renal outcome.

Methods:

In a 9 years study (mean follow up of 33.0± 17.1 months) in 111 patients, an association was sought between ultrasonography, PRA, split renal function (SRF), GFR and serum creatinine. Pyeloplasty was performed if patients became symptomatic or SRF fell more than 10% during follow up. Statistical Methods: included one way and repeated measures ANOVA and analysis was performed using STATA 9.0 (College Stations, Tx, USA).

Results: Four subgroups were identified: I- continued delayed drainage (n=58); II- improved drainage (n=17); III- continued good drainage (n= 23) and IV- delayed drainage after a period of good drainage (n= 13). Increase in PRA, renal length and decrease in SRF, GFR and pelvic volume were most pronounced with delayed drainage, esp., those requiring pyeloplasty (n=32). In the latter PRA increased by 75.5% between initial and preoperative values and became normal after surgery as against non-operatively followed patients. Rise in PRA was more pronounced in younger patients.

Conclusions:

Early surgery avoids continued obstructive stress to the kidneys and offers the maximum potential for renal recovery.

43

Surveillance of patients with bladder exstrophy epispadias complex: role of urinary biomarkers

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Abstract

Surveillance of patients with bladder exstrophy epispadias complex: role of urinary biomarkers

Abstract

Introduction-The incidence of bladder exstrophy epispadias complex(BEEC) is 1:50,000 live births. Patients born with BEEC have normal upper tracts at birth. They undergo either Complete Primary Repair of Exstrophy or Modern Staged Repair of Exstrophy. The morbidity of a small capacity bladder poses these patients at a risk of chronic kidney damage due to the back pressure changes, repeated reflux, and infection. The process further predisposes them to bladder cancer, the risk being more than 600 times higher in BEEC patients in adulthood as compared to the general population. Our study aims to identify the cohort of BEEC patients at a higher risk for chronic renal damage (CKD) and bladder cancer.

Aim-To study the urinary biomarkers TFF-1, TFF-3, Micro-albumin, and Neutrophil gelatinase associated lipocalin (NGAL) in BEEC as markers for renal injury, and to study BTA, BLCA-1, BLCA-4 and NMP-22 as markers of surveillance for bladder cancer in BEEC.

Methods:- Thirty five BEEC patients and 25 control patients were enrolled and their urinary samples were collected and stored. Standard sandwich ELISA tests were performed to see the levels of the 8 urinary biomarkers in both the groups. The Results: were tabulated and statistically analyzed using SPSS.

Results:- The difference in the median values of TFF-1, TFF-3, BTA and BLCA-4 in the BEEC group and the control group ($p<0.05$) was statistically significant. The ROC curve of TFF-3, had the AUC (98% CI) of 0.73 (0.59 to 0.87) at a cut off value of 7.59mug/ml showed a sensitivity of 73.53% and specificity of 70.83%. The BLCA-4, at a cut off value of 0.0015 or grater had an AUC (98%CI) of 1.00 (1.00-1.00) showed a sensitivity of 100% and a specificity of 95.83%. TFF-1 was significantly higher in those who underwent CPRE as compared to those with staged repair. Furthermore, the difference in BLCA-4 was significantly higher in patients who had undergone repair >10years ago, those who underwent augmentation and those with GFR<80ml/min/m2 for each, independently.

Conclusion-The BLCA-4 can be used as a surveillance marker for determining the at-risk group for bladder carcinoma in patients with BEEC. It may warrant a cystoscopy and biopsy in the high-risk groups.

44

Molecular characterization of human bladder cancer xenografts

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Abstract

Background: Significant advances in bladder cancer genomic profiling led to identification of molecular subtypes that show distinct clinical characteristics and sensitivities to frontline chemotherapy. Broadly all bladder cancers can be categorized into luminal or basal subtypes based on expression of basal and luminal signature genes. In this study, we sought to molecularly characterize nine human bladder cancer xenografts that were orthotopically developed in immunocompromised mice with the goal of assigning them to human bladder cancer subtypes.

Methods: Bladder cancer cell lines derived from bladder cancer patients were cultured in vitro and tagged with a luciferase construct. 200,000 cells were orthotopically implanted into the bladder wall of NSG mice and tumor development was monitored by luciferase imaging. Mice with significant weight loss, hematuria and urinary incontinence or moribund were sacrificed and tumor tissue was collected, fixed in formalin or flash frozen. Formalin fixed tissue was used for histology and immunohistochemical studies. RNA was extracted from flash frozen tissue and DNAase treated. Libraries were prepared and Whole transcriptome RNAseq was performed on Ion Gene Studio S5 (Thermo Fisher Scientific).

Results: All the nine bladder cancer cell lines tested (UC3, UC6, UC9, UC13, UC14, T24, ScaBER, RT4V6 and RT112) successfully engrafted in bladder wall and developed into palpable tumors monitored by increase in bioluminescence with time. H&E staining of xenografts was performed on end-stage tumors to identify cellular composition and architecture. Immunohistochemical staining for Ki67 staining was performed to evaluate the percentage positivity for each xenograft. Alpha smooth muscle actin expression and basal markers (p63, CK5 and CK14) expression was analyzed on each of these xenografts and we identified distinct patterns among these cell lines. RNAseq analysis and basal/luminal/epithelial-mesenchymal transition (EMT) marker expression revealed that among the 9 cell line xenografts, ScaBER was true basal cell line with high basal, low luminal and high EMT markers; whereas RT4V6 was true luminal cell line with high luminal, low basal and low EMT marker expression. UC13, T24 and UC3 were negative for both basal and luminal markers but showed increased expression of EMT markers suggesting that they perhaps represent the claudin-low sybtype. RT112, UC6, UC9 and UC14 were positive for basal, luminal as well as EMT markers.

Conclusion: We were able to use mRNA-based molecular classification to characterize human bladder cancer cell line xenografts into molecular subtypes. We further validated our findings using immunohistochemical analysis. Our studies will be impactful in rationally using these models in bladder cancer research.

45

Development of CTPC, a combined transcriptome dataset of prostate cancer cell lines

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Abstract

Background: Cell lines are the most used model system in cancer research. The transcriptomic data of established prostate cancer (PCa) cell lines help researchers to explore the differential gene expression across the different PCa cell lines and develop hypothesis. Methods: and Results: Through large scale datamining, we established a curated Combined Transcriptome dataset of PCa Cell lines (CTPC) which contains the transcriptomic data of 1840 samples of seven commonly used PCa cell lines including LNCaP, C4-2, VCaP, 22Rv1, PC3, DU145, and NCI-H660. The CTPC dataset provides an opportunity for researchers to not only compare gene expression across different PCa cell lines but also retrieve the experiment information and associate the differential gene expression with meta data such as gene manipulation and drug treatment information. Additionally, based on the CTPC dataset, we built a platform for users to visualize the data (https://pcatools.shinyapps.io/CTPC_V2/). Based on CTPC dataset, we further developed CTPC-ADT dataset which only contains the androgen deprivation treated samples and wildtype/ control samples with finely annotated treatment details. Through analysis of CTPC-ADT, we identified novel AR downstream targets and diagnose markers. Conclusion: It is our hope that the combined CTPC dataset and the user-friendly platform are of great service to the PCa research community.

46

EFFECTS OF ATORVASTATIN WITH OR WITHOUT DEGARELIX ON THE PROSTATE TUMOR IMMUNE ENVIRONMENT USING A MYC-CaP/AS MURINE MODEL

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[1]University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. [2]University of Wisconsin Carbone Cancer Center, Madison, WI, USA

Abstract

Background:

Recent studies investigating racial differences in the gene profiles of patients with prostate cancer demonstrate that Black men have immunosuppressive tumor microenvironments (TME). Differences between ethnicities are also seen in oncogenes where *myc* amplification is enriched in tumors from Black men with metastatic disease. Along with on-target, anti-tumor actions, ADT exerts immunomodulatory effects and induces a unique phenotypic response that accumulating data suggests may be improved with the addition of chemotherapy, androgen-signaling inhibitors or even standard medications. Prior data have shown that androgen deprivation therapy (ADT) and metformin or statins improves oncologic outcomes versus ADT alone. However, when factoring for race, Black men on ADT and statins had worse prostate cancer-specific mortality than White men (unpublished data). We hypothesize that this difference in outcome may be due to the role that statins play in immune modulation and the documented racial differences in the immune tumor microenvironment (TME). In this study, we investigated whether Atorvastatin ± ADT alters tumor growth, proliferation, apoptosis and the immune infiltrate within myc-CaP/AS murine models.

Methods:

FVB/NJ mice were injected subcutaneously with $1x10^6$ Myc-CaP/AS cells. Treatment was initiated at an average tumor growth of $400mm^3$. Groups were randomized into short term and long term groups for a total of 8 mice per group per time period. Mice were injected i.p. with: 1) 5µg/g Lipitor, q2 days x 3 doses (short term) and q2 days x 5 doses (long term) AND/OR 2) Degarelix 25µg/g x 1 dose OR 3) PBS i.p. for controls. The change in tumor volume was calculated as the difference in volume from pre- and post-treatment. Western blotting was used to measure the expression of PCNA and CC3. Immunohistochemical staining for CD4, CD8, FOXP3, GR-1/CD11b followed by flow cytometry analysis was performed.

Results:

ADT + Atorvastatin treated tumors demonstrated the greatest decrease in volume vs control (p=0.018) followed by Atorvastatin-only treated tumors (p=0.029) (Figure). Atorvastatin-treated tumors depicted the greatest CD8 T-cell expansion over time (p=0.022). MDSC infiltration decreased significantly over time for ADT and ADT + Atorvastatin-treated tumors (p=0.002-0.007). ADT and ADT + Atorvastatin decreased tumor proliferation while Atorvastatin monotherapy increased cellular apoptosis. Further, ADT+ Atorvastatin-treated tumors showed a moderate inverse correlation between tumor shrinkage and CD8+ T-cell density. No other treatment group demonstrated a higher concentration of CD8 T-cells with tumor shrinkage.

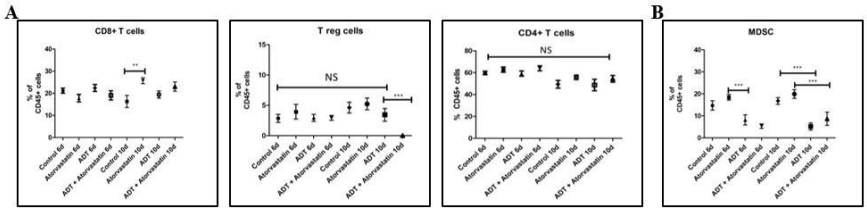

Figure. ***Flow cytometry quantification of T-cells and myeloid derived suppressor cells (MDSCs) post-castration and post-atorvastatin treatment.*** Single cell suspensions of harvest tumors post-treatment analyzed by flow cytometry. Data representative of 6-8 mice per group. **(A) T cell populations as a percentage of CD45+ cells in the TME.** T reg are defined as CD4+/FOXP3+/CD25+. For CD8 T-cells, one-way ANOVA with Bonferroni post-tests showed significant differences present between all groups (p=0.012) and between late Atorvastatin vs control (p=0.022). Atorvastatin-treated tumors depict greatest CD8 T-cell expansion over time. No statistically significant differences identified among the treatment groups for CD4+ cells. Two-tailed unpaired testing showed significant differences in T reg between ADT and ADT + Atorvastatin **(B) Additional population of interest: MDSCs (CD11b+ GR-1+).** One-way ANOVA with Bonferroni post-tests showed statistically significant differences present between all groups (p=0.0001) and between early ADT vs early Atorvastatin (p=0.002), late ADT vs control (p=0.003), late ADT + Atorvastatin vs late Atorvastatin (p=0.007) MDSC expression decreased significantly over time for ADT and ADT + Atorvastatin-treated tumors. ** = p <0.05, *** = p <0.01 NS = not significant

Conclusions and Impact:

Atorvastatin + ADT decreased tumor volume and tumor proliferation when compared to ADT or Atorvastatin alone. Atorvastatin alone increased CD8 T-cell presence, important for cell death, and in combination with ADT decreased MDSC and Treg expression both of which impede cancer cell death. Demonstrating synergy between androgen deprivation and immunomodulatory agents has the potential to shift the treatment paradigm in patients with high-risk prostate cancer.

47

Anxiety Behaviors are Affected in Juvenile Gli2+/- and Gli3+/Δ699 Mutant Mice

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Abstract

Background:

The hedgehog (Hh) pathway is a critically important signaling pathway in embryonic development and dysregulation of Hh signaling can produce defects in both urogenital and brain development. We postulated that mutations of the Hh transcription factors Gli2 and Gli3 in mice would also be associated with measurable changes in behavior.

Methods:

Postnatal day 25-26 (juvenile) Gli2+/- mutant (12M, 10F) and control mice (11M, 10F) and Gli3+/Δ699 mutant (M10, F11) and control mice (M10, F10) were evaluated for social interaction, play, and anxiety behaviors. Social investigation was measured as time spent grooming, sniffing, or following a test stimulus. Play-like behavior was assessed by measuring a "jerk and run" series of behaviors. Anxiety behavior was tested by automated detection of movement in an open field arena. Anogenital distances (AGD) were measured after euthanasia. Statistical analysis was performed by two-way ANOVA with Tukey post hoc comparison.

Results:

No genitourinary abnormalities were observed. AGD measurements were greater in males than females ($p < 0.001$) as expected and was not influenced by genotype. No significant effects of genotype were observed for play-like behavior or social interaction. However, there was a significant impact of genotype on juvenile anxiety-like behavior. Gli2+/-is a Hh loss of function mutation and males exhibited decreased anxiety-like behavior compared to wild type male controls ($p < 0.001$). In contrast, Gli3+/Δ699 is a Hh gain of function mutation and both male and female Gli3+/Δ699 mice exhibited increased anxiety-like behavior compared to wild type controls.

Conclusions

This is the first evidence for altered behavior in mice with genetic disruption of Hh-GLI signaling. Absence of changes in AGD with these mutations argues that the effects were independent of hormonal dysfunction. Previous studies of these heterozygous mutants have not revealed significant genitourinary defects, possibly because changes in Hh pathway activity were not sufficient to disrupt peripheral tissue development. Nonetheless, we found significant and opposite effects on anxiety-like behavior in the Gli2+/- loss of function and Gli3+/Δ699 gain of function mutant mice - arguing strongly for a genetic basis of the observed differences. The significance of these observations to the urologic community is to recognize that genitourinary and brain development share many developmental signaling pathways and that both may be affected by mutations in these pathways.

48

Physiologic Determinants of Urinary Frequency and Urgency in Overactive Bladder

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Abstract

BACKGROUND:

Humans generally exhibit wide variations in voided volumes over the course of 24 hours. This contrasts with the general perception that people void in response to a "full bladder" and prompts speculation that people often void for other reasons such as feeling the sensation of the need to void, urgency, or out of convenience. Patients with overactive bladder (OAB) complain of urinary urgency and increased frequency of voiding. We examined the relationship between the bladder filling rate, the urge to void, and urinary frequency in a study of bladder diaries (BD) of 113 patients with OAB.

METHODS:

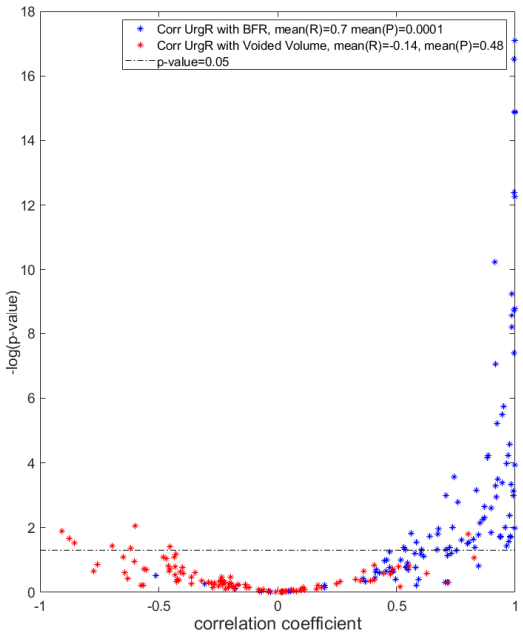
BDs contained timings and volumes of voids and urgency scores at the time of void (0 -no urge, 4-urgency incontinence episode). Bladder filling rate (BFR) and urgency growth rate (UrgR) were defined as voided volume and urgency score divided by the time interval from the previous voids. The frequency metric (Fr) was defined as reciprocal of the time intervals between voids. Note that BFR, UrgR, and Fr all vary among time intervals. Intra-patient Pearson correlation coefficients of these time-dependent variables were calculated for each BD.

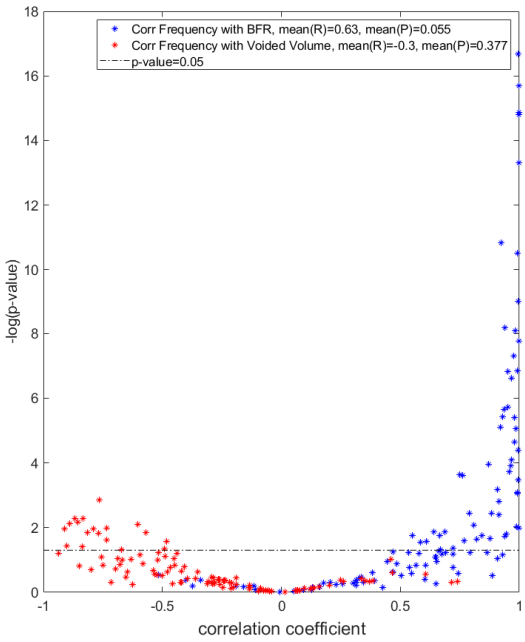
RESULTS:

Urgency growth rate and frequency metric are strongly correlated with BFR but not with voided volume. This is illustrated in volcano plots in Figure 1. Each dot represents correlation coefficient and p-value for a single patient. The vast majority of blue dots (correlations with BFR) are above the significance level, whereas most of the red dots (correlations with voided volume) are below. The poster will show sample bladder diaries with multiple instances of high urgency low volume voids and various values of slopes of UrgR vs BFR dependences suggesting different degrees of sensitivity of the OAB patients to the rate of bladder filling.

CONCLUSIONS

This analysis reveals BFR to be the critical determinant of voiding behavior in patients with OAB. It further suggests that the symptoms of frequency and increased urgency in patients with OAB may be the product of greater sensitivity to the rate of bladder filling.


Corr UrgR with BFR, mean(R)=0.7 mean(P)=0.0001
Corr UrgR with Voided Volume, mean(R)=-0.14, mean(P)=0.48
p-value=0.05
-log(p-value)
correlation coefficient


Corr Frequency with BFR, mean(R)=0.63, mean(P)=0.055
Corr Frequency with Voided Volume, mean(R)=-0.3, mean(P)=0.377
p-value=0.05
-log(p-value)
correlation coefficient

49

Allograft mouse models of bladder cancer representing basal-squamous, stroma-rich, and neuroendocrine molecular subtypes

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Abstract

Background: Bladder cancer is a heterogenous disease and molecular classification of bladder tumors could help drive treatment decisions based on classification. Pre-clinical models representing each classification are needed to test novel therapies. Carcinogen-induced cancer models represent heterogeneous, immune-competent, pre-clinical testing options with many features found in the human disease.

Methods: Invasive bladder tumors were induced with N-butyl-N-(4-hydroxbutyl nitrosamine (BBN) in the drinking water. Tumors were excised and serially passed by subcutaneous implantation into sex-matched syngeneic hosts. Eight tumor lines were developed and named Bladder Urothelium Roswell Park (BURP) tumor lines and characterized by applying consensus molecular classification to RNA expression (in the tumor line name), histopathology, and immune profiles. Two lines were further tested for cisplatin response.

Results: Most BURP tumor lines, 3 male and 3 female, have a basal/squamous (BaSq) molecular phenotype and morphology. BURP16-SR has a stromal-rich (SR) molecular phenotype, and a sarcomatoid carcinoma morphology and BURP19-NE has a neuroendocrine (NE)-like molecular phenotype and poorly differentiated morphology. The BURP tumor lines have unique immune profiles with fewer immune infiltrates compared to their originating BBN-induced tumors. BURP16-SR responded to cisplatin treatment, while BURP24-BaSq did not respond to cisplatin.

Conclusions: The BURP tumor lines represent several molecular subtypes, including basal/squamous, stroma rich, and NE-like. The stroma-rich (BURP16-SR) and NE-like (BURP19-NE) represent unique immunocompetent models that can be used to test novel treatments in these less common bladder cancer subtypes. With multiple basal/squamous established passable allografts, the BURP tumor lines have less heterogeneity than the carcinogen-induced tumors. The six basal/squamous models are in both male and female mice and can be used to evaluate the treatment response without the confounding mixed response often observed in heterogeneous tumors. Additionally, basal/squamous tumor lines were established and maintained in both male and female mice allowing these tumor lines to be used to compare differential treatment responses between sexes.

50

Developing Standard Operating Procedure for Organic Metabolites in Urine for Cancer Diagnosis

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Abstract

Cancer detection can be invasive, expensive, and unreliable. Thus, searching for alternative diagnostic Methods: is of clinical importance. While the area of urinary metabolomic profiling has received interest, there are factors that may affect the validity, specificity, and sensitivity of its applications for cancer diagnosis. The goal of the study was to investigate the influence of sample storage and processing conditions on the urinary volatile organic compounds (VOCs) profiles, and to develop a robust, reliable, and sensitive standard operating procedure (SOP) for such applications. We investigated five controlled independent variables, i.e., storage temperatures, duration, thaw-freeze cycle, sample collection condition, and sample amount. Chemical analysis of VOCs was performed by Stir Bar Sorptive Extraction coupled with Thermal Desorption-Gas Chromatography/ Mass Spectrometry (SBSE-TD-GC/MS). Compound identification was conducted by ChemStation with the National Institute of Standards and Technology Library (NIST17). Statistical analyses, such as linear regression modelling (LRM), partial least squares-discriminant analysis (PLS-DA), principal component analysis (PCA) multi-variate analysis of variance (MANOVA) was performed using R and Metaboanalyst 5.0. Based on our Results:, factors included in this study have no significant impact on urine metabolite profiles suggesting that flexible conditions can be both reliable and adequate within urine storage duration, amount, and temperature. As for Thaw-freeze cycle, the Results: found that temperature and thaw-freeze cycles showcase significant clustering within the urinary profile. Urine samples collected under fasting and non-fasting condition showed no significant impact on the urinary metabolite profile looking into fasting and non-fasting and temperature variables. SBSE-TD-GC/MS and statistical analyses provide a consistent strategy to investigate biomarkers for cancer diagnosis. The Results: showcase these techniques as viable analyses for detecting urinary target compounds for cancer screening and diagnoses. The result of this SOP has the potential to be translated into a standard analyses of urinary VOC metabolites for a non-invasive and sensitive cancer screening techniques and even a potential point of care method.

51

Identification of Protein Cargo in Extracellular Vesicles across Prostate Cancer Subtypes

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Abstract

Background: Prostate cancer is the most common form of cancer in men, and approximately 10-20% of cases will develop into lethal castration resistant prostate cancer (CRPC). Small particles known as extracellular vesicles (EVs) are important mediators of cell-cell communication and may play a role in establishing this resistance as well as provide key information to overcoming it.

Methods: We hypothesize that the proteins and phosphoproteins expressed in EVs can significantly impact treatment resistance via autocrine or paracrine transfer of signaling cargo in CRPC. We have isolated EVs from prostate cancer lines of different subtypes – 3 adenocarcinoma, 3 neuroendocrine, and 2 double negative – to define the EV proteomes by mass spectrometry.

Results: To date, we have prepared and submitted the proteome of the EVs for mass spectrometry analysis from one cell line per subtype. This identified 1,000+ proteins for each cell line, of which over 600 proteins were shared across all three subsets. This suggests substantial overlap in the EV cargo contents across PC lines, as well as unique differences. The adenocarcinoma line 22RV1 had 76 unique proteins, the neuroendocrine line NCI-H660 had 342 unique proteins, and the double negative line PC-3 had 464 unique proteins. We are currently preparing the EV proteomes from all 8 cell lines to compare and contrast EV cargo that may differentiate phenotypes.

Conclusions: We anticipate that the protein cargo of the EVs, particularly the unique proteins per subtype, will have functional roles in the creation or maintenance of resistance. Our overall objective is to understand the composition and function of proteins expressed in EVs and how they contribute to mechanisms of resistance with the long-term goal of identifying key proteins in EVs that drive resistance and can be therapeutically targeted in CRPC.

52

Chemotherapy induces migration in prostate cancer cells.

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Abstract

Background: Chemotherapy is normally the first line of treatment in cancer patients. Depending on the cancer types and the stages of the disease, this strategy could be effective and curative. However, the development of distant metastases is quite common in cancer patients. Recent studies have shown that chemotherapy can be responsible of inducing the cancer cells to escape from death and migrate to distant sites to form metastases. Early stage prostate cancer is localized and is treatable by surgery and radiation therapy; the prognosis in these patients is very good. However, the prostate cancers in later stages of the disease metastasize to other tissues and bone, reducing drastically the overall survival of patients. In prostate cancer patients, the most common treatment approach is androgen deprivation therapy (ADT), based on either inhibition of biosynthesis and/or action of androgens. However, these treatments ultimately lead to the development of castration-resistant prostate cancer (CRPC) and commonly, patients will develop metastatic prostate cancer. Previosly, we have shown the essential role of the heterotrimeric G-protein subunit alphai2 (Gαi2) in prostate cancer cell migration and how small molecule inhibitors targeting Gαi2 are able to reduce the migratory behavior of several cancer types. In this study, we evaluated the effects of anti-androgen drugs (enzalutamide and bicalutamide) on cell migration in AR+ LNCaP prostate cancer cells, and if the combination treatments with Gαi2 inhibitors can affect their migratory behavior.

Methods: We incubated the cells with and without the anti-androgens and the Gαi2 inhibitors, and we performed transwell migration assay, using LNCaP prostate cancer cell lines. We also performed western blot analysis, to evaluate if the treatments with anti-androgens were affecting the expression of Gαi2 protein.

Results: We observed that anti-androgens significantly increased cancer cell migration, compared to untreated cells. Furthermore, we observed that simultaneous treatment with Gαi2 inhibitors blocks prostate cancer cell migration induced by anti-androgens. We also observed that anti-androgens differently modulated the expression of Gαi2 protein.

Conclusions: These preliminary Results: show for the first time that anti-androgens may induce prostate cancer cell migration and a combination treatment with Gαi2 inhibitors could blunt the cell migration effects of anti-androgens.

53

Preliminary Validation of Angiotensin Receptor Type 1 and 2 Expression in the Bladder

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Abstract

Background: It is well documented that patients suffering from interstitial cystitis/bladder pain syndrome (IC/BPS) have increased immune cell infiltration, fibrosis, and inflammation, yet the pathophysiology of this disease remains poorly understood. In other organ systems (including the lung, liver, kidney, and heart) it is well known that angiotensin II, a signaling peptide, plays a critical role in fibrosis, inflammation, and oxidative stress. Tissue damage increases local renin-angiotensin II signaling, which then activates angiotensin receptor types 1 (AT1R) and/or 2 (AT2R). However, there is a lack of information on whether angiotensin plays a role in bladder diseases. The location and extent of angiotensin receptor expression in the bladder have not been thoroughly described. Therefore, we wanted to characterize the expression of AT1R and AT2R in bladder tissue under naive and disease-like conditions.

Methods: We utilized transgenic AT1aR-tdTomato and AT2R-eGFP reporter mice that express the fluorescent reporters under the Agtr1a and At2r promoters, respectively. We injected cyclophosphamide intraperitoneally to evoke bladder inflammation. We then performed immunohistochemistry and co-stained the bladders with bladder cell-type markers to evaluate which cell types express the reporter. We compared the histology between naive and disease-like conditions.

Results: Preliminary analysis indicates that urothelial, blood vessels, fibroblasts, leukocytes, and potentially interstitial-like cell types express AT1R under disease conditions in comparison to naive bladders. The data indicate that interstitial cells express AT1R based on cellular morphology and negative staining for various other cell markers. In addition, quantitative analysis of AT1R expression intensity increases under inflammatory conditions. Preliminary analysis also reveals AT2R expression in the bladder.

Conclusions: Various bladder cell types express both AT1R and AT2R. Reporter expression intensity also increases under inflammatory conditions. These findings warrant further investigation into the role of AT1R and AT2R signaling in bladder diseases. Improved understanding of normal and disease state bladder cellular signaling is critical for the development of effective therapies.

54

Epigenetic Regulation of SPDEF Gene Expression during Prostate Cancer (PCa) Progression and Resistance to Androgen Deprivation and AR-targeted Therapies.

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Abstract

Background: Prostate cancer progression and resistance to androgen deprivation and AR targeted therapies accounts for most of PCa deaths. SAM-pointed domain-containing Ets-like factor (SPDEF) has been identified as a possible suppressor of metastasis in castration-resistant prostate cancer (CRPC) cells in earlier investigations. However, the regulatory mechanisms underlying the loss of PDEF during prostate cancer progression is not completely understood. In the current study, we have investigated the role of epigenetic modulators (DNA methylation and histone modifications) in driving the loss of SPDEF during prostate cancer progression.

Methods: We analyzed data from TCGA (GDC TCGA Prostate Cancer, and TCGA Prostate Cancer (PRAD) for DNA methylation using Xena UCSC genome browser (https://xena.ucsc.edu/) and cell line histone ChIP-seq using cistrome project (http://cistrome.org/db/#/). PCa cells: LNCaP, RCC7/T and PC3 cells were grown in RPMI, DMEM and DMEF/F12 respectively supplemented with 10% FBS and antibiotics, and maintained at 37°C in a humidified incubator. Where indicated LNCaP cells were grown in charcoal stripped FBS and/or treated with Enzalutamide. We used bisulfite sequencing (BSP) to examine the differentially methylated PDEF gene in SPDEF proficient (LNCaP) and deficient (PC3 and RCC7/T) cells. We also employed the ChIP-qPCR to reveal the active and repressive key histone marks across the SPDEF gene, including H3K4me3, H3K27ac, and H3K27me3 histone marks.

Results: In these clinical cohorts, SPDEF expression showed a substantial inverse correlation with the degree of DNA methylation and epigenetic writer enzymes such as DNMT1 and EZH2 levels. BSP experiments revealed that the SPDEF CpG island in PC3 and RCC7/T cells is hyper-methylated compared to LNCaP cells. In highly metastatic PC3 cells, the cistrome project analysis of histone modifications revealed an elevated enhancer repressive mark H3K27me3 (marked by EZH2) and a decreased promoter active mark, H3K4me3, compared with less metastatic LNCaP cells. ChIP-qPCR analysis in RCC7/T and PC3 cells compared with LNCaP cells validated these findings. Moreover, androgen deprivation and enzalutamide treatment of LNCaP cells resulted in decrease in SPDEF expression. Treatment with 5-aza-2-deoxycytidine (DNMT inhibitor) and GSK-126 (EZH2 inhibitor) increased the SPDEF expressions levels and also restricted colony formation, migration and invasion in RCC7/T cells as well as PC3 cells. Moreover, combination of 5-aza-2-deoxycytidine and/or GSK-126 potentiated the effects of enzalutamide in LNCaP cells.

Conclusion: Overall, these findings suggest that DNA methylation and histone modifications play a crucial role in suppression of SPDEF expression during prostate cancer progression and may impact therapy resistance. DNMT and EZH2 inhibitors may help overcome therapeutic resistance by limiting suppression of SPDEF expression and prostate cancer progression.

55

Mechanical Characterization of Murine Bladder During 3D Ex Vivo Filling

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Abstract

Background: The urinary bladder is a highly distensible organ capable of holding a large amount of volume as it fills maintaining low luminal pressures. For mechanical analysis, the bladder is often modeled as an idealized spherical pressure vessel. In reality, the shape of the bladder is closer to an ellipsoid, and the true shape changes throughout filling due to the anisotropic and heterogeneous material properties of the soft tissue. The bladder has been shown to have different material properties in the longitudinal (i.e., apex-to-base) vs circumferential directions as well as distinctive properties between the dome, lateral bladder wall, and trigone. It follows that the urinary bladder has complex mechanical characteristics and accurately modeling the shape of the bladder during filling is necessary to better understand the unique mechanical features of this organ.

Methods: Bladders from male and female mice (age-matched) were dissected and mounted in our novel Pentaplanar Reflected Image Macroscopy (PRIM) System for simultaneous recording of bladder luminal pressure, volume, and shape during ex vivo filling. We also developed a novel method for reconstructing accurate 4D geometries/meshes from PRIM experimental data with a time resolution of 10 Hz. The method is highly automated and utilizes open-source software. 4D meshing of the bladder provides a vectorized organ representation from empty to full capacity (25 mmHg of intravesical pressure). The geometry and pressure measurements were then used to evaluate stress-stretch behavior in the lateral region (i.e., at the equatorial line) and in the dome.

Results: We were able to accurately reconstruct the bladder volume throughout filling in a highly automatized way. The accuracy was validated by comparing the volume result to a manual segmentation at points throughout the experiment. Through the mechanical analysis, we have found that bladder tissue in the lateral area behaves in an anisotropic manner: distensibility is higher in the circumferential compared to the longitudinal direction during filling. Conversely, bladder tissue in the dome behaves in an isotropic manner where stress-stretch behaviors are equivalent in all directions.

Conclusions: This study presents an accurate, automated, and fully open-source method for 4D bladder reconstruction and subsequent calculation of bladder wall mechanical properties. Using these Methods:, we uncovered that while the bladder generally distends anisotropically during filling, the mechanical characteristics of the wall do differ in different locations. Supported by NIH P20DK127554 (MC, BZ, SR, NT).

56

Gene expression profiling of prostate tumors from a race-matched military cohort identifies intratumoral mast cell depletion as a contributor to disease progression

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Abstract

Background: Prostate cancer (PCa) is expected to be the second leading cause of cancer-related death this year for US men, and racial disparities in PCa place African American (AA) men at a much greater risk of developing PCa and having a poorer outcome, compared to Caucasian American (CA) men. Biological factors, including complex differences in immune biology, may contribute to this disparity. The goal of this study was to identify immune differences influencing PCa health disparity, as this has not yet been studied in a military cohort where access to care is available to all qualified beneficiaries.

Methods: We evaluated 51 patient cases (26 AA, 25 CA), with available ex vivo, treatment-naïve tumor biopsies following radical prostatectomy (RP), obtained under IRB-approved protocols. Total RNA from fresh frozen tissue was amplified by PCR-based multiplexed target enrichment and resulting transcripts were evaluated using NanoString nCounter technology. Relative abundances of immune cell subsets were determined using previously published deconvolution algorithms. Immune-related genes as well as relative cell type abundances were evaluated for correlation with clinico-pathologic features and events.

Results: Differentially expressed genes between AA and CA tumors are characterized by genes that regulate cytokine function, innate immune response, and cancer pathways, including Wnt signaling. Among the entire cohort, high expression of NK cell receptor, *KLRC2*, is associated with worse biochemical recurrence (BCR)-free survival. Low abundance of intratumoral mast cells is also associated with worse prostate tumor pathology and poorer BCR- and metastasis-free survival.

Conclusions: Immunobiological differences we have observed based on race and other clinico-pathologic factors at time of RP are clinically relevant. These types of patient-centered studies will help guide precision medicine efforts inclusive of an increasingly diverse US and US military population, and these discoveries have the potential to guide treatment-related decisions, especially for immunotherapies targeting a specific cell type or function.

57

Benign Prostatic Hyperplasia: Signature Genes, Pathways and Novel Therapeutic Strategies

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Abstract

Background: Benign Prostatic hyperplasia (BPH) – a hyperproliferation of epithelial and stromal compartments, is a common pathological condition affecting older men that severely impacts the quality of life due to lower urinary tract symptoms (LUTS). Currently, therapy is limited to 5ARI and/or Alpha-blockers, both of which fail in about a third of patients. Therefore, there is an urgent need for identifying novel molecular-based therapeutic strategies for the effective management of BPH. Through integrating three RNA-Seq datasets from BPH patients, our goal was to identify common differentially expressed genes (cDEGs) that play key roles in BPH pathogenesis and 5ARI resistance.

Methods: Datasets and expression profiles were downloaded from Gene Expression Omnibus (GEO) and Genotypes and Phenotypes (dbGap) databases. DEGs were identified by DNASTAR and Array star and analyzed/represented using RStudio, GSEA, DAVID, STRING, Cytoscape, Immunohistochemistry (IHC), cell proliferation assays and organoid (3D) culture.

Results: We identified 85 cDEGs enriched for biological processes that are associated with BPH. By comparing the common genes with the 5ARI treatment groups, we found SLIT3 as one of the common candidate genes that is dysregulated in BPH. We subsequently validated SLIT3 over-expression at the protein level through immunohistochemistry. We next investigated the molecular functions of SLIT3 through genetic manipulation of SLIT3 in human BPH cell lines. Our studies revealed that when compared with the respective controls, reduced SLIT3 expression resulted in decreased proliferation of both epithelial and stromal BPH cells in culture. Furthermore, in agreement with these Results:, SLIT3 knockdown in benign prostatic cells resulted in smaller and fewer organoids in 3D culture.

Conclusions: Our Results: suggest that SLIT3 plays a key role in BPH pathogenesis.

58

TBX2 is a potential mediator of the switch from Androgen to Glucocorticoid Receptor Signaling in Castrate Resistant Prostate Cancer

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Abstract

Background: A major obstacle in the treatment of metastatic prostate cancer (PCa) is acquired resistance to androgen deprivation therapy (ADT). It is now recognized that ADT, particularly the next generation androgen receptor (AR) antagonists such as enzalutamide, orchestrate plasticity changes/molecular alterations leading to therapy resistance. These molecular alterations are driven by the loss/decrease of AR and a switch in signaling from AR to the glucocorticoid receptor (GR) - a transcription factor that can bind to the same DNA sequences as AR. Therefore, identifying the molecular mechanisms that drive the loss of AR and gain of GR signaling may be crucial in devising novel and effective therapeutic modalities against metastatic castrate resistant prostate cancer (mCRPC). We have previously reported that TBX2, a T-box transcription factor (TF) with both repressor and activator functions, is over-expressed in CRPC and that TBX2 drives PCa bone metastatic progression. In agreement with our findings, a recent report showed that TBX2 is a key TF that drives plasticity associated with CRPC. In this study, we investigated the molecular mechanisms by which TBX2 signaling regulates AR and GR signaling.

Methods: We genetically modulated TBX2 using two approaches: a) dominant negative, DN, to block TBX2 (TBX2DN), and b) overexpression, OE, to increase TBX2 expression (TBX2OE). RNA-seq was performed, and qRT-PCR, Western blot and immunohistochemical (IHC) analyses were used for validation. Further, we used chromatin immunoprecipitation (ChIP) and site directed mutagenesis (SDM) to confirm TBX2 binding on the AR promoter.

Results: Using publicly available databases, we observed a negative correlation between TBX2 and AR; and a positive correlation between TBX2 and GR. RNA-seq revealed an elevated AR signature in PC3 human PCa cells blocked for TBX2. These Results: were validated at the protein level using Western blot and immunohistochemical (IHC) analyses. Further, ChIP and SDM analyses confirmed that TBX2 directly binds and transcriptionally represses AR. Further, using both the DN and OE approaches in human PCa cells, we found a negative association between TBX2 and AR; and a positive association between TBX2 and the GR. In silico analyses revealed TBX2 binding sites on the GR promoter suggesting that TBX2 potentially binds and activates the GR at the transcriptional level.

Conclusions: Overall, our studies suggest that TBX2 could be acting as a switch in CRPC, wherein TBX2 represses AR and activates GR.

59

DNA methylation in driving neuroendocrine phenotype in advanced prostate cancer

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Abstract

Background: Upon prolonged anti-androgen treatment, castration resistant prostate cancer (CRPC) can progress to poorly differentiated, androgen-independent neuroendocrine prostate cancer (NEPC) expressing neural markers. Studies on clinical samples of CRPC and NEPC have shown dramatic differences in DNA methylation while their genomic Background: overlaps substantially. However, mechanistically, we still do not know how these epigenetic alterations arise and how best to leverage these alterations as a therapeutic opportunity.

Methods: To explore the molecular alterations in progressing CRPCs, transcriptome and methylome of histopathologically confirmed tumor foci from a novel genetically engineered mouse model (GEMM) with prostate- specific MYCN induction and Pten/Rb1 co-loss was studied. We performed similar analyses following acute and chronic androgen deprivation in LNCaP cells engineered with MYCN induction and Rb1 loss and patient- derived NEPC xenografts (PDX). Finally, we assessed the therapeutic potential of DNA methylation inhibition in vitro and in vivo using our human and murine models.

Results: In our murine model, we found transcriptome from adenocarcinoma or NEPC foci closely resembled clinical CRPC or NEPC transcriptome, respectively. We also found increased expression of DNA methylation regulators (DNMT1, DNMT3B and TET1) in NEPC foci. A clinically relevant methylome signature based on murine model was found to segregate clinical samples into CRPC and NEPC subtypes which was also validated in acute and chronic androgen- deprived LNCaP cells. Interestingly, AR-target genes (e.g. FKBP5 and TMPRSS2) were found to be hypermethylated and downregulated while cell fate decision regulators (e.g. FOXA2 and PROX1) were hypomethylated and upregulated in NEPC foci. Motif analyses on differentially methylated sites identified specific transcription factors that may contribute to the specificity of differential DNA methylation. Lastly, in vitro inhibition of DNMT with decitabine in GEMM organoids and LNCaP cells showed reduced cell proliferation. This corroborated with findings from short-term in vivo inhibition on NEPC-PDX where tumor growth was significantly reduced upon decitabine treatment for 8 days.

Conclusion: These Results: show that the methylation programs established in our human and murine pre-clinical models are clinically relevant and that DNMT inhibition may provide an alternative therapeutic approach for men with NEPC or CRPC that are progressing to NEPC.

60

Kinase GRK3 connects angiogenesis and neuroendocrine differentiation in prostate cancer progression by enhancing epigenetic activity of HDAC2

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Abstract

Background: Treatment-related neuroendocrine prostate cancer (NEPC) is an aggressive subset of castration-resistant prostate cancer (CRPC), found in ~20% of lethal CRPC. The mechanisms underlying the progression of prostate cancer to NEPC are largely unclear, and new drug targets are desperately needed. NEPC is known to be highly vascularized. Elevated expression of NE markers and increased angiogenesis are two prominent phenotypes of NEPC, and thus are expected to be linked. However, direct molecular links between these two phenotypes are still elusive, whose elucidation will substantially expand our knowledge in NEPC and enable the development of effective treatments for NEPC. Through RNAi & cDNA screening and functional validations, we previously discovered that GPCR-kinase 3 (GRK3) is essential preferentially for highly metastatic cancer cells as compared to lowly metastatic cancer cells. The mechanisms of GRK3 in prostate cancer progression were mostly unknown.

Methods: We assessed GRK3's expression in patient samples through IHC staining on TMA and data mining on public RNA-seq datasets with large cohorts. Using molecular and cell biology Methods:, we determined the impacts of GRK3 and HDAC2 overexpression and silencing on NE marker expression and angiogenesis in prostate cancer cells. Through biochemistry and mass spectrometry, we investigated the nature of GRK3-HDAC2 relation. By ChIP-PCR, we measured the impacts of GRK3 genetic and pharmacological modulations on HDAC2's epigenetic activity. Through compound library screening and biochemical characterization, we searched for novel GRK3 inhibitors to study GRK3 biology and to evaluate whether GRK3 is a suitable drug target for prostate cancer.

Results: GRK3 is significantly overexpressed in metastatic prostate tumors from patients, especially in NEPC. GRK3 promotes both angiogenesis and neuroendocrine differentiation in prostate cancer cells, indicating that it is a key missing link for these two phenotypes. Mechanistically, GRK3 enhances the epigenetic repressor activity of histone deacetylase 2 (HDAC2) to suppress some repressors of angiogenesis or NE phenotype. We have identified several compounds that block kinase activity of GRK3 much more potently than that of GRK2, the closest-related kinase to GRK3. Of note, our GRK3 inhibitors could substantially reduce angiogenesis and NE marker expression, as well as significantly inhibit NEPC cell growth in culture and in mouse xenografts.

Conclusion: Kinase GRK3 connects angiogenesis and neuroendocrine differentiation in prostate cancer progression. Its mechanism of actions is at least in part through enhancing HDAC2's epigenetic activity. Results: based on our novel GRK3 inhibitors suggest that GRK3 is a valuable new drug target for aggressive prostate cancer.

61

Elucidating the Role of Androgen Receptor Variants in Prostate Cancer Lipid Metabolism

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Abstract

Background: Castration-resistant prostate cancer (CRPC) is a lethal form of prostate cancer that develops through multiple mechanisms, including the expression of constitutively active androgen receptor (AR) variants (AR-Vs). Prostate cancer is highly dependent on lipid metabolism and obese men display more aggressive forms of prostate cancer at diagnosis and higher rates of recurrence after surgery. Although there is strong evidence for the regulation of lipid metabolism by the AR, the role of AR-Vs in this process remains unclear. The goals of this project are to understand the mechanisms by which lipid metabolism is regulated by AR-Vs and to identify potential therapeutic targets in this pathway in CRPC.

Methods: We used the isogenic LNCaP/LNCaP-95 and R1-AD1/R1-D567 cell line models of CRPC progression where androgen-dependent LNCaP and R1-AD1 cells express full-length AR and their isogenic androgen-independent sublines LNCaP-95 and R1-D567 express AR-Vs. We used hydrophilic interaction chromatography coupled to tandem mass spectrometry (HILIC LC-MS/MS) lipidomic analysis as well as RNA-sequencing with these cell lines to analyze the levels of specific lipid species as well as the expression of genes related to lipid metabolism. We evaluated the impact of lipid depletion and supplementation on the growth of prostate cancer cell lines using crystal violet staining.

Results: In the LNCaP/LNCaP-95 cell line model of CRPC progression, we identified differential lipidomic profiles and growth responses to lipid depletion and supplementation. Using RNA-seq, we observed differential expression of enzymes involved in the synthesis or degradation of these lipid species between LNCaP and LNCaP-95 cell lines. For example, lipoprotein lipase (LPL) is an enzyme involved in in the uptake of circulating lipids that was expressed in LNCaP95 but not in LNCaP. Further, lipid depletion of culture medium inhibited growth of LNCaP and LNCaP-95 cells, but addition of a set of 9 chemically-defined lipid species selectively rescued the growth of LNCaP-95 cells. Differential lipid requirements for growth and lipidomic profiles were extended to the isogenic R1-AD1/R1-D567 cell line model of CRPC progression.

Conclusions: Collectively, these data indicate changes in lipid requirements and metabolism as prostate cancer cells progress to CRPC. These data indicate a potential for differential roles of AR and AR-Vs in regulating lipid metabolic pathways and sensitivities to lipid manipulations. Understanding these differential roles could lead to the identification of new therapeutic targets in CRPC.

62

FOXA1 inhibits hypoxia programs through transcriptional repression of HIF1A

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Abstract

Background: Tumor hypoxia has been associated with castration-resistant prostate cancer (CRPC), a lethal disease. Hypoxia Inducible Factor 1 (HIF-1), which is composed of HIF1A and HIF1B subunits, is a heterodimeric transcription factor and master regulator of the cellular response to hypoxia. Notably, tumor hypoxia and HIF-1 signaling have been shown to promote tumor progression by inducing tumor angiogenesis, epithelial-mesenchymal transition (EMT), metastasis, and infiltration by immunosuppressive cells, such as tumor-associated macrophages. However, how HIF-1 signaling is regulated in prostate cancer (PCa) is incompletely understood. We have previously shown that FOXA1, an epithelial transcription factor, is downregulated in CRPC. Its loss contributes to aberrant signaling by the androgen receptor (AR) and induces EMT and cell motility through elevated TGFβ signaling. However, whether FOXA1 regulates hypoxia pathways of PCa tumors has not been previously studied.

Methods: We performed RNA-seq, ChIP-seq, qRT-PCR, western blot, and ELISA analyses to assess FOXA1 regulation of HIF1A and downstream pathways. We utilized *in vitro* macrophage migration and cell invasion transwell assays to examine, respectively, how the FOXA1-HIF1A axis regulates macrophage migration towards PCa cells and PCa cell invasion. Finally, we performed bioinformatic analysis of PCa patient datasets to confirm the clinical relevance of our findings.

Results: We found that FOXA1 is negatively correlated with hypoxia markers in clinical PCa samples, and its loss induces hypoxia transcriptional programs. Mechanistically, we found FOXA1 directly binds an intragenic enhancer of HIF1A to repress its expression and downstream hypoxia gene expression. Notably, we identified CCL2, a chemokine known to promote an immunosuppressive tumor microenvironment and cancer progression, as a key target of the FOXA1-HIF1A axis, showing marked upregulation upon FOXA1 depletion in a HIF1A-dependent manner. We found that loss of FOXA1 led to immunosuppressive macrophage infiltration and increased PCa cell invasion, which was abolished by genetic or pharmacological inhibition of the HIF1A-CCL2 axis. Future studies are needed to further elucidate the effects of FOXA1 on hypoxia signaling *in vivo* and evaluate its therapeutic targeting in additional preclinical models.

Conclusion: This study reveals a novel role of FOXA1 as a critical regulator of the PCa hypoxia program and suggests the HIF1A-CCL2 axis as a potential therapeutic target for the treatment of FOXA1-low CRPC.

63

Pudendal nerve injury and its contribution to stress urinary incontinence in a male rat model

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Abstract

Background: Urinary continence in men is maintained by a combination of the detrusor muscle, the proximal intrinsic sphincter, the external urethral sphincter (EUS) which is innervated by the pudendal nerve (PN), and the urethral suspensory mechanism provided by the pubourethral ligaments. However, individual contributions to continence for each of these contributors is unknown. The purpose of this study was to demonstrate the impact of PN injury on male continence both acutely and chronically using a rat model.

Methods: Six male Sprague-Dawley rats underwent acute terminal leak point pressure (LPP) and EUS electromyography (EMG) testing with each of the following interventions in the following order: intact PN, urethra exposed (UE), PN exposed bilaterally (NE), and PN transected (NT). Statistical comparisons were made with a Kruskal-Wallis test followed by a Dunn's multiple comparison test with all groups compared to the NE group ($p < 0.05$). For the chronic study, 67 male rats underwent either pudendal nerve crush (PNC), pudendal nerve transection (PNT), or sham pudendal nerve injury (sham). Sham and PNC rats underwent LPP and EUS EMG testing four days (4d), three weeks (3w), or 6 weeks (6w) after injury. PNT rats only underwent testing 6 weeks after injury. After terminal testing, the urethra and PN were dissected for qualitative histological and immunofluorescence assessment. Outcomes at 4d and 3w were compared with a t-test ($p < 0.05$). Outcomes at 6w were compared with an ANOVA followed by a Tukey multiple comparison test ($p < 0.05$).

Results: Acute PN transection significantly decreased LPP, as well as both EUS EMG amplitude and firing rate compared to the NE group, demonstrating the significant role of PN innervation in maintaining continence in males. There were no significant differences between the intact, UE and NE groups. Both LPP and EUS EMG firing rate were significantly reduced 4d, 3w, and 6w after PNC compared to sham. PNT also significantly decreased both LPP and EUS EMG firing rate compared to sham but not significantly more so than PNC. EUS EMG amplitude was significantly decreased 4d and 6w after PNC but not after 3w, compared to sham. Six weeks after PNT, EUS EMG amplitude was significantly decreased compared to both PNC and sham. Histology and immunofluorescence showed denervation of the EUS.

Conclusions: The PN contributes significantly to urinary continence in male rats. This model could be useful for preclinical testing of neuroregenerative therapies for postprostatectomy incontinence in which the PN is injured.

64

Sulfotransferase SULT2B1b: a novel tumor suppressor in prostate cancer

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Abstract

Background:

SULT2B1b transfers a sulfate group to its substrates, cholesterol and oxysterols, and its sulfated products have been shown to interfere with the normal functions and homeostasis of cholesterol. We have previously shown that SULT2B1b inversely correlates with human prostate cancer (PrCa) status, with abundant expression in normal prostates and decreased expression in tumors. Lowest SULT2B1b levels were found with the most advanced metastatic samples. The function of SULT2B1b in PrCa remains unknown. This study aims to determine whether SULT2B1b plays any active and critical functions in PrCa progression.

Methods:

Doxycycline-inducible SULT2B1b lines were established with both androgen receptor (AR)+ LNCaP and AR- PC3 human PrCa cells. SULT2B1b knockout (KO) clones were established with mouse MycCaP cells. Human cells with inducible SULT2B1b and MycCaP KO clones were implanted subcutaneously into immunodeficient NRG mice and syngeneic FVB mice, respectively. In addition, to investigate whether the immune microenvironment influences the function of SULT2B1b, MycCaP KO cells were also implanted into NRG mice. Tumor growth and animal survival were followed weekly. The growth properties of SULT2B1b-expressing and KO cells were also analyzed in vitro with proliferation and colony formation assays.

Results:

Both LNCaP and PC3 tumors with induced SULT2B1b were smaller in size compared to control tumors (LNCaP, 1015 vs. 1595 mm3, p=0.02; PC3, 1109 vs. 1704 mm3, p=0.003). Consistently, survival rate was significantly improved in mice with SULT2B1b-expressing tumors. In contrast, MycCaP KO cells formed larger tumors compared to parental cells (1366 vs. 526 mm3, p=0.003), accompanied with significantly reduced survival rate. The absence of functional immune cells did not affect the growth of SULT2B1b KO tumors, as these tumors grew at the same rate in NRG mice as in FVB mice and were also larger in size compared to controls (1629 vs. 591 mm3, p<0.0001). Interestingly, SULT2B1b did not have any effects on cell proliferation or colony formation in vitro.

Conclusions

These findings demonstrate that, consistent with its expression pattern in human PrCa, SULT2B1b inhibits prostate tumor growth whereas removal of SULT2B1b promotes tumor growth, suggesting SULT2B1b may function as a critical tumor suppressor in PrCa. However, it does not have a direct impact

on cell growth nor does it require a functional immune system. Whether it alters tumor cell's survival under adverse conditions (e.g. limited nutrients or oxygen) is currently under investigation.

65

Identification of key molecular players in the development of Radiation Cystitis through RNA sequencing

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Abstract

Background: Pelvic cancer survivors that were treated with radiation therapy are at risk for developing radiation (hemorrhagic) cystitis (RC) up to 15 years after completion of radiation therapy. Patients with RC suffer from lower urinary tract symptoms, including frequency, nocturia, pelvic pain and incontinence. At an advanced stage, patients can develop hematuria, which can become life-threatening if bleeding cannot be controlled. To date, no safe and reliable therapy exists to treat RC, in part due to the unavailability of bladder biopsies. Taking biopsies of an already fragile bladder is unethical. The objective of this study is to use our established preclinical model to gain insight into the molecular pathways that drive radiation-induced tissue changes in the bladder.

Methods: Female C57Bl/6 mice received a single dose of 40 Gy using the SARRP irradiator. Bladder physiology was assessed using void spot assay. Bladders were harvested at different time points (1 week, 4 weeks, 3 months and 6 months) after irradiation, RNA was harvested, and mRNA sequencing was performed at paired end 150bp on the Illumina NovaSeq6000 with a target of 30 million reads per sample. Following RNA sequencing, thorough bioinformatics analysis was performed. Findings of the RNA sequencing were validated using qPCR analysis.

Results: At 1-week post-irradiation, altered gene expression was detected in genes involved in DNA damage response, apoptosis, and transcriptional regulation. By 6-month post-irradiation, significant changes in gene expression were observed in vascular health, wound healing, inflammation, and apoptosis. Genes significantly up- or downregulated in these pathways are listed in table 1. Affected pathways include the Wnt signaling, p53, JAK-STAT, and PI3K-Akt pathways. These findings are currently being validated in vitro in human urothelial cells (SV-HUC-1), and in vivo in bladder tissues from our preclinical model.

	Early RC		Late RC		
	DNA Damage/Apoptosis	**Transcriptional Regulation**	**Wound Healing**	**Inflammation**	**Vascular Health**
Up-regulated genes	Rprm	Etv4	F7	IL12b	Col22a1
	Cdkn1	Wnt10a	F10	IL17	Itgax
	Ccng1	Wnt6		IL18	Cxcr2
	Gtse1	Ctnna2		H2-Aa	
	Polk			H2Ab1	
	Bax			H2-Eb1	
				H2-M2	
				Itgax	
Down-regulated genes	Igfbp3	Sox7	Pecam1	Pecam1	Pecam1
			Wnt10b	Ccl21a	Lrg1
				Ccl21c	Cldn5
					Tek

Table 1: Gene expression changes during different phases of RC

Conclusions: The outcome of these studies will have a tremendous impact on the field. This is the first study to determine the molecular changes in the bladder in response to radiation treatment. We hereby identify the physiological changes that drive the progression of radiation cystitis which will aid in identifying potential therapeutic targets.

66

Characterization of novel regulatory factors during the progression to neuroendocrine prostate cancer

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Abstract

Background:

The progression from castration-resistant prostate cancer (CRPC) to neuroendocrine (NE) prostate cancer (NEPC) is driven by several molecular events, including the amplification and overexpression of MYCN (encoding the transcription factor N-Myc). In a cohort of advanced prostate cancer patients, we found an enrichment for the co-occurrence of N-Myc overexpression and the loss of the tumor suppressor RB1. Moreover, patients harboring both genetic events have significantly reduced overall survival. We have previously showed that N-Myc overexpression in a genetically engineered mouse model (GEMM) of prostate cancer can induce a transcriptional profile similar to what is observed in the context of RB1 loss-of-function. However, the molecular programs underlying these changes are not understood.

Methods:

We have used single-cell based approaches, such as scRNA-seq and scATAC-seq, in conjunction with genome-wide N-Myc ChIP-seq, to fully characterize the development of NEPC in a novel GEMM with N-Myc overexpression in a Pten- and Rb1-null Background:. We have also compared the Results: from our GEMM to a cohort of clinical prostate cancer patients, including two scRNA-seq profiles from NEPC patient samples. For in vitro validation, we created isogenic LNCaP cell lines that overexpress N-Myc and carry a CRISPR-mediated deletion of RB1.

Results:

We performed scRNA-seq and scATAC-seq on GEMM prostates collected at 6 and 8 weeks of age. While many cells at these early time points were N-Myc+ and AR-, a distinct subpopulation of cells was present that were N-Myc+ and AR+ but showed low expression of AR target genes, suggesting a transition away from an AR-dependent state. Combined analysis of scRNA-seq and scATAC-seq data revealed the existence of population of cells expressing novel transcriptional regulators which may be implicated in the transition to an NE-like state. Moreover, RNA velocity-based approaches suggest this population of cells exists on a continuum between adenocarcinoma and NE cells. Overexpression of the identified regulatory factors during long-term androgen withdrawal in vitro led to decreased expression of luminal prostate epithelial markers and increased expression of E2F targets and NEPC-related genes.

Conclusions:

We have shown that N-Myc overexpression and Rb1 loss-of-function can synergize to dramatically accelerate tumorigenesis, promote castration resistance, and drive the acquisition of an NE-like state. We have also identified novel regulatory factors that are associated with the CRPC/NEPC transition. Future studies will determine the potential to target these factors therapeutically.

67

High-fat diet fosters c-MYC-driven prostate cancer progression through the accumulation of the oncometabolite lactate and tumor microenvironment remodeling

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Abstract

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

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

hallmarks with clinico-pathological features was investigated in independent human datasets (i.e., TCGA and META855).

Results: Obesogenic HFD accelerates the development of invasive PCa through metabolic rewiring. Although c-MYC alone modulates key metabolic pathways, interaction with HFD is required to induce PCa glycolytic phenotype and accumulation of the oncometabolite lactate. These metabolic effects are associated both with the activation of transcriptional programs linked to disease progression and therapy resistance and augmented infiltration of tumor-associated macrophages (TAM) and FOXP3+ regulatory T cells. Moreover, direct exposure to lactate stimulates neoangiogenesis and PCa cell migration. PCa from patients with high saturated fat intake displays glycolytic features, which promote M2-like TAM infiltration. Finally, high mRNA levels of lactate dehydrogenase correlate with disease recurrence and shorter survival in independent human cohorts.

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

68

Saturated fat and cholesterol promotes prostate cancer lineage plasticity in an autocrine and paracrine manner

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Abstract

Prostate cancer (PCa) incidence affect an estimated 248,530 new cases and 34,130 deaths in 2021. US has the highest incidence rates of PCa worldwide according to a recent report. High fat diet and obesity are associated with the progression and mortality of PCa. Our preclinical data suggested that high fat diet induced PCa cells metastasis to the liver and other soft tissues in mice. Our laboratory initially demonstrated the role of cancer associated fibroblastic cells could support sensitivity to androgen receptor signaling inhibition (ARSi) in cancer progression at an early stage. The premise of our work was the novel observation of a cross-talk occurs between PCa epithelia and associated stromal fibroblasts resulting in a synergistic response to saturated fat, palmitic acid, and cholesterol mediated lineage plasticity of the cancer cells. Objective: Investigate the mechanistic underpinning of how saturated fat and cholesterol reprograms independently contributes to PCa lineage plasticity and ARSi sensitivity, based on the hypothesis that signaling cooperativity involve an epigenetic reprogramming mechanism. Results: We found that exogenous palmitate activated SOX2, a known mediator of lineage plasticity. Histone actyl-K27 ChIP Seq analysis demonstrated differential chromatin activity at the SOX2 promoter. These findings were supported by respective bromo domain modification in response to saturated fat and master-regulator analysis of RNASeq analysis revealed ARID5A activity. The fat metabolite, cholesterol, having been associated with high-grade prostate cancer, could also promote SOX2 expression through the up regulation if cilium and Gli1 activity. The striking finding was that fat and cholesterol independently upregulated SOX2 expression and linage plasticity markers (NMYC, CHGA, NKX2) was revealed by neutralization of the cell surface saturated fat receptor, CD36, and cholesterol biosynthesis inhibition with simvastatin. Interestingly, similar treatment with palmitate co-cultures of PCa lines (ARCaPM or CWR22Rv1) and primary human prostatic fibroblasts resulted in greater SOX2 expression suggesting a paracrine signaling axis convergent on lineage plasticity reprogramming. Stroma-derived Wnt5a was a product of palmitate cross-talk with PCa cells that seemed potentiate cholesterol-mediated Hedgehog signaling. In mouse models, high fat diet promoted liver metastasis of prostate cancer with pronounced SOX2 expression in tumor cells. Conclusions: These studies revealed an interdependent mechanism for fat and cholesterol in epigenetic reprogramming of PCa cells in support of liver metastasis, a lethal manifestation of the disease.

69

The role of cytokines and PD-L1 in regulating EMT in renal cell carcinoma with implications for understanding sarcomatoid differentiation

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Abstract

Background: Sarcomatoid renal cell carcinoma (RCC) accounts for approximately 10 – 15% of all RCC and is thought to arise from an epithelial to mesenchymal transition (EMT) of the parental tumor cell type, most commonly clear cell RCC. While sarcomatoid RCC historically is associated with very poor outcomes, recent studies have shown these tumors to have high response rates to PD-1/PD-L1 directed therapy. We aim to test the hypothesis that cytokines from the microenvironment drive the EMT process in sarcomatoid RCC and that PD-L1 may play an important role in the transition.

Methods: Baseline expression of EMT related proteins and PD-L1 were measured by Western Blot in two clear cell RCC lines (Caki-1 and 786-O) and four sarcomatoid RCC lines (RCJ-41T1, RCJ-41T2, RCJ-41M, UOK-276). To evaluate the factors driving cytokine mediated EMT, the same proteins were measured in the clear cell RCC lines after treatment with TGFβ alone, IFNγ alone, and a combination of TGFβ and IFNγ. To evaluate the role of PD-L1 in EMT, PD-L1 was knocked down by siRNA and its effect on EMT related protein expression was measured by Western Blot.

Results: The clear cell RCC lines had very low expression of the mesenchymal marker N-cadherin and had low expression of EMT transcription factors SNAI1, SNAI2, ZEB1, and ZEB2. Conversely, the sarcomatoid RCC lines all expressed N-cadherin, and varying levels of EMT transcription factors, demonstrating an advanced EMT state in sarcomatoid RCC, although with variation between cell lines. PD-L1 expression positively correlated with increasing EMT state in all cell lines. Treatment of Caki-1 cells with TGFβ or IFNγ led to increased N-cadherin, but through different transcription factors. TGFβ increased SNAI1 and SNAI2, whereas IFNγ increased ZEB1 while decreasing SNAI1 and SNAI2. In combination, the two cytokines had diminishing effect on each other. Furthermore, IFNγ also upregulated PDL1 expression while TGFβ did not. PDL1 knockdown in UOK-276 and 786-O led to decreased expression of N-cadherin and EMT transcription factors.

Conclusions: This data supports the theory that sarcomatoid RCC arises from an EMT process and suggests that cytokines from the tumor microenvironment may drive EMT while also regulating PD-L1 expression. In the cell lines tested, PD-L1 appears to play a role in maintaining the mesenchymal state. These findings may help explain enhanced response to PD-1/PD-L1 directed therapy in sarcomatoid RCC.

70

Vitamin D sufficiency enhances epithelial differentiation of mouse prostate organoids and cancer cell lines

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Abstract

Background:

A large racial disparity exists between white and black men in age of prostate cancer (PCa) onset, disease aggressiveness and mortality. Black men are also at highest risk for vitamin D3 (VitD) deficiency as melanin inhibits cutaneous synthesis. Well-studied for its role in calcium homeostasis, VitD, a steroid hormone, also functions in cell fate decisions, proliferation and differentiation. Healthy epithelial differentiation is crucial for normal prostate morphogenesis and maintenance, a process which becomes dysfunctional in PCa where form/function of glandular structures are lost. Our lab previously showed that VitD accelerated differentiation of human prostate organoids, however, these have limited capacity for luminal differentiation. Here, we delve into the pro-differentiating effects of VitD using mouse prostate organoids (morgs) and VitD-adapted PCa cell lines.

Methods:

Wild type C57Bl-6 morgs were grown from isolated prostate cells and imaged to assess visual changes. Gene expression and subpopulations were assessed by RT-qPCR and scRNAseq. PCa cell lines (22Rv1 & MDA-PCa-2b) were adapted long-term to culture in VitD.

Results:

VitD treated morgs show accelerated growth, size, lumen formation, and secretions compared to controls, supporting enhanced luminal differentiation. VitD response and pro-differentiation genes increased in a dose-dependent manner in VitD treated morgs. scRNAseq of VitD treated morgs revealed unique epithelial subpopulations that express genes known to regulate cellular differentiation. Notably, scRNAseq showed a much stronger effect of VitD compared to androgens alone. Cell lines adapted to VitD show significant differences in expression of differentiation genes (AR, KLK3, & NKX3.1) compared to short-term treatment.

Conclusions

VitD enhances epithelial differentiation in morgs and PCa cell lines suggesting that it impacts overall prostate differentiation. Therefore, VitD deficiency may negatively affect prostate differentiation leading to increased risk of aggressive PCa. As long-term adaptation presents significant differences from short-term treatment in both VitD and androgen regulated genes, ongoing work includes total RNA sequencing of VitD adapted cell lines to assess major transcriptional changes and xenograft implantation to assess aggressiveness in vivo. As VitD is easily supplemented, our studies provide rationale to prevent VitD deficiency to maintain normal prostate epithelial differentiation, which may reduce risk of aggressive PCa.

71

A CELL-AUTONOMOUS MECHANISM PROPELLING TUMOR MUTATIONAL BURDEN AND HETEROGENEITY IN PROSTATE CANCER

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Abstract

Background: Intratumoral heterogeneity and mutational burden provides the fuel of resistance for many targeted therapies including AR targeted therapies in prostate cancer (PCa). Emerging evidence indicates that tumor cells could hijack the powerful mutagenesis machinery mediated by DNA deaminase APOBEC proteins to intensify mutagenesis, promote tumoral heterogeneity, and foster therapy resistance. However, the molecular details of this mechanism have yet to be fully characterized.

Methods: Through a multi-disciplinary approach integrating bulk and single cell RNA-Seq (scRNA-Seq), whole-genome exome-sequencing (WES), and CRISPR library screening, we revealed that ectopic activity of APOBEC-driven mutagenesis as a key molecular driver event promoting tumor mutational burden, heterogeneity, and AR therapy resistance.

Results: For the first time, our Results: demonstrate a cell-autonomous mechanism, through which PCa cells hijack the APOBEC-driven mutagenesis machinery to propel mutational burden, tumor heterogeneity and resistance. We also demonstrated that this ectopic APOBEC-driven mutagenesis as one of the major sources of mutations observed in key oncogenic driver genes, including FOXA1 and EP300. Our single cell transcriptomic analysis of around 40,000 cells, combined with somatic mutation calling from WES analysis, revealed previously unparalleled details of the evolution and dynamics of temporal tumoral heterogeneity, as well how this heterogeneity fuels AR targeted therapy resistance. Contrary to traditional beliefs, we show that the predominately resistant subclones are not the tumor cells that carry AR mutations, but rather the cells that carry FOXA1 mutations which evolutionarily outcompete AR mutated cells and confer resistance in an AR-independent manner.

Conclusions: These findings expose the molecular mechanism responsible for controlling and activating APOBEC-driven mutagenesis, which is primed to increase tumor mutational burden, tumoral heterogeneity, and AR therapy resistance. These Results: also suggest that inhibition of APOBEC functioning is a potential therapeutic avenue to overcome resistance.

72

Identification of FKBP51 and FKBP52 interactors implicated in androgen-mediated castration resistant prostate cancer

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Abstract

Identification of FKBP51 and FKBP52 interactors implicated in androgen-mediated castration resistant prostate cancer

BACKGROUND: Prostate cancer (PCa) is a hormone-sensitive malignancy that relies on androgen receptor (AR) signaling for proliferation and survival. Current PCa treatments exploit this dependence by targeting androgen synthesis and signaling, but resistance mechanisms invariably lead to the development of Castration Resistant Prostate Cancer (CRPC). Considering that AR function requires the collaboration of co-activators and chaperone molecules, there is a growing interest in the role of chaperones in androgen-dependent CRPC progression. Previously, FK506-binding proteins, FKBP51 and FKBP52, have been implicated in AR-dependent mechanisms crucial for prostate carcinogenesis. Accordingly, our lab showed that FKBP52 directly interacts with β-catenin to promote AR co-activator recruitment and signaling. Therefore, better understanding the signaling pathways and protein-protein interactions regulated by FKBP51 and FKBP52 influencing AR function may provide novel therapeutic targets. Here, we identified novel protein interactions involved in AR-dependent CRPC.

METHODS: To determine changes in gene expression profiles influenced by FKBP52, the AR-dependent cistrome and transcriptome was assessed by ChIP-seq and RNA-seq in 22RV1 CRPC cells with or without FKBP52 and hormone. To identify auxiliary proteins involved in FKBP51- and FKBP52-mediated AR activity, an interactome analysis was conducted by tandem affinity purification and mass spectrometry in 22RV1 cells. Protein interactions of interest were then validated via reciprocal co-immunoprecipitations and purified recombinant protein pull-downs.

RESULTS: A previous pathway analysis of genes affected by FKBP52 demonstrated dysregulation of oxidative phosphorylation pathways in fkbp52-deficient 22Rv1 cells. Consistent with these findings, heatmaps representing interacting proteins for FKBP51 and FKBP52 show that members of the peroxiredoxin family, implicated in oxidative stress protection and resistance to treatment, are among the top 100 protein interactors.

CONCLUSION: Our preliminary analysis suggests that FKBP proteins interact with the peroxiredoxin family of proteins, and that the FKBPs may have a role in protecting PCa cells from oxidative stress. This study contributes to the growing list of functionally diverse proteins that interact with the FKBPs, which may underlie aberrant AR activation in prostate-tumor cells and can serve as novel therapeutic targets to disrupt FKBP regulation in prostate cancer.

73

Bladder Wall Biomechanics Differ between Male and Female Mice

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Abstract

Background: The high compliance of the bladder wall allows for the storage of relatively large volumes of urine, while simultaneously providing enough structure so that the detrusor muscle can generate forceful voiding contractions. Changes in the structure of the urinary bladder wall and alterations in bladder compliance are present in numerous urinary bladder pathologies. Yet, we know very little about how mechanical properties of the bladder wall differ between sexes even though bladder dysfunction is more prevalent in females than males. Thus, we measured and compared the mechanical properties of normal bladders from male and female mice.

Methods: Voiding behavior was measured from age-matched male and female mice using the void spot assay. Whole mouse bladders were then dissected and mounted in our novel Pentaplanar Reflected Image Macroscopy (PRIM) System for simultaneous measurement of intravesical pressure, infused volume, and bladder shape. These measurements were used to calculate bladder wall stress and stretch during *ex vivo* bladder filling as intravesical pressure increased from 0 to 25 mmHg.

Results: No significant differences in quantity of void spots, void spot volume, or primary void volume were noted between sexes. However, bladder wall compliance was significantly increased in male mice *versus* female mice.

Conclusions: This study uncovered that the mechanical properties of the bladder wall differ between sexes, even in the absence of bladder dysfunction. These data suggest that the relationship between the mechanical properties, structure, and function of the bladder wall are key in understanding how bladder dysfunction can develop in both sexes. These data also suggest that “normal” clinical bladder compliance may differ between sexes. Future studies will investigate how the arrangement and expression of extracellular matrix proteins differ between male and female mice.

74

Therapeutic efficacy of AURKA and PARP co-targeting in Prostate Cancer

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Abstract

Background: Prostate cancer (PC) is the most commonly diagnosed non-skin cancer among males in the US. The primary reason of PC mortalities is metastatic castration-resistant PC (mCRPC). mCRPC accumulates genomic mutations in homologous recombination (HR) genes such as BRCA1/2, causing deficiency in proper DNA double-stranded breaks (DSBs) repair. Poly (ADP-ribose) polymerase (PARP) is an enzyme that regulates DNA repair, and PARP inhibition (PARPi) causes lethal DSBs that are not repaired in HR-deficient tumors. However, only 19.3% of mCRPC patients are HR mutants and benefit from PARPi.

Aurora Kinase A (AURKA) is a kinase that is upregulated in mCRPC and associated with poor prognosis. AURKA regulates mitotic entry during cell division. When overexpressed, it is able to override cell cycle arrest upon genomic mutations caused by HR deficiency and/or PARPi. Moreover, AURKA has been shown to regulate protein stability of MYC oncogene, which inter alia induces HR gene expression. This suggests that AURKA inhibitors (AURKAi) might be useful for both HR-deficient and -proficient PC and may synergize with PARPi.

Methods: We evaluated viability of HR-deficient and HR-proficient PC cell lines with cell titer-glo assay. We used Incucyte live-cell imaging to assay the therapeutic effect of AURKAi and PARPi Olaparib, either alone or in combination. We used immunoblotting to survey DSB, DNA damage response, and apoptosis. Whether AURKAi impairs HR was assayed by pLCN DSB reporter system. Finally, in vivo efficacy of AURKAi, PARPi was evaluated on CRPC xenograft model.

Results: Here we tested a new-generation AURKAi with high selectivity towards AURKA and favorable safety profiles as shown in early clinical trials. We found that AURKAi remarkably reduced AURKA activation in PC cells at concentration as low as 10nM. By contrast, AURKB and AURKC remained unaffected. Critically, as a single agent AURKAi inhibited the growth of both HR-deficient cells such as LNCaP, C4-2B, and 22Rv1, and HR-proficient VCaP cells with IC50 less than 0.35uM. Mechanistically, we observed that AURKAi induced DSBs, DNA damage response, and apoptosis, along with reduced MYC protein levels that may be associated with HR suppression. As controls, the HR-deficient cells showed high sensitivity to PARPi Olaparib, whereas VCaP is unaffected by RAPR inhibition. However, we observed as strong synergistic effect with the combination of AURKAi and PARPi in both HR-proficient and -deficient cells, further suggesting that AURKAi might lead to HR deficiency de novo.

Conclusion: We report that a new-generation high-selective and safe AURKAi creates de novo HR deficiency, is effective in suppressing the growth of a wide-range of PCa cells and shows synergy with Olaparib. Our data suggest that AURKAi might allow PC patients with both HR-proficient and -deficient tumors to benefit from PARP inhibitors.

75

ZBTB7A as a novel vulnerability in neuroendocrine prostate cancer

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Abstract

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer. NEPC is characterized by the loss of androgen receptor (AR) signaling and transdifferentiation toward small-cell neuroendocrine (SCN) phenotypes, which Results: in resistance to AR-targeted therapy. NEPC resembles other SCN carcinoma clinically, histologically, and genomically. We have recently reported the enrichment of RET kinase activity and its overexpression in NEPC cell lines and tumors, suggesting RET as a promising therapeutic target in this lethal variant. Thus, we aimed to find other viability regulators in NEPC by exploring a broader set of SCN-like cancer cell lines using RET as a marker of NEPC. We discovered ZBTB7A, a transcription factor, as a candidate promoting the progression of NEPC. ZBTB7A has been reported to play oncogenic or tumor suppressive roles in several human cancers depending on the tissue and cancer type. However, its function in NEPC has not been investigated to date.

Methods: We leveraged SCN phenotype scores of cancer cell lines and gene depletion screens from DepMap to identify vulnerabilities in NEPC. To identify a potential role of ZBTB7A in NEPC, we explored gene networks in patients with castration-resistant prostate cancer using informatic modeling of whole transcriptome sequencing (WTS) data from tumors of patients. We examined the potential differences of ZBTB7A function that are robust in NEPC versus prostate adenocarcinoma (AdCa) using Gene Set Enrichment Analysis (GSEA). We performed genetic perturbation using shRNAs to validate the effect of ZBTB7A on the cellular growth of an NEPC cell line, NCI-H660. The impact of silencing ZBTB7A on cell cycle progression and apoptosis was evaluated using flow cytometry.

Results: Cancer cells with high SCN phenotype scores showed a strong dependency on RET kinase suggesting the NEPC-like phenotype of the cells. Then, we observed a strong correlation between the dependencies of RET and ZBTB7A in the same cells. Gene network analysis of WTS data revealed the distinct gene networking patterns of ZBTB7A in NEPC versus prostate adenocarcinoma. Specifically, we observed a robust association of ZBTB7A with genes promoting cell cycle progression in NEPC, and this result was confirmed through GSEA. Furthermore, we showed a potential interaction of ZBTB7A with apoptosis-regulating genes. Silencing ZBTB7A in NCI-H660 cells confirmed the dependency of cells on ZBTB7A for cell growth, and the role of ZBTB7A in regulating G1/S transition in the cell cycle and inducing apoptosis.

Conclusions: Collectively, our Results: highlight the oncogenic function of ZBTB7A in NEPC, and emphasize the value of considering ZBTB7A when developing therapeutic strategies for targeting NEPC tumors.

76

Genetic alterations induce distinct histone post-translational modifications during the transition to castration-resistant prostate cancer.

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Abstract

Background: Epigenetic alterations can impact gene expression and have been implicated in tumorigenesis. One process is the post-translational modification (PTM) of histone proteins governed by histone modifying enzyme (HME) activity. There is growing evidence that dysregulation of HMEs play an essential role in cancer initiation and progression. In this study, we hypothesized that alterations in histone PTMs and their associated enzymes drive the development of castration-resistant prostate cancer (CRPC) from hormone-sensitive prostate cancer (HSPC). To address this, we screened several histone PTMs in a series of prostate cancer patient-derived xenografts (PDXs) before and during the transition to CRPC to identify changes in HMEs. We validated our findings in human prostate cancer samples and cell lines. This novel study provides an unbiased assessment of unique epigenetic states during the development of CRPC.

Methods: Levels of ~100 histone PTMs were analyzed in 10 paired HSPC and CRPC PDXs using quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The histone PTM patterns were subcategorized based on common genetic alterations found in prostate cancer. The RNA-sequencing data for the PDXs and publicly available human prostate cancer datasets were used to characterize the expression of several HMEs. Co-expression of CHD1 and NSD2 was examined in prostate cancer patient tissue microarrays (TMAs) (n=49). *CHD1*-knockout (KO) clones were generated in CRPC cell line DU145 using CRISPR-Cas9 technology. Levels of histone PTMs and NSD2 were assessed by LC-MS/MS, Western blot, and RT-qPCR. The effect of knockdown of NSD2 using shRNAs was examined in *CHD1*-wildtype (WT) and -KO DU145 cells.

Results: A comparison of all PDX tumors revealed a unique signature of histone PTMs. We observed increased histone H3K14 and H4 acetylation in CRPC tumors relative to HSPC tumors ($p < 0.05$). We noted distinct histone PTM patterns associated with genetic alterations in these PDXs. When compared to tumors with *CHD1*-WT (n=4), tumors with *CHD1* loss (n=6) showed a marked decrease in histone PTM H3.3K36me2 ($p < 0.05$). *CHD1* is a chromatin remodeler commonly deleted in ~ 15% of primary human prostate cancers. Screening the differential expression and activity of HMEs in these PDXs revealed that histone lysine methyltransferase NSD2 correlates with *CHD1*. The human CRPC dataset SU2C/PCF 2019 showed a strong positive correlation between CHD1 and NSD2 ($r=0.44$, $p < 0.0001$) in contrast to the HSPC TCGA Firehose Legacy dataset ($r=0.20$). Human CRPC TMAs showed decreased expression of NSD2 ($p < 0.05$) and its histone mark H3K36me2 ($p < 0.01$) with reduced levels of CHD1 ($p < 0.01$). *CHD1*-KO in the CRPC cell line DU145 showed a similar pattern of loss of H3.3K36 methylation (decrease in H3.3K36me2) and a 0.5-fold decrease in NSD2 expression ($p < 0.05$). The dual loss of *CHD1* and NSD2 in DU145 suppressed the cell proliferation more relative to *CHD1*-KO only ($p < 0.01$).

Conclusions: We have identified a unique epigenetic signature associated with the genetic loss of *CHD1* during the transition to CRPC. The expression of NSD2 is altered in the CRPC state in a *CHD1* status-dependent manner at mRNA and protein levels. This research raises the possibility of a potential link between *CHD1* and NSD2 and their role in epigenetic dysregulation in CRPC. Finally, this study provides a

putative role of *CHD1* as a biomarker for predicting responses to inhibition of NSD2 and NSD2 regulated pathways in CRPC.

77

Survey of B and Plasma Cells in Bladder Cancer Across Tumor Microenvironments

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Abstract

Introduction:

Tumor infiltrating B cells in bladder cancer (BLCA) have been associated with cancer invasion, but little is understood of their roles in the tumor microenvironment. In this study, we analyzed data from a large single cell (SC) RNA sequencing cohort of tumor (n=18) and PBMC (n=16) specimens from 26 BCa patients and PBMC samples from healthy donors (n=3) to profile B cells and plasma cells (PCs) in relation to BCa sample types.

Methods:

SC data was imported into R (v4.03) using Seurat (v4.0.1). Genes expressed in fewer than 3 cells, cells with <200 or >2500 unique genes, and cells with >15% mitochondrial genes were discarded. Data normalization, variable gene identification, integration, scaling, dimensionality reduction and graph-based clustering were performed.

Results:

We identified 11,254 naïve and memory B cells and PCs by known marker genes (Fig 1A-B). Profiling these populations across sample types, we found a proportional decrease in naïve B cells in tumor and patient PBMC compared to healthy PBMCs, whereas PCs exhibited the opposite trend (Fig 1C). In addition, PCs from patient PBMC had upregulated expression of MHC class II genes and PTPRC (CD45), whereas those from tumors had higher expression of IGHG1/2/3/4 and CXCR4, suggesting that tumor PCs represent a more mature, longer-lived subtype (Fig 1D). Clinically, we found that TCGA patients with high IgG1 or IgG3 expression in the setting of high numbers of tumor-infiltrating NK cells (captured by the NK-specific marker NCR1) have significantly longer survival compared to patients with low IgG1 or IgG3 (Fig 1E-F).

Conclusions:

In our study of a large BLCA SC cohort, we observed significant proportional differences in B cell and PCs across BLCA tumors, PBMCs, and healthy PBMCs that could possibly be attributed to a stall in maturation or competing maturation pathways between PCs and memory B cells from GC B cells in BLCA

tumor. We also observed significant upregulation of immunoglobulin genes in tumor relative to BLCA PBMCs, and showed that high IgG1/3 in the setting of a large number of tumor-infiltrating NK cells improves survival. This benefit could be attributed to enhanced tumor killing from upregulation of antibody dependent cellular cytotoxicity, a process that is highly dependent on activation of the CD16 receptor on NK cells by IgG1/3.

Figure 1

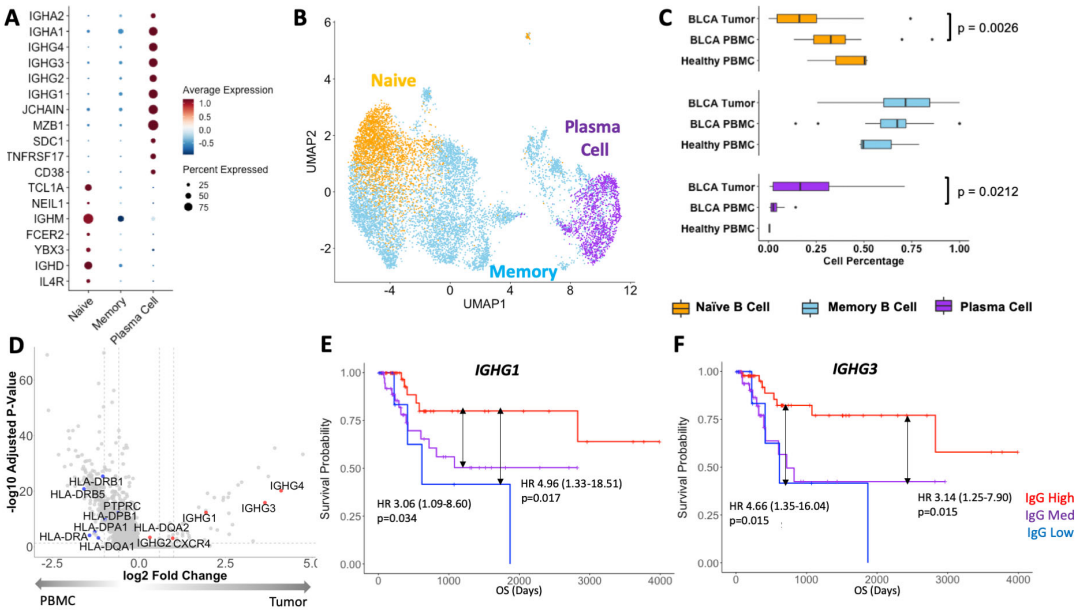

Figure 1

(A) B cell and PC marker expression in naive B, memory B, and PCs.
(B) UMAP visualization of B cells and PCs from scRNA-seq analysis of bladder cancer and healthy PBMC samples.
(C) Proportional abundance of naïve and memory B cells and PCs across bladder cancer tumor and PBMC and healthy PBMC.
(D) Volcano plot of differentially expressed genes between PCs from bladder cancer tumor compared to PBMC. Significant genes (adj. $p < 0.05$) are highlighted.
(E-F) Survival of TCGA bladder cancer patients with high tumor-infiltrating NK cells stratified by IgG1 (E) and IgG3 (F) levels.

78

A First-in-Class Synthetic Inhibitor of Skp1-Skp2 Protein-Protein Interaction Has Potent Preclinical Efficacy against Metastatic Castration-Resistant Prostate Cancer

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Abstract

Background: Metastatic castration-resistant prostate cancer (mCRPC) directly contributes to prostate cancer mortality. It is an urgent and unmet medical need to identify new molecular targets and develop novel therapeutic agents against lethal mCRPC.

Methods: We developed a novel small molecule anticancer compound named GH501 via a “molecular hybridization” approach and evaluated the *in vitro* cytotoxicity of GH501 in the NCI-60 human cancer cell panel and in established human mCRPC cell lines. The mechanism of action of GH501 was investigated using in silico docking, biolayer interferometry (BLI), cellular thermal shift (CETSA) assay, and molecular and cellular approaches. The *in vivo* anticancer efficacy of GH501 against mCRPC was evaluated in animal models.

Results: GH501 effectively inhibited the proliferation of NCI-60 human cancer cell lines and established mCRPC cell lines at nanomolar potency via the induction of cell cycle arrest and apoptosis. Mechanistically, GH501 bound S-phase kinase-associated protein 1 (Skp1) and disrupted the physical interaction between Skp1 and S-phase kinase-associated protein 2 (Skp2), thereby affecting multiple oncogenic signals implicated in mCRPC progression, including Skp2, E2F1, c-Myc, enhancer of zeste homolog 2 (EZH2), ATF3, ATF4, β-catenin, cyclin D1, p21, p27, RUNX2, and survivin. As a monotherapy, GH501 effectively inhibited the skeletal and subcutaneous growth of multiple xenografts from human mCRPC cell lines and patient-derived xenografts.

Conclusions: These preclinical Results: support that pharmacological interruption of Skp1-Skp2 interaction is a promising therapeutic strategy for mCRPC.

79

Role of the microRNA miR-888 cluster in prostate cancer cell plasticity and castration-resistant disease

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Abstract

Background: Prostate cancer (PCa) is the second leading cause of male cancer-related deaths. Androgen deprivation therapy (ADT) is a first-line therapy for advanced disease. Prolonged ADT treatment Results: in castration-resistant prostate cancer (CRPC), an aggressive form of PCa. A clinical association exists between ADT and treatment-emergent neuroendocrine PCa (T-NEPC), a subset of CRPC characterized by tumor acquisition of neuroendocrine (NE) characteristics and down-regulation of androgen receptor (AR). As CRPC is a lethal disease with a mean survival of 2-3 years, new therapeutic targets are needed. We hypothesize that the noncoding RNA miR-888 cluster promotes PCa progression and NE transdifferentiation. Our lab identified the miR-888 cluster as preferentially elevated in prostatic fluids from high-grade PCa patients. Members of this cluster, notably miR-888 and miR-891a, promote prostate cell growth and invasion *in vitro* and accelerate prostate tumor load in mice. Cluster members are predicted to suppress AR. Using a human prostate LNCaP cell culture assay for NE transdifferentiation, we aimed to test if the miR-888 cluster influences PCa plasticity by quantifying neurite outgrowth and stemness marker expression.

Methods: miR-888 and miR-891a expression were modulated in LNCaP cells using lentiviral miRNA mimic overexpression (OX) vectors or generating CRISPR-specific miRNA knockout (KO) cell lines. Cells were deprived of hormones by culturing in phenol-free RPMI media with 10% charcoal-stripped fetal bovine serum. KO cells were harvested at 0 days and weekly for 8 weeks, and OX cells were harvested at 0 days and every 48 hours for 3 weeks. Cells were stained with neurite marker beta-3-tubulin and neurite length was measured using NeuronJ. Total RNA and protein lysate from these timepoints were analyzed for stem cell markers (H19, SOX2, NANOG, BRN2) and NE markers (SCGN) by qRT-PCR and western blot.

Results: LNCaP cells overexpressing miR-888 and miR-891a displayed significantly accelerated neurite outgrowth after 9 days compared to controls. miR-888 and miR-891a deleted cells showed neurite outgrowth delay of 5 weeks compared to controls. Stem cell marker studies are in progress.

Conclusions: Our Results: indicate that the miR-888 cluster accelerates NE transdifferentiation. These findings could lead to novel anti-miRNA therapies for CRPC.

80

MED12 Mutation Leads To a Dysregulated Transcriptome Through Altered Enhancer-Promoter Looping

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Abstract

Background:

The Mediator Complex Subunit 12 (MED12) is recurrently mutated in 2-5% of prostate cancer (PCa) patients. Specifically, the L1224F mutation is commonly observed. Despite a clear clinical relevance, the impact of these mutations has not been studied. MED12 plays a role in transcription by linking the transcription factors bound at enhancers to the transcription machinery at the promoters. These links are established by the looping of the chromatin as enhancers are often kilobases away from promoters. We hypothesize that the mutation leads to dysregulation of the transcriptome caused by impaired enhancer-promoter looping.

Methods:

We integrated various chromatin binding, transcriptomics and 3D chromatin contact information to fully dissect MED12 and mutant's function. Specifically, MED12, H3K27ac, H3K4me3, and RNA Pol II ChIP-Seq, RNA-Seq and Hi-C were performed in four conditions: control, MED12 knockdown, MED12 wildtype re-expression and MED12 L1224F mutant re-expression.

Results:

We show that MED12 knockdown and the L1224F mutant leads to a large dysregulation of the transcriptome as expected with its role in transcription. At upregulated genes upon knockdown or mutant repression, there is increased Pol II binding accompanied by increased H3K27ac. At downregulated genes, the reverse is observed. Interestingly, this mutation does not impact the composition of the Mediator complex.

Conclusions

Our Results: shed light on the fundamental process of transcription in PCa and the corporation between the transcription factors at the enhancers and the transcription machinery at the promoters and how this process can be disrupted with a clinically relevant mutation. This study is also applicable for other biological systems as the Mediator is a integral part of any transcription process.

81

POTENTIAL ROLE OF S-NITROSO GLUTATHIONE IN AMELIORATING THE EPIGENETIC LANDSCAPE OF NEUROENDOCRINE PROSTATE CANCER

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Abstract

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

METHODS: For the in-vivo study, castrated SCID mice were grafted with H660 cells to generate NEPC murine models. Once the tumors became palpable, the animals were divided into two groups. Group 1 received vehicle (Control) and group 2 received GSNO treatment at the dose of 10mg/kg/day intraperitoneally (ip). Treatment was performed daily for two weeks, and tumor growth was measured twice a week for a month after completion of treatments. Tumors were harvested to study the expression of well-established neuroendocrine and cancer progression markers. Cytokine array analysis was also done with tumors in both groups.

RESULTS: In-vivo Results: highlighted that treatment with GSNO reduced the tumor burden by showing a significant decrease ($p<0.05$) in the tumor volume compared to the untreated control. The tumor and spleen weight were also reduced. The western blot analysis showed a reduction in neuendocrine markers, chromogranin A and synaptophysin, compared to control. Furthermore, the pro-tumorogenic cytokines were reduced in the GSNO-treated group which indicates its efficacy in manipulating the tumor microenvironment (TME). Further studies are underway to investigate the epigenetic markers of NEPC and how GSNO treatments affect them.

CONCLUSION: This study shows that GSNO decreases NEPC tumor burden by altering the tumor microenvironment, which could serve as a potential regulator of the epigenetic changes involved with this type of cancer.

82

Role of Engrailed-2 (EN2) as a Disease Stratifier in Prostatic Tumorigenesis

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Abstract

BACKGROUND:

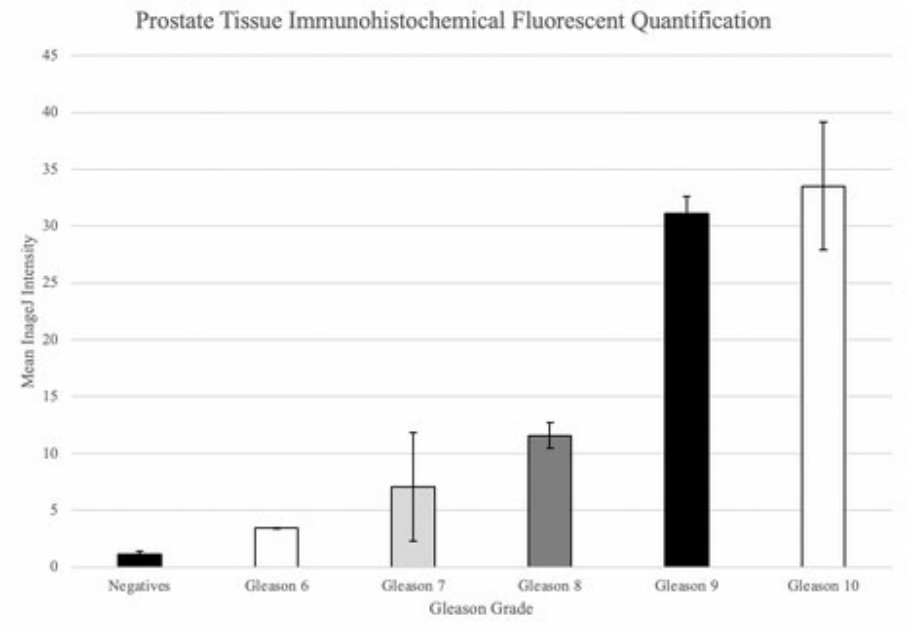
Prostate cancer (PC) poses one of the most significant disease burdens among males in the US. Although PSA is approved for diagnosis and monitoring of prostate cancer, its sensitivity and specificity can limit its use. A 2015 study by McGrath et al. purported that Engrailed-2 (EN2), a homeodomain-containing transcription factor, could serve as a novel biomarker with urine ELISA levels being predictive of prostate cancer with improved sensitivity and specificity. More recently, Li et al (2020) found that there was a statistically significant correlation between AJCC clinical staging and the EN2 immunohistochemical score of various prostate cancer samples. With supporting literature being sparse, our study will attempt to corroborate these findings. We hypothesize that there will be a positive association between EN2 expression patterns and increasing Gleason grade clinical samples and that the homeobox gene EN2 has compelling potential to serve as a disease risk-stratifier.

METHODS:

We conducted immunohistochemical staining using the EN2 antibody to compare gene expression in negative prostate tissue samples and samples of increasing Gleason grade and analyzed the correlation between mean staining intensity via ImageJ and Gleason grade. Expression patterns amongst two PC cell lines (PC3 and DU145) and one normal prostate epithelial cell line (PWR1E) were also compared using immunofluorescence and RT-PCR techniques. Colony forming assays were then performed amongst all three cell lines after EN2 siRNA-induced transfection to evaluate differences in growth potential.

RESULTS:

There was a strong positive correlation between increasing Gleason grade and mean immunohistochemistry fluorescent staining intensity ($r=0.752$, $p<0.01$) with Tukey's HSD tests demonstrating statistically significant differences ($\alpha=0.05$) between eight of fifteen total comparison groups. This result was supported by differences in immunofluorescent intensity amongst cell lines with normal PWR1E epithelial cells exhibiting a mean signal of 4.48 units compared to 11.70 units in moderate metastatic potential DU145 cells ($p<0.01$) and 17.10 units in high metastatic potential PC3 cells ($p<0.01$). Similarly, RT-qPCR assay demonstrated marked differences in gene expression. Relative to GAPDH, the PWR1E line had a transcription accumulation index of 1.19 whereas DU145's was 4.79 ($p<0.01$) and PC3's was 5.73 ($p<0.01$). After transfection with 25 pmol EN2 siRNA, PC3 and DU145 cells demonstrated much larger percent-wise decreases in colony forming capacity (64% and 58.5% respectively, $p<0.01$) compared to a smaller 42.5% decrease in colony formation capacity ($p<0.05$) in PWR1E cells.



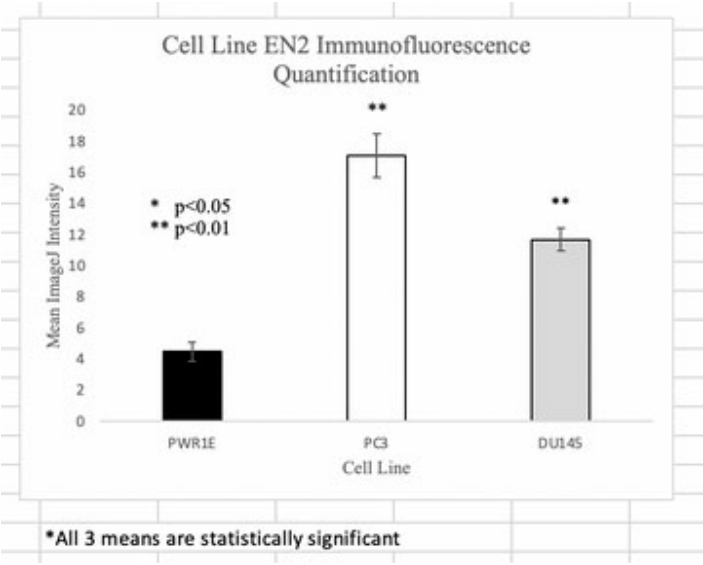
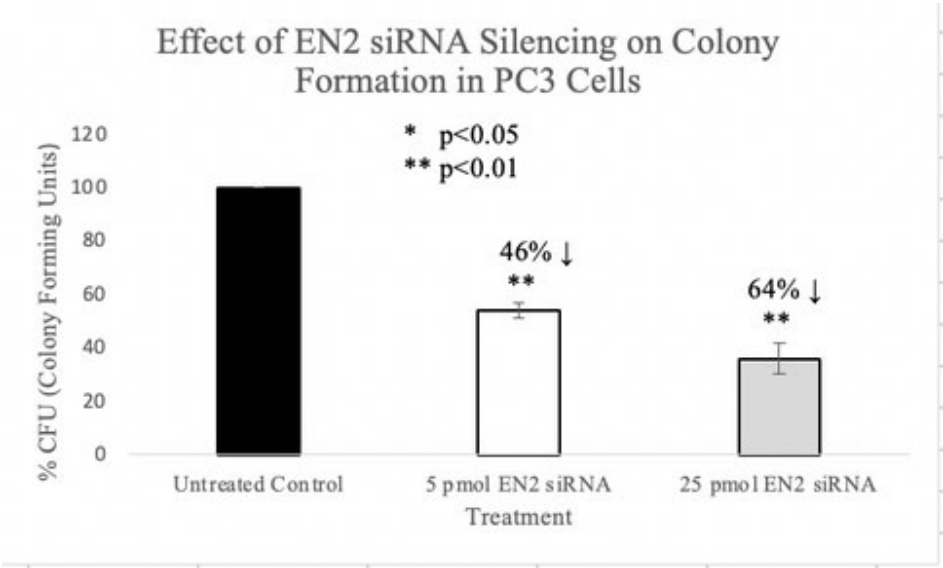
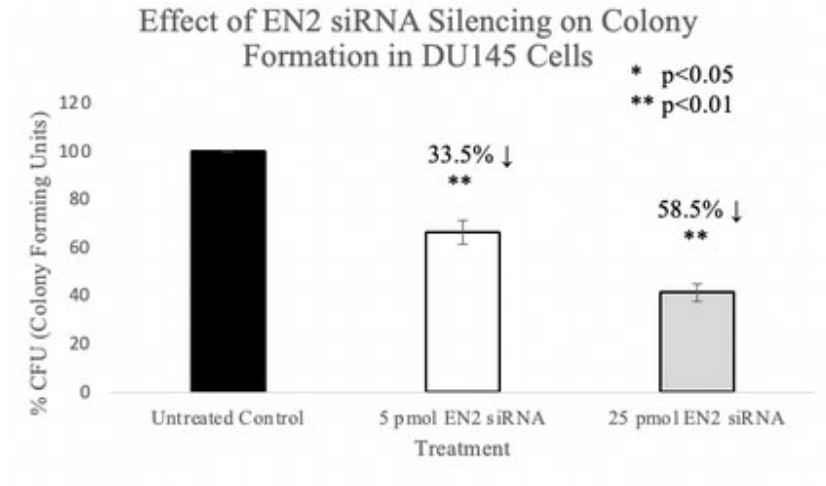


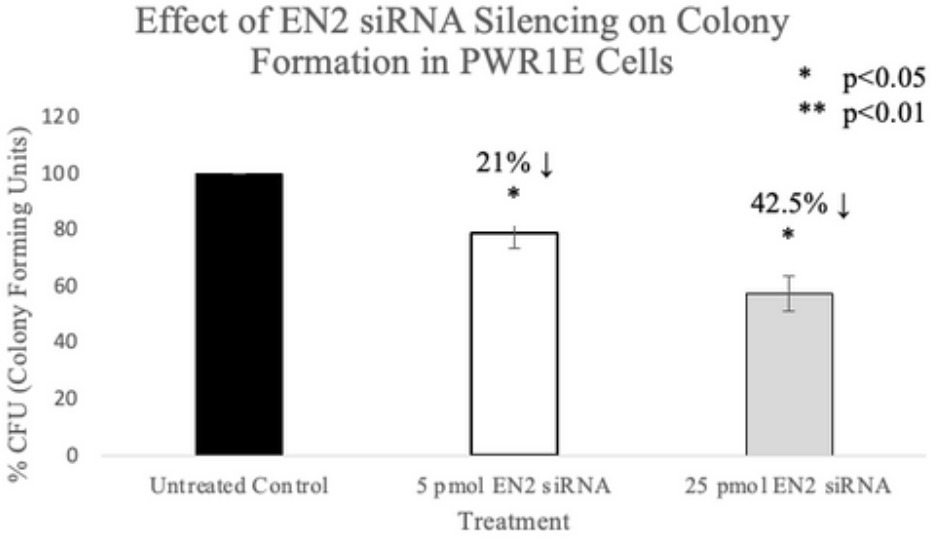
Figure 2. Tukey's HSD Comparison of Prostate Tissue Fluorescent Intensity Means

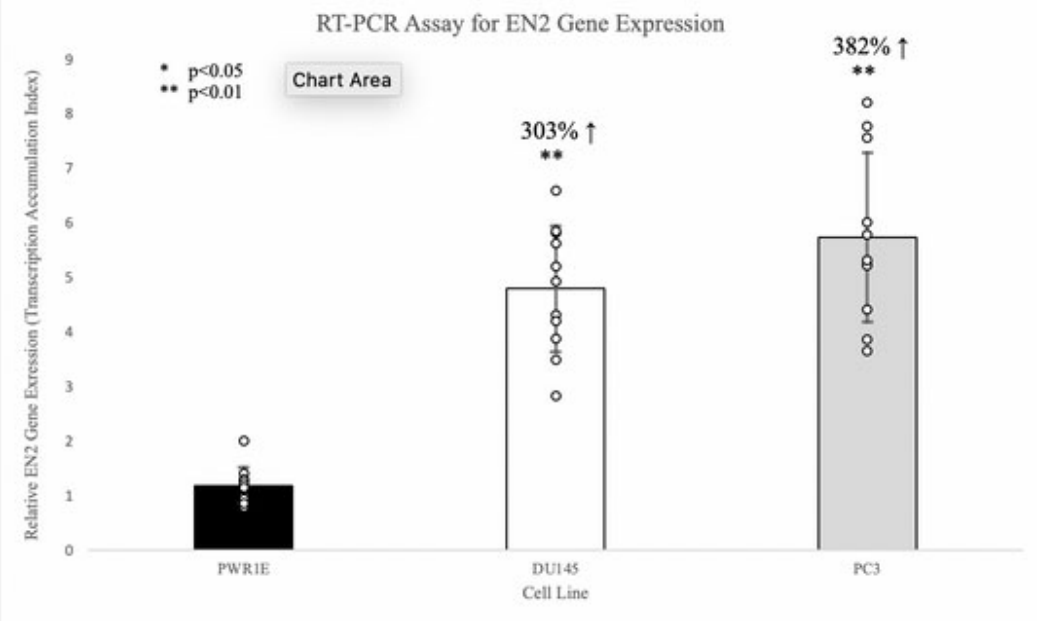
	Negatives	Gleason 6	Gleason 7	Gleason 8	Gleason 9	Gleason 10
Negatives		p= 0.90	p= 0.48	p= 0.10	*p< 0.01	* p< 0.01
Gleason 6			p= 0.83	p= 0.22	*p< 0.01	* p< 0.01
Gleason 7				p= 0.69	*p< 0.01	*p< 0.01
Gleason 8					*p< 0.01	* p< 0.01
Gleason 9						p= 0.90
Gleason 10						

*Each box denotes Tukey's HSD p-value when comparing 2 means with an α of 0.05 set as significance level


Effect of EN2 siRNA Silencing on Colony Formation in PC3 Cells
* p<0.05
** p<0.01
46% ↓
**
64% ↓
**
% CFU (Colony Forming Units)
120
100
80
60
40
20
0
Untreated Control
5 pmol EN2 siRNA
25 pmol EN2 siRNA
Treatment


Effect of EN2 siRNA Silencing on Colony Formation in DU145 Cells
* p<0.05
** p<0.01
33.5% ↓
**
58.5% ↓
**
% CFU (Colony Forming Units)
120
100
80
60
40
20
0
Untreated Control
5 pmol EN2 siRNA
25 pmol EN2 siRNA
Treatment


Effect of EN2 siRNA Silencing on Colony Formation in PWR1E Cells
* p<0.05
** p<0.01
21% ↓
*
42.5% ↓
*
% CFU (Colony Forming Units)
120
100
80
60
40
20
0
Untreated Control
5 pmol EN2 siRNA
25 pmol EN2 siRNA
Treatment



CONCLUSIONS

Our findings demonstrate a trend of increasing EN2 expression patterns and staining correlating with worsening Gleason grade and metastatic potential, indicating that EN2 could serve as a novel gene for stratifying disease risk and prognosis. Further studies regarding targeting of EN2 for therapeutic purposes may be warranted.

83

FTO Inhibition Protects Against Ferroptosis-Induced Kidney Cell Death In Vitro and In Vivo

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Abstract

Background: Ferroptosis, an iron-dependent regulated form of cell death caused by lipid peroxidation dysregulation and reactive oxygen species (ROS) accumulation, is one of the main drivers of renal fibrosis following acute kidney injury (AKI). Fat mass and obesity-associated (FTO) protein plays a role in many cellular processes, including ferroptosis, and it has been shown to be significantly upregulated following AKI. As an mRNA demethylase, FTO enables transcription of the glutamine transporter SLC1A5, a crucial component of the ferroptosis pathway. We hypothesize that FTO inhibition protects kidney cells from AKI-induced ferroptosis and renal fibrosis through downregulation of ROS production.

Methods: Human kidney epithelial HK2 cells and mouse primary kidney epithelial cells were treated with Erastin to induce ferroptosis in the presence or absence of FB23-2, a small-molecule FTO inhibitor. Cell viability and ROS production assays were performed to determine whether FTO inhibition protected the cells from ferroptosis. In vivo, acute kidney injury was induced in mice via unilateral ureteral obstruction (UUO). Mice with UUO were treated with or without daily injections of FB23-2 or a ferroptosis specific inhibitor, Liproxstatin-1 for 2 weeks. Kidneys were surgically removed, fixed and immunohistochemical staining (IHC) was performed for markers of ferroptosis including transferrin receptor (TfR), 4-hydroxynonenal (4-HNE) and fibrosis markers α-SMA and Collagen Ⅲ. Fibrosis collagen deposition was evaluated by Hydroxyproline assay and Sirius Red staining. FTO expression was evaluated by western blot and IHC in the Sham and UUO mice.

Results: Erastin treatment significantly reduced viability in HK2 cells and mouse primary kidney epithelial cells, which was completely reversed by FB23-2. In line with these Results:, erastin-treated HK2 cells produced significantly higher levels of ROS compared to cells treated with both erastin and FB23-2. Kidney tissue samples from UUO mice treated with FB23-2 showed decreased collagen deposition, 4-hydroxynonenal (4-HNE), and transferrin receptor (TfR) expression, all indicative of reduced fibrosis and ferroptosis, compared to untreated UUO samples. Consistent with these Results:, kidney tissues from FB23-2 treated UUO mice showed decreased hydroxyproline levels and iron production compared to untreated UUO mice.

Conclusion: FTO inhibition protects cells from undergoing ferroptosis following renal injury, unveiling a new target in preventing renal fibrosis.

84

STMN1 expression is elevated in neuroendocrine prostate cancer

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Abstract

Background: Prostate cancer (PCa) is the most common non-skin cancer and the second leading cause of cancer-related death among men in the United States. Neuroendocrine prostate cancer (NEPC) is a highly aggressive and non-curable variant of PCa and is considered as “treatment induced” PCa since it may de novo arise from PCa treated with androgen deprivation therapy as a mechanism of resistance. Stathmin1 (STMN1) is known to involve in tumor progression, but its expression and mechanism in NEPC are still unknown.

Methods: To assess the expression of STMN1 in prostate cancer, we analyzed the publicly available RNA-Seq data obtained from the cBioportal, the Cancer Genome Atlas (TCGA), and the Cancer Cell Line Encyclopedia websites (CCLE). To verify the expression pattern of STMN1, we performed western blot (WB), immunohistochemical (IHC), and Immunofluorescence (IF) staining on PCa cell lines and tissue samples of human PCa specimens and mouse models.

Results: The bioinformatics transcriptomic analysis indicated that the expression of STMN1 was significantly increased in high-grade tumors (Gleason score (GS) >=8), compared to immediate-grade (GS = 7) or low-grade tumors (GS=6) ($p<0.01$), and was reversely correlated with clinical outcomes in PCa patients, including overall survival and disease-free survival rate ($p<0.01$). Additionally, the expression of STMN1 in NEPC samples was significantly higher than that in adenocarcinoma prostate cancer (AdPC) samples ($p<0.01$). RNA seq data analysis and western blot on PCa cell lines samples were consistent with this finding, STMN1 expression levels were higher in NEPC cell line NCI-H660 than AdPC cell lines including VCaP, LNCaP, C42B, 22RV1, PC3, and DU145. IHC and IF staining on human and mouse NEPC model samples further supported the elevated expression of STMN1 in NEPC. Furthermore, increased STMN1 expression was also observed in proliferating cancer cells indicated by the diffusely positive staining of Ki67 immunomarker.

Conclusion: Increased expression of STMN1 in NEPCa and proliferating cancer cells provides a potential mechanism for the emergence and progression of NEPC. Consequently, STMN1 may serve as a prognostic marker for advanced PCa and facilitate the identification of tumor stage, and be considered as a potential therapeutic target for the treatment of both NEPC and advanced PCa.

85

Immune cell single-cell RNA sequencing analyses suggest a role for age-associated T cell subset in symptomatic benign prostatic hyperplasia

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Abstract

Immune cell single-cell RNA sequencing analyses suggest a role for age-associated T cell subset in symptomatic benign prostatic hyperplasia

Meaghan M Broman, Nadia A Lanman, Renee E Vickman, Gregory M Cresswell, Juan Sebastian Paez Paez, Gervaise Henry, Douglas Strand, Simon W Hayward, Timothy L Ratliff

Introduction and objective

Age-related immune dysfunction is known to impact a variety of chronic conditions. Benign prostatic hyperplasia (BPH) is among the most common age-associated conditions in men. Likewise, increased prostatic immune cell infiltration is frequently observed with aging coincident with BPH; however, the relationship between age-related immune changes, prostate enlargement, and associated symptoms has not been defined. We seek to define immune cell types and their associations with BPH.

Methods:

scRNA-Seq was performed on immune cells isolated from 10 small (<60g) and 3 large (>70g) prostates from aged men (>50 years). Subtypes were clustered based on differential gene expression. These data were combined with previously published scRNA-Seq data from three young organ donor prostates. T/NK cell and macrophage clusters were subclustered to identify and compare subsets between sample types. Velocity analysis predicted the trajectory of macrophage differentiation in small and large prostates.

Results:

Analysis of prostate immune cells revealed a T cell-dominant leukocyte phenotype with a relative shift in the proportions of various T cell subsets in BPH compared to normal prostates from young men. Interaction analyses suggest enhanced ligand-receptor interactions among T cells and macrophages, and gene expression profiles of macrophage subsets reveal a mix of M1 and M2-associated inflammatory profiles. Also, velocity analysis revealed an overall reversal in the differentiation trajectory of a suspected resident macrophage subset between small and large prostates. Notably, we identified a CD8+ granzyme K (GZMK) high, granzyme B (GZMB) low T cell subset that has been previously associated with immune aging and is positively correlated with International Prostate Symptom Score (IPSS).

Conclusion

Overall, these data demonstrate a dominant T cell inflammatory response in prostate tissue and suggest a link between specific T cell subsets and symptomatic BPH. Also, a shift in macrophage differentiation

between small and large prostates may suggest altered microenvironmental cues related to macrophage differentiation and potentially function in symptomatic BPH. Investigation into functional impact of T cell and macrophage subsets in BPH is ongoing.

86

ONECUT2 Activates Diverse Drug-resistant Phenotypes in Prostate Cancer

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Abstract

BACKGROUND:. Prostate cancer (PC) cells can escape from lineage confinement and be reprogrammed to a drug-resistant, high-plasticity state, however the mechanism underlying this process is not understood. Our group identified the HOX/CUT transcription factor ONECUT2 (OC2) as a mediator of metastatic castration-resistant PC (mCRPC) that can be inhibited with a novel family of small molecules. OC2 was shown to be a lineage plasticity driver that promotes neuroendocrine prostate cancer (NEPC). In this study, we sought to uncover the mechanism whereby OC2 exerts effects on lineage plasticity.

METHODS:. RNAseq, CUT&RUNseq, ssRNAseq, forced expression, shRNA, CRISPR-Cas9, co-precipitation/western blot, PCR, mutagenesis, human PC, PC models, computational modeling.

RESULTS:. We found that OC2 is capable of activating multiple androgen receptor- (AR)-indifferent, drug-resistant lineages, including programs not associated with NEPC, such as upregulation of the glucocorticoid receptor (GR). Consistent with the GR findings, OC2 regulated genes on the AR cistrome previously shown to be regulated by GR in CRPC, including KLK3/PSA. OC2 activated bivalent promoters of master regulator genes via epigenetic modification, and reprogramed multiple super-enhancers, including those associated with TMPRSS2 and SRRM4. OC2-expressing cells in human CRPC included populations that express AR, NE+/stem-like+, NE-/stem-like+, and “double negative” (NE-/AR-) phenotypes. OC2 also transcriptionally upregulated UDP-glucuronosyltransferase enzymes UGT2B15 and UGT2B17, which inactivate DHT, suggesting that the emergence of phenotypic plasticity also arises from AR suppression by ligand depletion. Consistent with this, low levels of androgen correlated with OC2 activation in human CRPC tumors. Enforced OC2 promoted both enzalutamide and docetaxel resistance in PC models.

CONCLUSIONS. These findings identify OC2 as an upstream mediator of many lineage-defining factors that govern drug resistance, including those associated with adenocarcinoma. Because OC2 can be inhibited pharmacologically, these Results: suggest that suppressing this target may inhibit the emergence of a wide range of drug resistance mechanisms arising from loss of lineage confinement.

87

Identification of TYMS as a Potential Therapeutic Target for Advanced Prostate Cancer

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Abstract

Background: Metastasis is the main cause of prostate cancer-associated deaths, highlighting the urgent clinical need to identify novel, effective therapies for advanced prostate cancers (PC). The DNA replication and repair is one of the major pathways that drive PC aggressiveness, making it a promising pathway to target for advanced PC. Thymidylate synthase (TYMS) is an enzyme that is an important component of DNA synthesis, replication, and repair. We demonstrated that TYMS is elevated in a new model of castration-resistant PC (CRPC) and neuroendocrine PC (NEPC) driven by overexpression of Trop2. This study aims to assess the anti-cancer effects of TYMS in advanced PCs.

Methods: Protein levels of TYMS were assessed by immunohistochemistry (IHC) and western blot (WB) in cancer xenografts and cancer cell lines. TYMS mRNA expression was analyzed in published patient datasets to assess the association between TYMS and disease progression and invasiveness. A prostate tumor tissue microarrays (TMAs) containing cores from normal prostate and localized PC, as well as a TMAs with cores from LuCaP CRPC patient-driven xenografts (PDXs) and NEPC PDXs were used to assess TYMS protein expression. Cell viability and colony formation assays were performed to test the anti-cancer effects with two TYMS inhibitors, Raltitrexed and Pemetrexed in vitro. Inhibition of TYMS by Raltitrexed (7.5mg/kg daily via i.p.) was further tested on tumor growth in NEPC xenografts in vivo.

Results: TYMS mRNA is elevated in metastatic patient tissues relative to benign and localized PC samples, indicating that TYMS mRNA level associates with the progression of the disease. TYMS is up-regulated in 62% of CRPC and NEPC PDX tissues (68 out of 111) relative to 3% in localized PCs (1 out of 38) and 4.5% in normal prostate tissues (1 out of 22) from patient TMAs. TYMS inhibition exerts anti-cancer properties through the reduction of PC viability, proliferation, and colony-forming ability. In vivo, TYMS inhibition by Raltitrexed significantly delayed tumor growth in a TD-NEPC model.

Conclusions: Our study suggests that TYMS is a novel molecular indicator for advanced PC and TYMS could serve as a potential novel therapeutic target for metastatic PCs.

88

CYCLIN K downregulation induces AR gene intronic polyadenylation, AR variant expression and PARP inhibitor vulnerability in castration-resistant prostate cancer

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Abstract

Androgen receptor (AR) mRNA alternative splicing variants (AR-Vs) are implicated in castrate-resistant progression of prostate cancer (PCa), although the molecular mechanism underlying the genesis of AR-Vs remains poorly understood. The CDK12 gene is often deleted or mutated in PCa and CDK12 deficiency is known to cause homologous recombination repair gene alteration or BRCAness via alternative polyadenylation (APA). Here, we demonstrate that pharmacological inhibition or genetic inactivation of CDK12 induces AR gene intronic (intron 3) polyadenylation (IPA) usage, AR-V expression, and PCa cell resistance to antiandrogen enzalutamide (ENZ). We further show that AR binds to the CCNK gene promoter and upregulates CYCLIN K expression. In contrast, ENZ decreases AR occupancy at CCNK gene promoter and suppresses CYCLIN K expression. Similar to the effect of CDK12 inhibitor, CYCLIN K degrader or ENZ treatment promotes AR gene IPA usage, AR-V expression, and ENZ-resistant growth of PCa cells. Importantly, we show that targeting BRCAness induced by CYCLIN K downregulation with PARP1 inhibitor overcomes ENZ resistance. Our findings identify CYCLIN K downregulation as a key driver of IPA usage, hormonal therapy-induced AR-V expression and castration resistance in PCa. These results suggest that hormonal therapy-induced AR-V expression and therapy resistance are vulnerable to PARP1 inhibitor treatment.

Significance

Expression of AR-Vs is implicated in development of castration-resistant PCa (CRPC). Others have shown that androgen depletion or antiandrogen treatment induces AR-V expression in PCa cell lines, xenografts and patient samples although the underlying mechanism remains unclear. Our findings reveal that hormonal therapy-induced CYCLIN K downregulation represents a key mechanism that drives IPA usage in AR gene, AR-V expression and castration resistance in PCa and that this mechanism of action can be therapeutically targeted by PARP1 inhibitor.

89

The Two Faces of Cancer: Janus Kinase (JAK) Signaling Promotes Stem-like Lineage Transition

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Abstract

Background: Despite the clinical success of targeted therapies directed towards driver oncogenes in many cancers, resistance to these therapies often emerges quickly, culminating in poor clinical outcomes. Although emerging evidence has revealed the crucial role of lineage plasticity in driving therapy resistance, the exact molecular mechanism and kinetics of acquiring lineage plasticity is not clearly understood. More importantly, therapeutic approaches targeting lineage plasticity-driven resistance are not currently available, underlying the unmet clinical urgency to identify druggable targets which drive lineage plasticity and resistance.

Methods: Through a multi-disciplinary approach integrating 3D organoid modeling, bulk and single cell RNA-Seq (scRNA-Seq) analysis, we illuminate the once hidden intratumoral heterogeneous subclones, which express multilineage, stem-like, and lineage plastic transcriptional programs, as the driving force of AR therapy resistance.

Results: We reveal that the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling is a crucial executor in promoting lineage plasticity-driven AR targeted therapy resistance in prostate cancer. Importantly, ectopic JAK/STAT activation is specifically required for the resistance of stem-like subclones expressing multi-lineage transcriptional programs, but not subclones exclusively expressing the neuroendocrine (NE)-like lineage program. Both genetic and pharmaceutical inactivation of JAK-STAT signaling restored the AR dependency of prostate cancer cells or 3D-cutlured organoids and re-sensitized them to AR targeted therapy, giving hope that JAK-STAT inhibitors could be a novel avenue to overcome resistance.

Conclusions: Together, these data revealed the novel and crucial function of JAK-STAT signaling in promoting a stem-like and lineage plastic state and presented a novel therapeutic approach to reverse AR targeted therapy resistance using JAK1 inhibitor.

90

Ectopic JAK/STAT Activation Enables the Transition to a Stem-like and Multi-lineage State Conferring AR-targeted Therapy Resistance

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Abstract

Ectopic JAK/STAT Activation Enables the Transition to a Stem-like and Multi-lineage State Conferring AR-targeted Therapy Resistance

Background: Emerging evidence indicates that various cancers can gain resistance to targeted therapies by acquiring lineage plasticity. Previous studies have shown that in metastatic castration-resistant prostate cancer (mCRPC), lineage plasticity-driven resistance co-occurrents with loss-of-function of TP53 and RB1, which is then accompanied by ectopic activation of SOX2. However, the molecular mechanism that promotes lineage plasticity in many mCRPC subtypes, especially in the context of TP53/RB1-deficiency, is not fully understood.

Methods: Transcriptional alternations that drive resistance to targeted therapy were identified through RNA-seq. CRISPR-base loss of function studies, as well as pharmaceutical approaches were carried out in both in vitro and in vivo models to validate candidate genes and dissect molecular mechanisms behind acquired resistance. Single cell RNA-seq (scRNA-seq) analysis was used to reveal cell lineage change at single cellular level.

Results: We find that the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling was altered concomitantly with TP53, RB1 and SOX2. Ectopic JAK/STAT activation in TP53-RB1-deficent cells is both required and sufficient to promote lineage plasticity and AR targeted therapy resistance and can be reverse with JAK/STAT signaling inhibition. Furthermore, ectopic JAK/STAT activation correlates with poor clinical outcomes. On single cellular level, JAK/STAT signaling maintains stem-like and multi-lineage subclones, but not subclones exclusively expressing NE-like lineage.

Conclusion: Our findings suggest a crucial role of JAK/STAT signaling in promoting lineage plasticity-driven AR targeted therapy resistance in prostate cancer. Ectopic JAK/STAT activation is specifically required for the resistance of stem-like subclones expressing multi-lineage transcriptional programs, but not subclones exclusively expressing the neuroendocrine (NE)-like lineage program. Both genetic and pharmaceutical inhibition of JAK/STAT signaling re-sensitizes resistant tumors to AR targeted therapy. These findings provide rationales of combined therapy in subgroup of mCRPC patients with TP53/RB1 loss.

91

Differential role of PTEN/AKT/TSC1/2 pathway in prostate cancer

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Abstract

Background: The PI3K/AKT/mTOR signaling pathway is essential for the initiation and progression of prostate cancer. However, there has been no a comprehensive comparison of the role of these signaling nodes in prostate tumor initiation and progression.

Methods: With genetically engineered animal models, we compared the impact of prostate-specific deletions of Pten, Tsc1, and Tsc2 and activation of Akt1 on tumor initiation and progression. Also, we assessed the expression and genetic alterations of PTEN, AKT1, TSC1, and TSC2 in human primary prostate cancers.

Results: For the genetically engineered mice, prostate-specific deletions of Pten, Tsc1, and Tsc2 led to initiation and progression of mouse prostatic neoplasia hyperplasia (mPIN). Tsc1 or Tsc2 prostate conditional knockout (cKO) led to slower prostate tumor initiation and progression than for mice with cKO of Pten. Mice with Tsc1/Tsc2 double-cKO developed more malignancy and tumor progression than mice with Tsc1 or Tsc2 single-cKO. Functional analyses showed that Pten single-cKO and Tsc1/Tsc2 double-cKO induced cell proliferation more than Tsc1 or Tsc2 single-cKO, but only Pten single-cKO reduced epithelial adhesion. Also, Pten single-cKO and Tsc1/Tsc2 double-cKO enhanced the phosphorylation of pAKT (S473) and pS6 (S235/236) more than Tsc1 or Tsc2 single-cKO. Akt1 transgenic mice developed more aggressive mPIN than that for mice with Tsc1 or Tsc2 single-cKO, but the effect was more moderate than that for Pten single-cKO mice. In human prostate cancers, PTEN, but not AKT1, TSC1, or TSC2 had frequent genetic alterations. However, as key signaling nodes, AKT1, TSC1, and TSC2 may be responsible for PTEN loss-mediated tumor initiation and progression.

Conclusion: Our Results: for genetically engineered mouse models address the role of the PI3K/AKT/mTOR signaling nodes in prostate cancer initiation and progression, leading to the identification of potential new targeted therapies for prostate cancer.

92

Single-cell comparative biology of MYC-driven murine and human prostate cancer

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Abstract

Background: While it is known that the tissue microenvironment in prostate cancer is altered, how prostate cancer cells and associated precursor lesions may alter the local tissue microenvironment is not well understood.

Method: In this study, we used single-cell RNA-sequencing (scRNA-seq) Methods: to assess the transcriptional profiles of the tissue microenvironment in prostate tissues from prostatectomies from men diagnosed with prostate cancer (N = 10). For each subject, tissues were collected from each zone of the prostate (peripheral, central, and transition), as well as from areas with visible tumors. Additionally, we carried out scRNA-seq from each prostate lobe (anterior, dorsal, lateral, and ventral) of the MYC-driven mouse model of prostate cancer (Hi-MYC) in both FVB/NJ (N = 2) and C57BL/6J (N =2) mouse strains, as well as age-matched wild type animals (FVB/NJ N =2, C57BL/6J N = 3).

Results: Dimensionality reduction and cluster analysis of prostatectomy and mouse prostate samples allowed visualization of cell types, including benign and cancer cells. Strikingly, gene set enrichment analysis (GSEA) comparing benign luminal epithelial cells with prostate cancer clusters showed that Hallmark MYC targets V1 (NES = 7.2, adj p-value = 3.8e10-39) was the top upregulated pathway in prostate cancer cell clusters across the majority of subjects, suggesting that MYC activation is a common denominator across prostate cancer molecular subsets. Inspection of the single-cell transcriptomes of prostates from 6-month-old Hi-MYC mice compared to age-matched WT animals revealed robust transcriptional changes beyond the MYC-expressing luminal cells, including Ly6d-expressing basal and luminal cells, Timp1/Irf7-expressing fibroblasts and TREM2-expressing macrophages. GSEA and inference of ligand-receptor pair interactions indicated that interferon response pathways activated in MYC-expressing luminal cells led to the expansion of these unique cell subsets in the microenvironment. These Results: suggest that interferon signaling from MYC-expressing cells may lead to resculpting of microenvironmental cellular phenotypes during tumorigenesis.

Conclusions: These data shed unprecedented molecular insights into how oncogene activity in prostate cancer cells and precursor lesions may alter the local tissue microenvironment.

93

PRELIMINARY RESULTS: FROM THE CAIRIBU PROGRESS SURVEY: PART OF A COLLABORATIVE EVALUATION OF A CONSORTIUM OF NIDDK-FUNDED RESEARCH PROGRAMS FOCUSED ON NON-MALIGNANT UROLOGIC DISEASES AND DISORDERS

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Abstract

BACKGROUND: In 2020, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded a U24 “Interactions Core” (IC) to facilitate collaboration between Centers and Programs in CAIRIBU (Collaborating for the Advancement of Interdisciplinary Research in Benign Urology), an umbrella organization of NIDDK-funded investigators investigating non-malignant genitourinary (GU) diseases and disorders. CAIRIBU includes the George M. O’Brien (U54) Cooperative Urology Research Centers, P20 Exploratory Centers for Interdisciplinary Research in Benign Urology, and 2 types of K12 Career Development Programs: urology epidemiologic research (KUroEpi) and multidisciplinary urologic research (KURe). CAIRIBU IC objectives are to build a community that promotes: (1) information- and resource- sharing, (2) collaborative research activity that advances non-malignant GU science, and (3) training the next generation of GU research leaders. NIDDK funding for all U-grants (except U01 grants) significantly increased from 2010-2020. However, there is very little published evaluation of these large research initiatives.

METHODS: We developed a 7-step plan to evaluate CAIRIBU IC efforts: (1) identify the mission, (2) identify objectives, (3) develop logic models for each objective, (4) define measurable outcomes, (5) present preliminary evaluation plan to stakeholders, (6) identify data sources and timeline, and (7) finalize the plan. The CAIRIBU Progress Survey was one part of the plan. Data on personal, professional, and scientific accomplishments as well as numbers of grants applied for and awarded are collected. The survey was disseminated to CAIRIBU investigators and trainees in 11/2021 with a follow-up in 3/2022.

RESULTS: Initial survey response rate (25%) improved to 47% in March. Results: demonstrated high satisfaction with CAIRIBU IC initiatives and favorable impact on collaborative research activity. Results: will provide baseline measures for future data collection and comparisons. Lessons learned: standardize the survey process to ensure uniform data collection over time, provide investigators’ responses to earlier surveys to abet accurate reporting within in each recall period, and reduce survey frequency to twice/year.

CONCLUSION: The CAIRIBU Progress Survey is one aspect of the CAIRIBU IC’s evaluation plan. Results: will contribute to ongoing evaluation efforts that assess CAIRIBU progress toward its objectives. Results: will also allow the CAIRIBU IC to restructure internal processes to be more effective and redirect/change course as needed to better support CAIRIBU investigators and trainees.

94

BUILDING AN INTERACTIVE POSTER SESSION INTO A VIRTUAL UROLOGY RESEARCH MEETING DURING THE COVID-19 PANDEMIC: SUCCESSES, CHALLENGES, AND LESSONS LEARNED

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Abstract

BACKGROUND: The COVID-19 pandemic caused a sudden switch from traditional in-person scientific meetings to large-scale virtual processes in order to safely continue the dissemination of research. However, because they were new to virtual meeting platforms, organizers struggled with how to include traditional interactive elements such as poster sessions, which are traditionally a forum for one-on-one discussion, dissemination of science, and networking. We piloted a model for highly interactive virtual poster sessions in virtual scientific meetings during the COVID-19 pandemic.

METHODS: The U24 Interactions Core for CAIRIBU (Collaborating for the Advancement of Interdisciplinary Research in Benign Urology), an umbrella organization of Centers and Programs funded by NIDDK to investigate non-malignant genitourinary research, used Zoom to virtually host the 2020 and 2021 annual CAIRIBU meetings. Meetings included poster presentations for which presenters were required to submit their abstracts, PDF posters, and 1-minute pre-recorded presentations. Recorded presentations were strung together and streamed one after the other during each meeting. Individual breakout rooms were opened for each presenter after the presentations; meeting attendees were able to freely flow from one breakout room to another for discussion with poster presenters. Full-text abstracts and PDF posters were available on the CAIRIBU website in a virtual “poster hall” during the entirety of each meeting. Feedback from post-meeting surveys and conversations with CAIRIBU leaders was solicited and compiled.

RESULTS: The number of registrants for the 2020 virtual meeting (n=199) grew in 2021 to 238 as did the percentage of attendees unaffiliated with CAIRIBU (34 to 42%). Feedback from the 2020 virtual meeting, for which posters (n=48) were presented in one long session, suggested shorter sessions. Thus, in 2021 posters (n=49) were distributed in 3 sessions over the 2-day meeting. Post-survey comments and feedback from CAIRIBU leaders was positive.

CONCLUSIONS: For trainees and early-stage investigators, poster sessions at scientific meetings afford the opportunity to meet others in their fields and to practice dissemination skills. By soliciting and acting on feedback from attendees of the 2020 virtual meeting, improvements to the poster presentations in 2021 were made. Virtual poster sessions that emulate the interactive nature of in-person poster sessions are possible. Attention to the length of sessions, the number of presentations within each session, and the ability of attendees to freely flow from one room to another are important.

95

Suppression of prostate cancer growth by targeted engineering mRNA 3'UTR length

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Abstract

Background:

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

Methods:

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

Results:

3'UTR globally shortens during prostate cancer progression to castration-resistance. Through blocking the proximal PASs, 3'UTRCES efficiently and specifically reverses the 3'UTR shortening of novel APA-linked, clinically-relevant prostate cancer oncogenic mRNAs, such as *SPSB1*, leading to reduced *SPSB1* mRNA translation and prostate cancer cell proliferation. Importantly, intratumor injection of LLN encapsulating 3'UTRCES RNA molecules effectively and safely inhibits prostate tumor growth in engrafted and transgenic mouse models.

Conclusions:

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

96

Prostate cancer progression and metastasis: Regulation of YAP1/TAZ-TEAD transcriptional networks by PDEF

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Abstract

Background: The Hippo TEAD-transcriptional regulators YAP1 and TAZ play essential role in cancer cell growth and metastasis. However, the function of YAP1 and TAZ in prostate cancer is not well characterized. We discovered that expression of PDEF is decreased during prostate cancer progression and re-expression of PDEF limits prostate cancer metastasis in part by promoting luminal epithelial phenotype, but mechanisms of PDEF action are not completely understood. In the present study, we evaluated expression of YAP1, TAZ and SPDEF in clinical cohorts of prostate cancer patients. We also evaluated the effects of the SPDEF on YAP1/TAZ levels and expression of YAP1/TAZ regulated genes in prostate cancer (PCa) cells. Material & Method: We analyzed publicly available gene expression data in several prostate cancer cohorts. Prostate cancer (PC3 and DU145) cells were transfected with PDEF or respective vector control. Protein levels were analyzed by western blotting. Gene expression was monitored by microarray analysis/RNA seq and confirmed by RTPCR assays. Cell migration and invasion were measured by scratch wound healing and by trans well migration though Matrigel assays respectively. Results: Analysis of publicly available PCa data sets revealed a significant increase in TAZ mRNA levels in prostate cancer tissues as compared to normal prostate tissues (p=4.95E-08). Furthermore, we also found a significant gradual increase in TAZ mRNA expression and concomitant decrease in YAP1 mRNA during disease progression and metastasis and in neuroendocrine PCa. We observed that the two well-characterized metastatic PCa cells (PC3 and DU145 cells) express both YAP1 and TAZ, but do not express SPDEF. Expression of PDEF in PC3 and DU145 cells resulted in an increased phospho-YAP1 and phospho-TAZ protein levels and inhibition of YAP1/TAZ target genes, as compared to the respective vector control , directly demonstrating that PDEF plays a critical role in modulating YAP1/TAZ-TEAD transcriptional activity, and by extension in the regulation of the Hippo pathway. Conclusions: Taken together these data suggest that SPDEF limits prostate cancer metastasis in part by targeting YAP1/TAZ driven transcriptional output.

97

A cellular atlas of the normal adult human female urethra

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Abstract

Introduction: Urinary voiding dysfunction is highly prevalent in aging women. A deeper understanding of the cellular composition of female urethra in the non-diseased state could provide insights into the etiology of dysfunction.

Methods: Urethra from female organ donors aged 18-45 (N=4) was collected fresh from surgery and enzymatically digested into a single cell suspension. Single cell RNA sequencing was performed using 10x Genomics V3 chemistry. Data were integrated with previously published male prostatic urethra in Seurat for cell type annotation. Immunohistochemical validation of cell types was performed on FFPE sections using anchor genes derived from the data.

Results: The female urethral lumen is lined with a stratified squamous urothelium composed of basal, intermediate and luminal epithelial layers. The Skene's ducts and glands contain basal, hillock and club cells similar to the prostatic urethra. The urethral lumen is surrounded by two discrete layers of fibroblasts and circumferential smooth muscle and skeletal muscle.

Conclusions: The identification of club cells in female urethral ducts could yield new insight into the regulation of infection and inflammation, which is the underlying cause of most urethral dysfunction.

98

Targeting Androgen Receptor by Extracellular Vesicles Based Delivery of CRISPR Machinery

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Abstract

Targeting Androgen Receptor by Extracellular Vesicles Based Delivery of CRISPR Machinery

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Background: Prostate cancer (PCa) is the most diagnosed malignancy in the United States in men. About 10-20% of PCa patients will develop castration resistance. Androgen receptor (AR) signaling is still maintained in the majority of PCa. CRISPR/Cas9 machinery mediated genomic editing provides a genetic approach to remove AR and silence AR signaling, but an efficient and safe delivery of CRISPR machinery to PCa cells has not been established.

Methods: The spCas9 was genetically modified by the fusion with the octapeptide derived from the N-terminus of Src kinase. 3T3 cell line stably expressing luciferase (3T3-Luc) was infected with lentivirus expressing Cas9, myristoylated Cas9 (mCas9), or mCas9 (G2A)/sgRNA-Luc. 293T overexpressing AR (293T-AR) cell line was infected by mCas9/sgRNA-AR lentivirus. Single-cell colony with mCas9/sgRNA-AR expression was isolated and expanded as stable extracellular vesicles (EVs)-producing cell line. VSV-G was also co-expressed in the EVs-producing cells. Cells were analyzed for Cas9 expression and for luciferase or AR knockout efficiency. Functionality of Cas9/sgRNA were examined by T7 endonuclease I assay and Sanger sequencing. EVs were analyzed for size distribution, concentration, morphology, and protein characterizations.

Results: We proved the principle that the genetically modified mCas9/sgRNA-luciferase was encapsulated into EVs and functional to knock out luciferase. Next, the mCas9/sgRNA-AR was capable of knocking out ectopically expressed and endogenous AR gene in the 293T-AR cells and tested PCa cells. The indel of AR gene was illustrated by the T7 endonuclease assay and Sanger sequencing. We further characterized the EVs isolated from the EVs-producing cells, which was about 150 nm in diameter and typical cup-shaped vesicles. Additionally, the encapsulated Cas9 protein accounted for 0.5% of total EV protein, and was resistant from the protease digestion. Further study will determine if EVs encapsulating mCas9/sgRNA-AR RNP can knock out AR in PCa cells.

Conclusions: N-myristoylated CRISPR-Cas9 is functional and encapsulated into EVs. The mCas9/sgRNA-AR leads to the indel of AR gene in target cells. This study will potentially develop a technology for treatment of CRPC by removing AR at the genomic DNA level.

99

A kinome-wide CRISPR screen identifies CK1α as a novel target to overcome enzalutamide resistance of prostate cancer

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Abstract

Title: A kinome-wide CRISPR screen identifies CK1α as a novel target to overcome enzalutamide resistance of prostate cancer

Background: Androgen deprivation therapy is the first line of therapy for localized prostate cancer (PCa), but most patients will eventually develop castration-resistant PCa (CRPC). Enzalutamide (ENZA), a second-generation androgen receptor (AR) antagonist, has significantly increased CRPC patient survival. However, resistance remains a prominent obstacle in treatment, illustrating the urgent need to develop new approaches to increase ENZA efficacy.

Methods: A Kinome-wide CRISPR-Cas9 screen was used to identify the targets related to ENZA response. *In vitro* cell viability and *in vivo* cell line or patient-derived xenografts were used to verify inhibiting CK1α overcoming ENZA resistance. RNA sequencing was used to address the functions of CK1α in ENZA resistance. *In vitro* kinase assay was used to study ATM phosphorylation by CK1α. Immunohistochemistry, immunofluorescence, immunoblot and immunoprecipitation assays were used to study the functions of CK1α in regulation of ATM and DNA damage response signaling. Bioinformatics analysis was used to confirm these findings with clinical patients' database.

Results: Utilizing a kinome-wide CRISPR-Cas9 knockout screen, we identified casein kinase 1 alpha (CK1α) as a novel therapeutic target to overcome ENZA resistance. Depletion or pharmacologic inhibition of CK1α with a novel compound in clinical trial significantly enhanced EZNA efficacy in ENZA-resistant cell lines and patient-derived xenografts. Mechanistically, CK1α phosphorylates the serine residue S1270 and modulates the protein abundance of ataxia-telangiectasia mutated (ATM), a primary initiator of DNA double-strand break (DSB)-response signaling, which is compromised in ENZA-resistant cells and patients. Inhibition of CK1α stabilizes ATM, resulting in the restoration of DSB-response signaling, and thus increases ENZA-induced cell death and growth arrest in an ATM-dependent manner.

Conclusions: Our study details an innovative therapeutic approach for ENZA-resistant PCa and characterizes a novel perspective for the function of CK1α in the regulation of DNA damage response signaling through phosphorylation of ATM S1270.

100

Urothelial cell stimulation is sufficient to evoke bladder contractions

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Abstract

Background: Pathological changes to urothelial cells and their signaling to sensory neurons have been observed in many different bladder diseases, including interstitial cystitis/bladder pain syndrome and overactive bladder. While we know that bladder sensory afferents receive signals from urothelial cells, it is challenging to determine the exact role of these cells in sensory signaling and, thus how this signaling changes in disease conditions. These challenges persist because it is difficult to specifically stimulate urothelial cells to study the effects on sensory nerve activity and bladder physiology. To overcome this challenge, we have developed a mouse model where we can specifically stimulate urothelial cells using optogenetics.

Methods: We have crossed a uroplankin 2 cre mouse (UPK2-Cre) with a mouse that expresses the light-activated cation channel Channelrhodopsin 2 (ChR2) in the presence of cre. This mouse, UPK2-ChR2, is used to study the effects of urothelial stimulation on bladder physiology. We used cell culture, cystometry, and in vivo electrophysiology to evaluate the effects of optogenetic urothelial simulation on bladder physiology.

Results: Optogenetic stimulation of urothelial cells cultured from UPK2-ChR2 initiates cellular depolarization and release of adenosine triphosphate (ATP). Cystometry recordings demonstrate that optical stimulation of urothelial cells increases bladder pressure and pelvic nerve activity. Severing the pelvic nerve or infusion of a P2XR antagonist can attenuate these contractions.

Conclusions: Collectively, our data suggest that urothelial cells can initiate a bladder contraction dependent on sensory nerve signaling. This data supports a foundation of literature demonstrating communication between sensory neurons and urothelial cells. Using this optogenetic tool, we can further dissect this signaling, its importance for normal micturition, and how it may be altered in pathophysiologic conditions.

101

Therapeutic Efficacy of YM155 to Regulate an Epigenetic Enzyme in Major Subtypes of RCC

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Abstract

Background: Renal cell carcinoma (RCC) is the most common type of kidney cancer and includes more than 10 subtypes. Because clear cell RCC (ccRCC) is the dominant phenotype accounting for more than 75% of total RCC, the underlying mechanism and therapeutic options for oncogenesis of the other subtypes, including papillary RCC (pRCC) and chromophobe RCC (chRCC) are limited.

Methods: We analyzed the public databases for ccRCC, pRCC, and chRCC to find factors that can multiple target RCCs. BIRC5 was commonly overexpressed in a large cohort of pRCC and chRCC patients as well as ccRCC and was closely related to the progression of RCCs. Through loss and gain of function studies and YM155, a BIRC5 inhibitor, we investigated the potential of BIRC5 as a therapeutic target for these major three types of RCCs.

Results: Loss and gain of function studies showed the critical role of BIRC5 for cancer growth. YM155 induced a potent tumor-suppressive effect in the 3 types of cells and xenograft models. To determine the anti-tumor effect of YM155, an analysis of epigenetic modification in the BIRC5 promoter was indicated that histone H3 lysine 27 acetylation (H3K27Ac) is highly enriched on the promoter region of BIRC5. Chromatin-immunoprecipitation (ChIP) analysis identified that H3K27Ac enrichment was significantly decreased by YM155. Immunohistochemistry experiment for xenografted tissue was confirmed that overexpressed BIRC5 is an important component of malignancy in the RCCs, and high expression of P300 is significantly associated with progression of RCCs.

Conclusions: Our findings reveal the P300-H3K27Ac-BIRC5 cascade in three types of RCC and provide a therapeutic possibility for major 3 types of RCC.

Figure 1

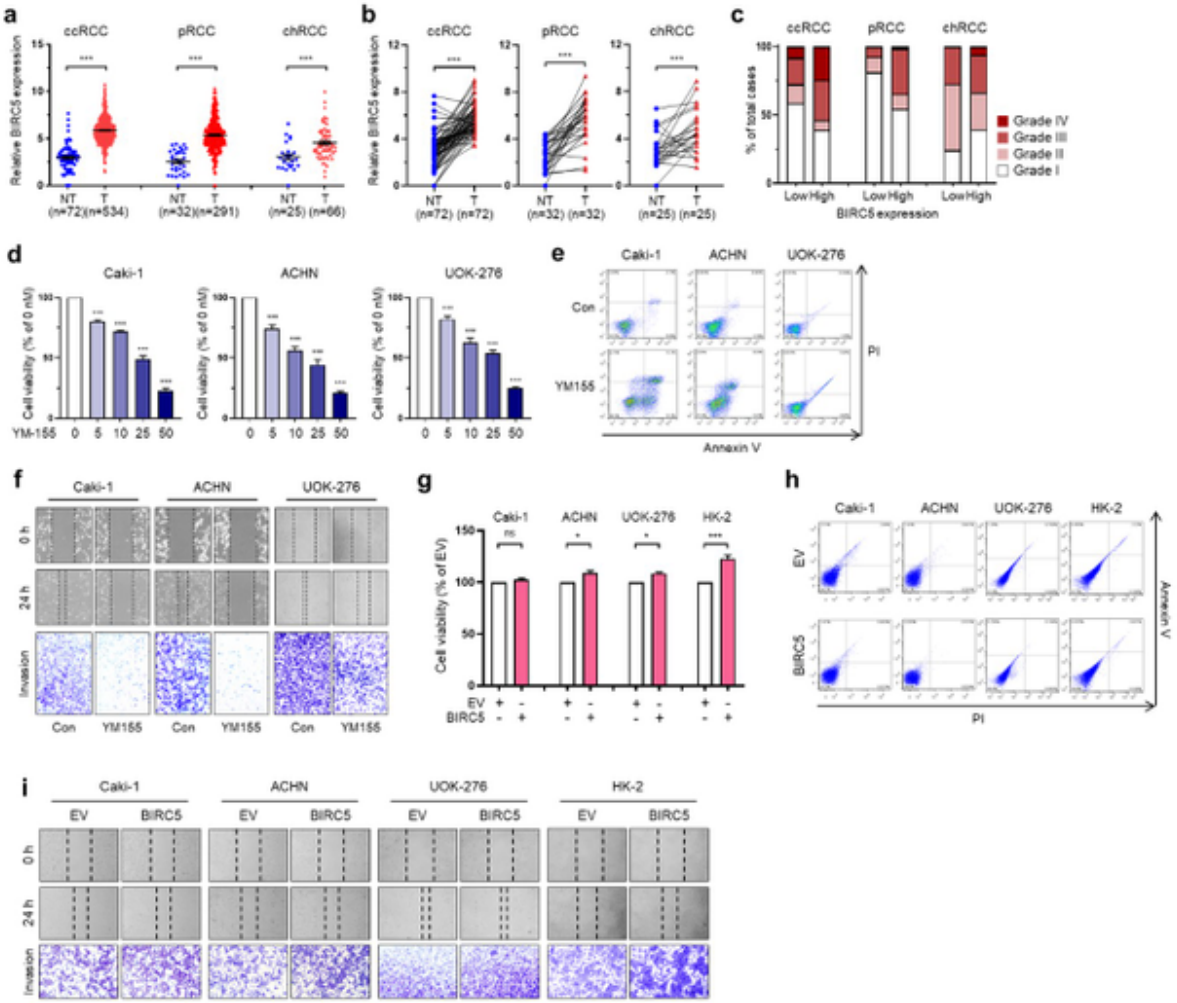

a
ccRCC
pRCC
chRCC
Relative BIRC5 expression
NT (n=72) T (n=534)
NT (n=32) T (n=291)
NT (n=25) T (n=66)
b
ccRCC
pRCC
chRCC
Relative BIRC5 expression
NT (n=72) T (n=72)
NT (n=32) T (n=32)
NT (n=25) T (n=25)
c
ccRCC
pRCC
chRCC
% of total cases
Grade IV
Grade III
Grade II
Grade I
Low High
BIRC5 expression
d
Caki-1
ACHN
UOK-276
Cell viability (% of 0 nM)
YM-155 0 5 10 25 50
e
Caki-1
ACHN
UOK-276
Con
YM155
PI
Annexin V
f
Caki-1
ACHN
UOK-276
0 h
24 h
Invasion
Con
YM155
g
Caki-1
ACHN
UOK-276
HK-2
Cell viability (% of EV)
EV
BIRC5
h
Caki-1
ACHN
UOK-276
HK-2
EV
BIRC5
Annexin V
PI
i
Caki-1
ACHN
UOK-276
HK-2
EV
BIRC5
0 h
24 h
Invasion

Figure 2

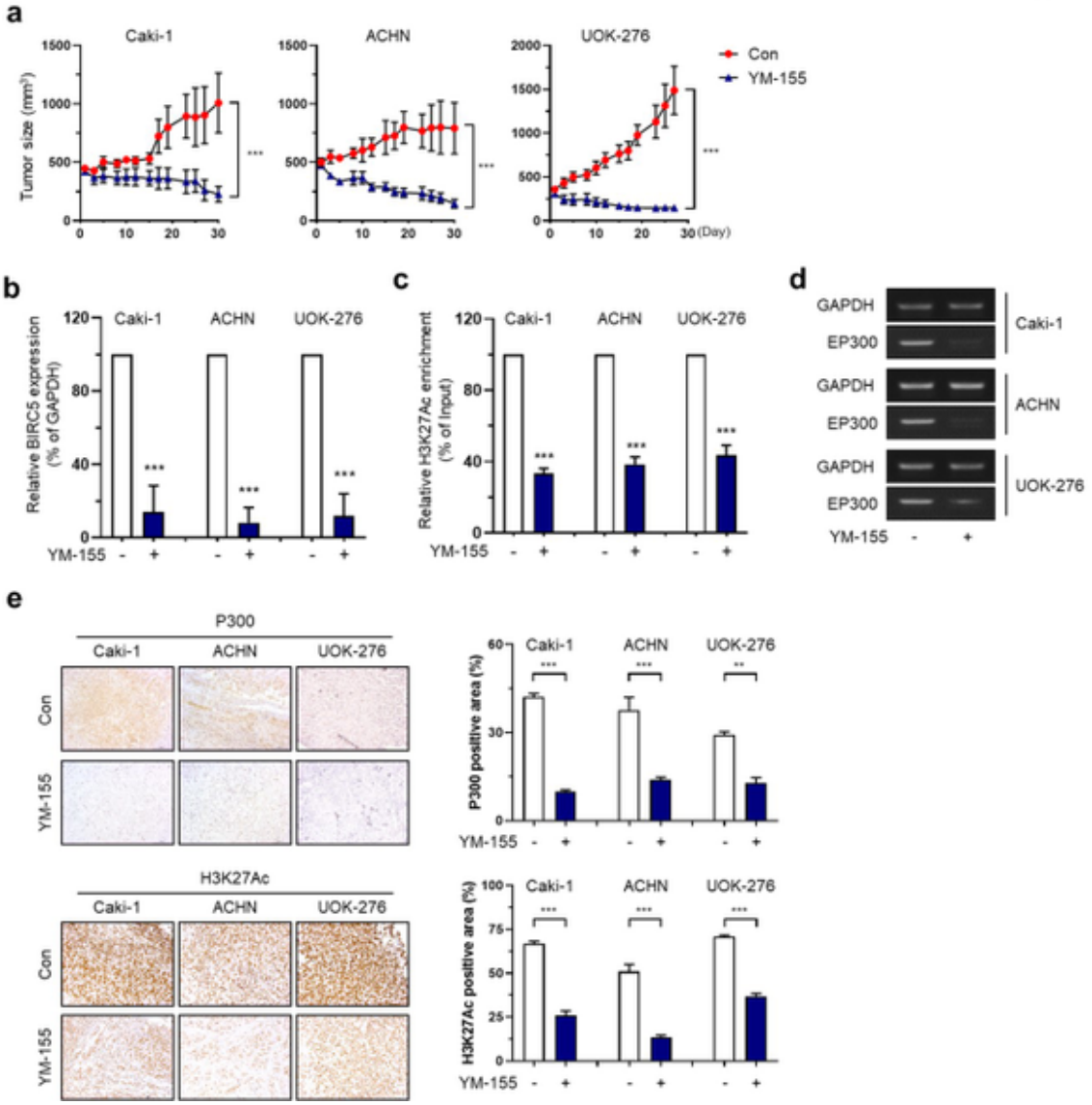
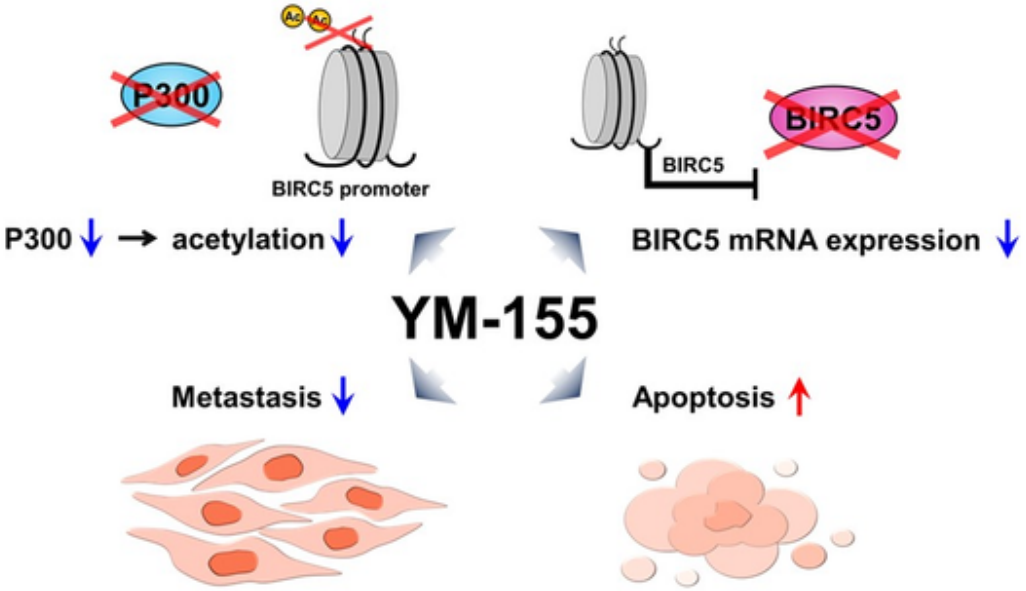

a
Caki-1
ACHN
UOK-276
Con
YM-155
Tumor size (mm³)
(Day)
b
Relative BIRC5 expression (% of GAPDH)
YM-155
c
Relative H3K27Ac enrichment (% of Input)
d
GAPDH
EP300
e
P300
H3K27Ac
Con
P300 positive area (%)
H3K27Ac positive area (%)

Figure 3


P300
Ac
Ac
BIRC5 promoter
BIRC5
BIRC5
P300 → acetylation
BIRC5 mRNA expression
YM-155
Metastasis
Apoptosis

102

Comparative RNA-Seq of Prostate Zonal and Seminal Vesicle Stromal Cells

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Abstract

BACKGROUND: Age-related benign hyperplasia of the prostate gland (BPH) and prostate cancer are a significant health problem, and new strategies are needed to prevent disease initiation and treat progressive disease. Prostate cancer predominantly arises in the peripheral zone (PZ), while BPH arises in the transition zone (TZ). In contrast, the Seminal Vesicle (SV) is an androgen-regulated organ from which disease rarely originates. Thus, understanding differences between prostate and seminal vesicle, and between prostate zones, has the potential to prioritize new pathways to enhance our understanding of prostate disease etiology and identify new targets for prevention and treatment. Here we conducted comparative RNA-seq between primary human stromal cells from prostate and seminal vesicle to identify distinct genes and pathways that warrant further investigation as mediators of disease initiation.

METHODS: Fresh human prostate specimens from peripheral zone (PZ), transition zone (TZ), central zone (CZ), and seminal vesicle (SV) were obtained using an IRB-approved protocol. Samples were minced and dissociated using collagenase. Dissociated cells were plated into RPMI1640/FCS/PenStrep to grow stromal cultures. RNA was isolated at passages 2 and 3. RNA-Seq libraries were prepared and sequenced on an Illumina® platform. Raw sequence data was trimmed using the Trimmomatic-0.36 package with default parameters. After quality control, each sequence was aligned to the human genome dataset hg38 using Kallisto. Outputs were analyzed using EdgeR - Bioconductor package. Gene set enrichment analysis (GSEA) was subsequently conducted using pairwise comparison. Normalized enrichment score (NES) and false discovery rate (FDR) were calculated.

RESULTS: No pathways were significantly prioritized between prostate zones, only between prostate and seminal vesicle. In SV the exclusively top enriched pathways were coagulation (NES = 0.84), tgf beta signaling (NES = 0.84), hedgehog signaling (NES = 0.83), notch signaling (NES = 0.79), apical junction (NES = 0.76), and complement (NES = 0.72); the most de-enriched pathways in SV exclusively were IL6/JAK/STAT3 signaling (NES = - 0.76), mtorc1 signaling (NES = - 0.59), and pancreas beta cells (NES = - 0.58). For PZ, the highest upregulated genes were FOXD2, ABCB1 and EYA2, and the most downregulated genes were GREM1, FLT1, FDE1C, VAT1L, FOXL2NB, FOXL2, LPAR3, PTER, HOXD9, JAKMIP2, WT1, ITGA1 (LFC > 1.5, FDR < 0.2). For TZ, most upregulated genes were CADM1, SBSPON, SPART, COLEC10, PLXDC2, PRUNE2, CCND2, GABBR2, RPL36AP37, SPON1 and most downregulated genes were KIAA0040, ADAMTS16, KDR, PCDH7, LOC102723566, HLA-A, NFKBIL1, SPINK13, RPL6P10, LZTS1 (LFC > 1.5, FDR < 0.1). For CZ, highest upregulated genes were PTGER3, GFRA1, S100A4, SIX2, LPAR3, HSD17B2, GABRA2, KCNS3 and the most downregulated genes were ALDH1A2, WT1, PAPPA-AS1 (LFC > 1.5, FDR < 0.2).

CONCLUSIONS: Cancers rarely originating in SV could be due to the de-enrichment of cancer related pathways: IL6/JAK/STAT3 signaling and MTORC1 signaling. Furthermore, the top upregulated genes in PZ, FOXD2, ABCB1 and EYA2, could contribute to the development of prostate cancer and may be potential targets for prostate cancer prevention.

103

Bladder Cancer Cell Lines Produce the Th1-inhibitory protein IL-18BPa but not IL-12p40 on exposure to INFγ

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Abstract

Introduction/Background:

A Th1-type immune response to BCG characterized by elevated production of INFγ has shown to be effective against non-muscle invasive bladder cancer (NMIBC). Macrophage and other antigen presenting cell-produced IL-12 and IL-18 mediate Th1 polarization. Interleukin 12 is a heterodimer of the p40 and p35 chains linked by disulfide bonds. IL-18 was originally termed INFγ inducing factor. IL-18 cannot induce Th1 cell development as can IL12, but has the capacity to activate established Th1 cells to produce INFγ in the presence of IL-12. Recent data has shown that IL-12p40 (monomer) is an anti-autoimmune cytokine that inhibits IL-12RB1 internalization, counteracting the effects of IL-12, IL-23, and p40 (homodimer). Furthermore, p40 (monomer) had a negative effect on IL-12 mediated up-regulation of CD4+IFNγ+ T cells and IFNγ production. Comparably, IL-18 Binding Protein, specifically isoforms a and c (IL-18BPa and IL-18BPc), inhibit the activity of IL-18 by binding to IL-18 and blocking its interaction with the IL-18 receptor. The balance between Th1 and Th2 polarizing cytokines, it thought to explain the varied response to BCG in bladder cancer. Changes in IL-18BPa levels in urine, as a response to BCG treatment, has been shown to predict disease recurrence and/or progression. In an effort to understand how bladder cancer cell lines respond to an INFγ-mediated Th1 immune attack, we measured IL-18, IL-12p70, IL-18BPa and IL-12p40 levels in the culture medium of each cell line before and after exposure to INFγ.

Methods:

Six bladder cancer cell lines, three high grade: T24m, TCCsup and HT-1197 and three low grades: RT4, 5637 and SW780 were harvested, counted and seeded in 12 well plates in DMEM medium containing 10% fetal calf serum. The LNCaP prostate cancer cell line was used as control since prior research has shown that it expresses IL-12p40 monomer, albeit, in serum free medium. At 24 hours, media were replaced and cells were exposed to 3 different concentrations of INFγ 1, 10 and 50ng/ml. At 48 hours, conditioned media were harvested and commercially available ELISA were used to measure IL-12p70, IL-12p40, IL-18 and IL-18Pa. Experiments were carried out twice in duplicates.

Results:

Our preliminary Results: showed that all six human bladder cancer cell lines expressed IL-18BPa at >20pg/ml over 48 hours per 100,000 cells in 2ml of serum containing medium in vitro when exposed to >1ng/ml of INFγ. An increasing trend of expression of IL-18BPa was observed in all cell lines with exposure to increasing concentrations of INFγ in vitro. Cell line T24 exhibited the highest levels of IL-18BPa followed by cell line RT4. Both cell lines had a higher baseline expression of IL-18BPa without exposure to INFγ compared to the other 4 cell lines. (see Table 1). Control prostate cancer cells (LNCap) only showed production of IL-12p40 in serum free media with or without INFγ. Neither IL-12p70, IL-12p40 nor IL-18 were detected in LNCaP culture medium with serum. No significant amounts of IL-12p70 or IL-12p40 were detected in the medium of any of the bladder cancer cell lines with or without INFγ in the presence of serum. Minimal IL-18 expression was identified upon exposure to INFγ (<15pg/ml from 1000,000 cells in 2ml medium over 48hours) in the bladder cancer cell lines.

Table 1

cell line	INFγ*			
	0 ng/ml	1 ng/ml	10 ng/ml	50 ng/ml
RT4	184	857	1356	1611
T24	242	1945	4008	4232
TCCsup	6	22	67	151
5637	4	652	1341	869
HT-1197	3	139	316	343
SW780	6	88	199	274
LNCaP	8	18	29	23

*Average of 2 experiments done in duplicate

Conclusions

Preliminary experiments with 6 human bladder cancer cell lines suggest that a significant amount of IL-18BPa is produced by bladder cancer upon exposure to INFγ in vitro. On the other hand, all 6 bladder cancer lines produced minimal amounts of IL-18. Although neutralization of IL-12p40 has been shown to lead to shrinkage of prostate tumors in mice, unlike prostate cancer cell lines, some of which produce IL-12p70 and IL-12p40, these cytokines were not detected in the conditioned media of any of the 6 bladder cancer cell lines tested in the presence of serum. To the best of our knowledge, this is the first report to provide evidence of IL-18BPa being produced by bladder cancer cell lines in response to INFγ. IL-18BPa expression by bladder cancer may result in immune modulation that favors cancer cell survival by decreasing BCG-mediated Th1 immune responses.

104

Evaluating SOX2 as a biomarker for radiation treatment outcome in localized prostate cancer

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Abstract

The first line of treatment for prostate cancer patients with primary localized tumors is one of three options: radical prostatectomy, radiation therapy, and active surveillance. In most instances, the decision between the three ultimately comes down to physician-patient shared decision. Currently, there are tumor expression profiles that score homologous recombination deficiency available for patients, but this is typically used to determine if PARP inhibitors are an additional treatment option. One potential biomarker we have identified, SOX2, was linked to increased Gleason Grade at diagnosis and decreased time to metastasis after biochemical recurrence. Previously, SOX2 was found to promote therapy resistance and lineage plasticity in prostate cancer. Further, SOX2 has binary expression in prostate cancer making it an ideal biomarker. This project aims to determine the impact of SOX2 expression on radiation induced DNA damage, and thus establishing it as a predictive marker for radiation outcome.

We used CRISPR/Cas9 editing to remove SOX2 from CWR-R1 cells. We observed reduced cellular growth with the loss of SOX2. Analyses of RNA-sequencing from these cells and patient data and found bioinformatic enrichment of DNA damage pathways. Further, both protein analyses and γ-H2AX foci showed there was an increase in the DNA damage marker γ-H2AX in the SOX2 knock-out cells. To further investigate this, we xenografted CWR-R1 SOX2 edited cells into athymic nude mice and irradiated their tumors via x-ray radiation. We measured tumor growth post radiation.

We aim to further investigate the role of SOX2 on DNA damage response and repair through overexpression cell lines. We also plan to analyze DNA damage response and repair pathways post radiation in tumor xenografts. Through comparison between in vitro, in vivo, and patient data, our objective is to determine whether SOX2 can serve as a predictive marker for radiation therapy outcome.

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

105

Pparg Signaling Promotes Renal UPK-UC Formation and Protects the Kidney During Urinary Tract Obstruction

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Abstract

Background: Urinary Tract Obstruction (**UTO**) is a leading cause of chronic and end stage kidney disease and in children. While no current treatments prevent obstruction-induced kidney disease, future therapies may be identified by understanding protective adaptations. We previously reported that uroplakin-expressing urothelial cells (**UPK-UCs**) protect the kidney during experimental UTO. The molecular program that governs UPK-UC formation in the renal urothelium is unknown. In bladder, the *Pparg* signaling pathway drives urothelial differentiation. Thus, we hypothesized that *Pparg* drives renal UPK-UC formation during UTO.

Methods: UTO was modeled surgically, using unilateral ureteral obstruction. Lineage analysis was performed using $K5^{iCre}$ and $Upk2^{iCre}$ mice crossed to stop-floxed reporter lines. Immunofluorescent Analysis and RT-qPCR following Fluorescent Activated Cell Sorting (**FACS**) were used to profile the *Pparg* pathway. UPK-UCs were depleted using $Upk2^{iCre}$;$R26^{DTR}$ mice, and Pparg was conditionally disrupted using Upk^{iCre};$Pparg^{fl/fl}$ (**Pparg-cKO**) mice. Renal ultrasound was used to evaluate UTO-induced parenchymal thinning.

Results: Lineage analysis studies showed that UPK-UCs, not K5-UCs, were the major source of UPK-UCs following UTO. UPK-UCs expressed Pparg and transcriptional targets, Grhl3 and Fabp4, following UTO, while Pparg and Fabp4 were not detected in sham-operated kidneys. FACS corroborated de novo expression of *Fabp4* in Upk-UCs in UTO kidneys. The *Pparg* pathway was abrogated following UTO in mice with genetic depletion of Upk cells. Finally, we found that Pparg-cKOs had fewer UPK-UCs and significantly reduced renal parenchyma (25.9% vs. 38.0%, P=0.0008) during UTO.

Conclusion: Our Results: indicate that UPK-UCs activate Pparg following UTO to preserve renal parenchyma. Future studies will investigate whether Pparg activation can protect the kidney during UTO. Our findings advance our understanding of renal adaptation to UTO and reveal a potential mechanism with therapeutic utility for mitigating obstructive kidney disease in children.

106

Identifying the Function of M2 Macrophage Subtypes and Cytokine Release Associated with Improved Long-Term Healing Outcomes in Patients with Stress Urinary Incontinence

Research associate ILAHA ISALI MD, Research associate Phillip McClellan PhD, Student Thomas R.Wong N/A, Research associate David Fletcher BS, Associate Professor Tracy Bonfield PhD, Professor Ozan Akkus PhD, Professor Adonis Hijaz MD

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Abstract

Background: Regenerative cells are a crucial component required to improve tissue function for benign urologic conditions, such as stress urinary incontinence and pelvic organ prolapse. They can be delivered to the target region either surgically or through needle injection. Cellular therapy-mediated M2 macrophage polarization has been shown to promote tissue remodeling in the treatment of injured tissues, including the myocardium, spinal cord, and skeletal muscle. It was proposed that downstream effects are influenced significantly, and these effects are dictated by the phenotype and overall function of macrophages. Understanding how macrophages respond to tissue injury could significantly impact the manner in which urogenital problems are treated in regenerative medicine. Macrophages are often divided broadly into two groups, M1 (pro-inflammatory) and M2 (anti-inflammatory). The prolonged presence of pro-inflammatory M1 cells can result in insufficient healing, whereas M2 macrophages are linked to interstitial collagen deposition and the wound healing resolution. We propose the beneficial anti-inflammatory and pro-regenerative characteristics of M2 macrophages are mostly a result of one of the M2 subtypes. This research aims to identify M2 subtypes originating from bone marrow and investigate variations in cytokine production from each known subtype.

Methods: Bone marrow was harvested from femurs of eight-week-old female Sprague–Dawley (SD) rats. Monocytes were harvested from rat bone marrow and polarized to four M2 subtypes (M2a, M2b, M2c, and M2d) in order to examine differences in cytokine production by means of a Luminex, bead-based, multiplex assay. Macrophage subtypes were identified by flow cytometry and immunocytochemistry using antibodies against CD68, CD163, CD86, Arginase 1, and iNOS.

Results: M2a cells produced nearly 1000x more IL-4 and 600x more IL-13 ($p < 0.05$) than other subtypes. M2c cells generated 30x more IL-10 and 6x more TGF-β1 ($p < 0.05$) compared to other groups. M2d produced 2x more VEGF-A ($p < 0.05$) and only 1/3 as much TIMP-1 ($p < 0.05$) compared to other subtypes.

Conclusions: These findings emphasize the significance of choosing strategies to favor macrophage subtypes that could enhance long-term healing outcomes of regenerated tissues that require structural augmentation, such as stress urinary incontinence.

107

Examining the Role of Intracellular Sex Hormone-Binding Globulin in Prostate Cancer

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Abstract

Background: Prostate cancer (PCa) is the most diagnosed non-cutaneous cancer and disproportionately impacts African American men, who tend to be diagnosed earlier and at more advanced stages. Transformation and proliferation of prostate cells is known to be mediated by androgens, which are carried in the blood by sex hormone-binding globulin (SHBG). According to the long-held "free hormone hypothesis," androgens and other steroid hormones must disengage from their carrier proteins, including SHBG, to diffuse into cells and exert their biological effects. Intracellular SHBG has been reported, but very little is known about its function. Additionally, multiple polymorphisms of the SHBG gene associate with race, prostate cancer risk, and resistance to androgen deprivation therapies. An intracellular role for SHBG stands in contrast to the free hormone hypothesis, which does not suggest a function for SHBG outside the bloodstream. Here we examine the presence of SHBG within prostate cells and tissues to determine its intracellular function and, ultimately, its potential contribution to PCa disparities.

Methods: Gene and protein expression of SHBG was examined in three prostate cancer cell lines, LNCaP, LAPC-4, and MDA-PC-2B, patient-derived primary epithelial and stromal prostate cancer cell lines, and a hepatocellular carcinoma cell line, HepG2. Gene expression was quantified by RT-qPCR and protein expression by western blot. Paraffin embedded samples from patients undergoing radical prostatectomy were sectioned, mounted, and stained with anti-SHBG antibody by immunohistochemistry.

Results: We found expression of SHBG gene and protein in benign prostate and prostate cancer cells. SHBG was highest in the MDA-PCA-2B cell line, which was derived from an African American (AA) patient. Tissue staining of human prostate showed that SHBG was detected in luminal epithelium and basal cells.

Conclusions: SHBG is expressed at a gene and protein level in human prostate cells. Ongoing work includes quantification of SHBG protein in a tissue microarray to compare between ancestry and disease state. Future studies will seek to further elucidate the role of intracellular SHBG in the androgen response.

108

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

Miss Emily Stanczak PhD Candidate[1], Miss Tara Fuller PhD Candidate[1], Mr. Hanyu Xia PhD Candidate[1], Dr. Irene Heredero Bermejo PhD[2], Dr. Douglas Strand PhD[3], Dr. Gustavo Arrizabalaga PhD[1], Dr. Travis Jerde PhD[1]

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Abstract

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

Emily F. Stanczak[1], Tara Fuller[2], Hanyu Xia[2], Irene Heredero Bermejo[2,4], Douglas Strand[3], Gustavo Arrizabalaga[1,2], Travis J. Jerde[1,2]

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

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

Results: Mice infected with *T. gondii* were significantly more likely to exhibit increased number of urine spots and increased smaller spots (<1 cm area) than control mice. These mice exhibit prostatic inflammation, reactive hyperplasia, and the formation of glandular nodular formations with microglandular hyperplasia. Men with BPH had a *T. gondii* positivity rate of 38% versus 9% in the donor controls. Within the BPH group, 100% of prostates from seropositive men exhibited glandular nodules with microglandular hyperplasia versus 33% in the seronegative samples. In addition, we saw a significantly higher amount of microglandular hyperplasia in seropositive BPH patients versus seronegative donors.

Conclusions: Systemic *T. gondii* infection induces inflammation and epithelial nodule formation in mouse prostates that is associated with an increased voiding phenotype. Seropostivity to *T. gondii* is associated

with increased BPH-LUTS in men and is associated with inflammation and the formation of epithelial nodules with microglandular hyperplasia.

109

Title: Understanding the Role of the Alzheimer's Disease Associated Gene BIN1 in Prostate Development and Prostate Cancer

Mr. Collin McColl B.S., Ms. Darlene Skapura B.S., Dr. Maria Elisa Ruiz Echartea Ph.D, Ms. Cammy Mason B.S., Ms. Jenny Deng B.S., Dr. Brian Simons D.V.M., Ph.D., Dr. Cristian Coarfa Ph.D, Dr. Salma Kaochar Ph.D

Baylor College of Medicine, Houston, Texas, USA

Abstract

Background: The ACS estimates that there will be 268,490 new cases of prostate cancer (PC), leading to 34,500 deaths in 2022. AR regulates the expression of several genes involved in cell proliferation and differentiation, making it an ideal target for PC treatment. Coincidently, BIN1 is frequently deleted in PC patients (~5%). Notably, BIN1 deletion frequency is higher in prostate cancer patients harboring Speckle-type POZ protein (SPOP) mutation. In human prostate cancer, the SPOP mutant subclass has been associated with increase AR activity. Furthermore, we have previously illustrated that SPOPMT;MYCHigh transcriptomic response, defined by the overlap between the SPOPMT and c-MYC transcriptomic programs, is associated with inferior clinical outcome in human PCs. Our observation of frequent BIN1 deletion in the SPOP mutant subclass leads us to hypothesize that BIN1 deletion may result in enhanced AR signaling.

Methods: We analyzed baseline protein and mRNA levels of BIN1 in 13 PC cell lines via quantitative polymerase chain reaction (qPCR) and immunoblot. To understand the role of BIN1 in PC cells, we overexpressed BIN1 and evaluated changes in cell proliferation, migration, and AR target gene expression. To elucidate the role of BIN1 in vivo prostate development, we also generated prostate-specific knockout of BIN1 and investigated changes in prostate development and AR signaling axis over time.

Results: We found that none of the 13 PC cell lines had strong protein expression of BIN1 at baseline. However, all the cell lines had detectable mRNA via qPCR at baseline. Interestingly, the three cell lines with the highest mRNA expression were AR negative (DU-145), AR independent (LN95) or castration resistant (MDVR). We next overexpressed BIN1 and observed significant decrease in mRNA expression of key downstream AR target genes KLK3 (PSA) and NKX3.1. We further confirmed inhibition of AR signaling by BIN1OE using a KLK3-Luc reporter assay. We also found that overexpression of BIN1 decreased invasive phenotype in PC cells, measured by reduced migration through Matrigel membranes. Since BIN1 deletion was enriched in SPOP mutant PC clinical data, we evaluated changes in cell proliferation in the wildtype and mutant SPOP cell lines with and without BIN1OE and found that BIN1OE hindered growth of SPOP mutant PC cells. Analysis of the TCGA primary PC dataset illustrate that PC patients harboring BIN1 deletion had higher AR activity compared to PC patients expressing wildtype BIN1. Importantly, mice with prostate specific BIN1 deletion had larger prostate mass at 2 months of age compared to age matched controls.

Conclusions: Herein, we illustrated for the first time BIN1 functions as an inhibitor of AR signaling axis. Our novel findings help elucidate the clinical significance of BIN1 deletion in PC patients and highlights AR-signaling axis as a targetable signaling axis for Bin1 deleted PC.

110

The Role of Immune Cells in *Toxoplasma gondii*-Induced Prostatic Hyperplasia

Ms. Tara Schmidt PhD Candidate

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Abstract

The Role of Immune Cells in *Toxoplasma gondii*-Induced Prostatic Hyperplasia

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By the age of sixty, 50% of men will experience BPH through lower urinary tract symptoms (LUTS), including urinary retention that sometimes leads to death. Currently existing therapies treat symptoms associated with BPH-LUTS, but do not halt or reverse BPH progression. Therefore, there is a critical need to understand the underlying mechanisms that contribute to severe BPH. Highly symptomatic, advanced BPH specimens exhibit both stromal and epithelial glandular nodular features, the latter being made up of ringlike microglandular hyperplasia. Inflammation is the most tightly associated histological finding associated with symptom severity in BPH-LUTS, and recent studies show that T cells are the most expanded immune cell population. However, the immunological mechanisms that underlie inflammation and microgland formation in BPH are not completely understood and have never been fully characterized. An early CD4+ T helper 1 (Th1) response is known to progress to a later CD4+ T helper 2 (Th2) response in prostate hyperplasia. Further, published data shows that Th2 cytokine IL-4 promotes cell growth and induces cell phenotype changes in prostate cells, possibly contributing to microgland formation. Our lab developed a novel mouse model of prostate hyperplasia induced by the intracellular parasite Toxoplasma gondii (T. gondii) that recapitulates key BPH features, including inflammation, epithelial glandular nodule-like formations, and reactive hyperplasia. Our objective is to define and delineate the CD4+ T cell response and its contribution to microgland formation. We identified a mixed Th1/Th2 response that subsides by 60 days post-infection, corresponding with the time at which microglands are seen in our mouse model and in agreement with Th2 cells limiting the Th1 response. We have confirmed our Results: through a multiplex array established previously in our lab that shows an increase in BPH-related cytokines in our model, as well. Future studies will address the contribution IL-4 plays in epithelial glandular nodule formation through blocking IL-4 in the prostate and histologically assessing microgland formation.

111

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

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Abstract

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

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

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

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

112

Elucidating the role of E2F1 in neuroendocrine prostate cancer.

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Abstract

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

Methods: We performed gene expression analyses and ChIP-sequencing in human and mouse models of advanced prostate cancer engineered with *MYCN* overexpression and *RB1* loss, in addition to patient-derived organoids and xenografts. Transcriptional differences between the models were validated using RNA-sequencing data from a CRPC and NEPC patient cohort. ChIP-sequencing data from these models was compared to publicly available E2F1 ChIP data and to additional NEPC driver gene cistromes, to highlight NEPC-specific E2F1 gene activity and potential cooperative activity. E2F1 depletion was carried out using siRNA.

Results: Specific E2F transcription factors, *E2F-1*, *-2*, *-7* and *-8* were significantly upregulated in N-Myc overexpressing, *Rb1* null GEMMs, in concordance with their upregulation in NEPC patient samples. Chronic androgen withdrawal in LNCaP cells highlighted increased E2F1 expression specifically in cells with both N-Myc overexpression and *RB1* loss. Novel characterization of the E2F1 cistrome in an NEPC PDX model revealed binding at NE gene promoters (e.g. *INSM1*, *NEUROD1* and *ASCL1*), which was absent from E2F1 ChIP performed in less advanced prostate cancer models, suggesting a redirection of E2F1 during progression to NEPC. Knockdown of E2F1 in patient-derived NEPC cell lines led to reduced expression of NE marker genes, further highlighting the potential role of E2F1 in driving the NEPC phenotype.

Conclusions: Upregulation of E2F1 is a potential mechanism that underlies the synergy between N-Myc overexpression and *RB1* loss in NEPC, and specific redirection of E2F1 binding towards NE gene promoters may help drive development of the NE phenotype. Further characterization of the specific role of E2F1 in NEPC will highlight areas for therapeutic investigation, to ultimately improve patient outcome.

113

Impact of combined type I, II and III Interferon receptors knockout on bladder carcinogenesis with squamous differentiation

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Abstract

Impact of combined type I, II and III Interferon receptors knockout on bladder carcinogenesis

Background:

Squamous differentiation occurs in up to 40% of muscle-invasive bladder cancers and is associated with poor clinical outcomes. Several studies have identified an association between chronic inflammation in the bladder and squamous bladder cancer. However, the mechanistic link between specific inflammatory insults and the development of squamous disease is elusive. Basal-squamous bladder cancers are enriched for gene targets activated by the inflammatory mediators, interferons (IFN). Therefore, we hypothesized that IFN signaling is required for the development of basal-squamous bladder cancer.

Methods:

To generate mice deficient in IFN signaling (*ifn* TRKO), *Ifnar1-/-Ifnlr1-/-* C57BL/6J mice were crossed with miceharboring knockout for the *Ifngr1* gene. To determine the impact of *ifn* TRKO on immune cells, we harvested spleens from age/strain-matched experimental and control mice and performed mechanical dissociation to prepare single-cell suspensions for flow cytometry. Bladder tissues were also collected from both groups at 8, 12, and 24 weeks of age to determine the impact of *ifn* TRKO on gross and microscopic bladder morphology. Separately, we exposed experimental and control mice to the bladder-specific carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) for 16 weeks. Bladder tissues were characterized via western blotting, PCR and immunohistochemistry for markers of basal and luminal bladder cancer.

Results:

Successful ablation of *Ifnar1*, *Ifngr1* and *Ifnlr1* was confirmed via genotyping and expression studies. Flow cytometry identified no significant differences in macrophages, dendritic cells, T cells and NK cells. Compared to controls, bladders obtained from *Ifn* TRKO mice appear normal at 8 and12 weeks of age. However, bladders dissected from 24 weeks old *Ifn* TRKO mice exhibit severe abnormalities. Specifically, bladders from *ifn* TRKO mice have a larger volume with smooth muscle hypotrophy and urothelial atrophy at the microscopic level. BBN treatment of control mice for 16 weeks resulted in the development of FOXA1 negative bladder carcinoma in situ (CIS) with evidence of squamous differentiation. However, we observed reduced incidences of CIS and squamous differentiation in Ifn TRKO mice. Surprisingly, FOXA1 expression was detected in *Ifn* TRKO mice.

Conclusions

Our findings suggest mice lacking functional IFN receptors develop morphological bladder abnormalities. In addition, our findings suggest *Ifn* TRKO Results: in reduced squamous differentiation in mouse models of basal-squamous bladder cancer.

114

Co-Expression Network Identifies Gender-Specific Prognostic Genes in Muscle Invasive Bladder Cancer

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Abstract

Background: Significant sex-based disparities can exist in disease incidence, tumor biology, treatment response, and patient prognoses in a variety of cancers. In our study, we used the muscle invasive bladder cancer (MIBC) cohort from the Cancer Genome Atlas (TCGA) to construct a gene co-expression network to identify gender-specific, prognostically impactful genes as a first step in uncovering sex-specific differences in MIBC.

Methods: mRNA expression and clinical data of MIBC patients (n=433) were obtained via the Genomic Data Commons (GDC) Data Portal, and underwent covariate correction for batch, race, and age, and low expressed genes were discarded to leave 16,962 genes for analysis. A co-expression network was generated using the MEGENA R package, and network visualizations were done using Gephi (v0.9.2). Differentially expressed genes (DEGs) were identified using the DESeq2 R package, and survival analysis was performed using the survival R package.

Results: MIBC gene co-expression network analysis identified gene modules enriched in processes including “Immune System Process”, “Cell Cycle”, and “RNA Binding” (Fig. 1A). 1,898 genes with high network connectivity are identified as hub genes, and gender-specific survival analysis revealed 73 female-specific and 134 male-specific hubs that significantly impacted overall survival (OS). Female MIBC patients with high expression of genes for B-cell mediated immunity (IGKC, IGKV3-20, IGKV3-11, IGLCC2, IGLV2-23), plasma cells (MZB1), and natural killer cells (NKG7, CCL4) have significantly better OS (Fig. 1B, Table 1), though these genes are not prognostically significant for male patients (Fig. 1C, Table 1). Interestingly, immunoglobulin-related genes are upregulated in stage III and IV male MIBC compared to stage II (IGHG1/2/3/4 6.13e-5 < adj. p < 2.1e-3), suggesting that high immunoglobulin production may be associated with advanced disease and poor survival in male MIBC.

Conclusions: In our study of a MIBC gene co-expression network, we highlighted evidence of sex-specific differences in OS. Overall survival of female patients is significantly impacted by hub genes related to B cell and antibody-related immunity as well as immune-mediated cytotoxicity. However, male patients with elevated expression of these genes did not exhibit a survival advantage. The co-expression network shows that these prognostically significant hub genes are upstream of genes encoding immunoglobulins, cytolytic enzymes, and pro-inflammatory chemokines. This suggests that overexpression of genes that facilitate antibody- and immune-mediated cytotoxicity may impart a survival benefit in female MIBC patients (Fig. 1D).

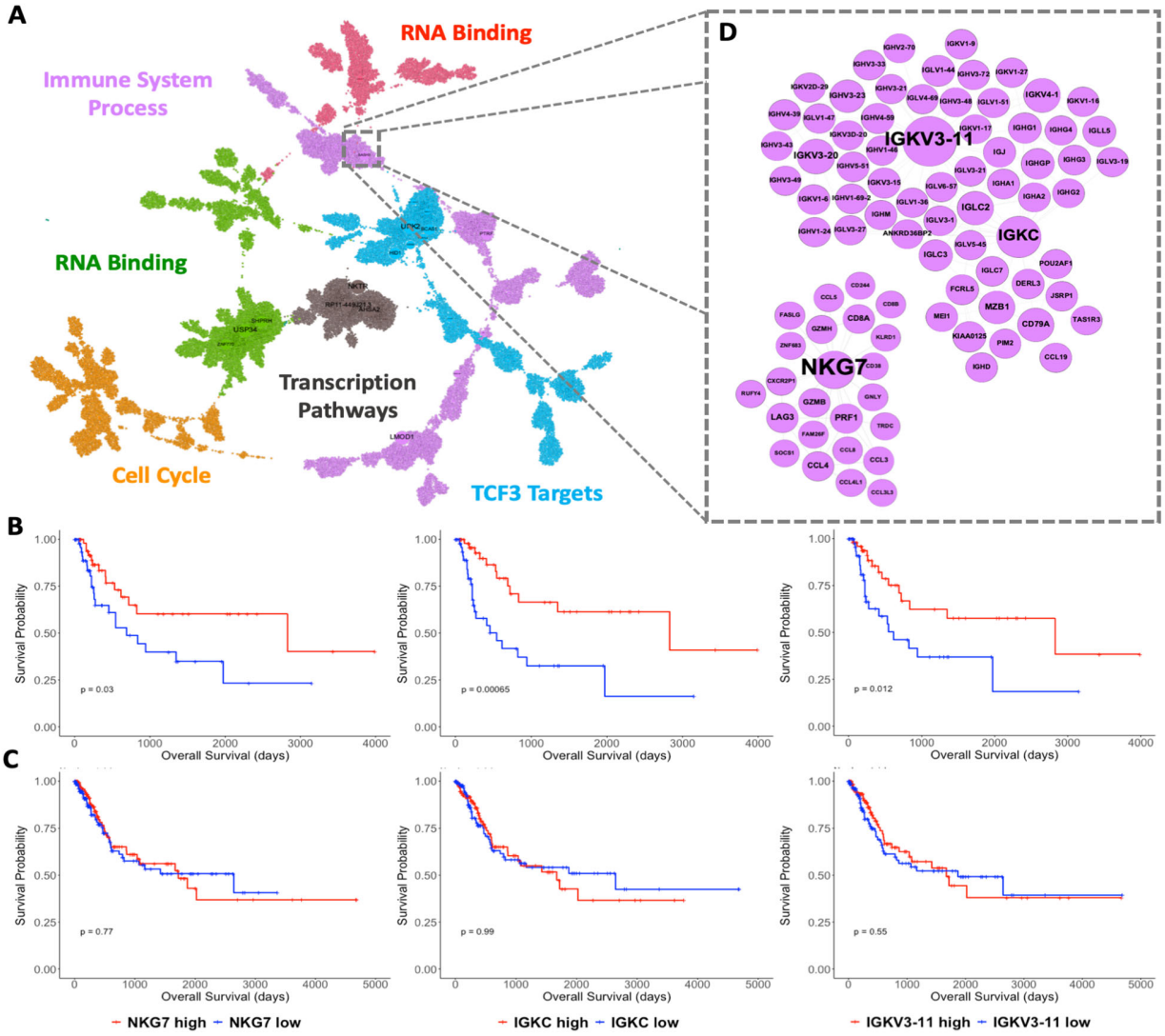

Figure 1: MEGENA gene co-expression network and impact of network key driver genes on patient survival
(A) MEGENA mRNA gene co-expression network of the TCGA MIBC cohort.
(B and C) Kaplan-Meier survival curves of TCGA MIBC (B) female and (C) male patients separated by *NKG7, IGKC, and IGKV3-11* gene expression.
(D) Sub-networks of the Immune System Process gene module, where node and text size correspond to the degree of connectivity within the network.

Gene Symbol	Female KM p-value	Male KM p-value
IGKC	0.00065496	0.986398
MZB1	0.00237504	0.710158
IGKV3-20	0.00246096	0.673094
IGKV3-11	0.01198768	0.547897
IGLC2	0.01292345	0.328574
IGLV2-23	0.01884018	0.156955
NKG7	0.03017698	0.72183
CCL4	0.03118297	0.761257
CD79B	0.04845873	0.222545

Table 1: KM p-values of female and male MIBC patients by gene expression

115

Giα2 plays a vital role in Rac1 dependent activation of Wave2 axis and essential for cell migration in prostate cancer cells.

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Abstract

Background: Giα2 is part of a heterotrimeric G proteins, classified into Gs, Gi/o, Gq, G12 in the form of activated Gα-GTP and Gβγ subunits, required for signal transduction in response to activation of G-protein coupled receptors (GPCRs). Previously we have shown the essential role of Giα2 protein in prostate cancer cells migration, in response to both oxytocin and EGF, acting via GPCR and PTKR, respectvely. Cell migration is regulated by many intracellular proteins; the most important proteins belong to the Rho family of GTPases, such as RhoA, Rac1 and Cdc42. Rac1 plays a central role in cell motility and its activation is required for actin polymerization and branching and formation of lamellipodia at the leading edge of the migrating cells. Previously, we have also shown that Giα2 acts downstream of activation of EGF activated PI3K signaling. The knockdown of Giα2 does not cause any reduction in basal Rac1 activity in both PC3 and DU145 prostate cancer cells and had minimum effects on EGF-induced increase in Rac1 activity. In the PC3 cells expressing constitutively active form of Rac1, Giα2 knockdown attenuated cell migration indicating Giα2 may be involved in the regulation of cell motility and invasion at a step which is independent or downstream of Rac1 activation. We hypothesize that the novel effects of Giα2 is required for the activation of the downstream proteins of Rac1 leading to lamellipodia formation at the leading edge of migrating cells.

Methods: we transfected PC3 empty vector and PC3 expressing constitutively active form of Rac1 with control siRNA or Giα2 siRNA, to knockdown endogenous Giα2 expression. In a parallel experiment we overexpressed constitutively active Giα2 in PC3 cells and determined its effect on cell migration and wave2 phosphorylation.

Results: The western blot analysis showed in the absence of Giα2 cells overexpressing constitutively active Rac1showed a decrease in activation of Wave2 and Arp2/3. The cell migration assay showed significantly increased motility in PC3 cells overexpressing constitutively active Giα2 compared to cells transfected with empty vectors.

Conclusion: Based on these Results: we conclude that Giα2 is playing a crucial role in the activation of proteins downstream of Rac1. These proteins are important for cell migration in prostate cancer cells.

116

GAPDH upregulation is critical for AR antagonist-induced glycolysis in CRPC cells

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Abstract

Background: Anti-AR treatment-induced neuroendocrine progression (t-NEPC) in castration-resistant prostate cancers is the major obstacle in the clinic without adequate treatment. Increased glycolysis was reported previously in NEPC cells and tumors. We have shown that AR antagonist Enzalutamide enhanced GAPDH expression in CRPC cells in SBUR and AUA annual meetings last year. In this report, we sought to determine the functional role of GAPDH up-regulation after ADT and anti-AR treatment in CRPC cells.

Methods: The CRPC C42-B cells were utilized for the entire study. GAPDH gene knockout was conducted using the CRISPR/Cas9 plus HDR system. GAPDH gene reinstallation was achieved using a retroviral vector harboring the wild-type, R234K, and R234F mutants. GAPDH promoter-driven reporter assay was utilized to examine cell signal pathways involved in AR antagonist-induced GAPDH gene expression. The global gene expression changes after GAPDH knockout were analyzed using the next-generation RNA-seq approach. Metabolomic alteration after GAPDH knockout was determined using a GC-MS method. A subcutaneous xenograft tumor model was established using the parental and GAPDH-KO C4-2B cell lines.

Results: GAPDH expression knockout was established in C4-2B cells as verified at the protein using an immunoblotting assay, and GAPDH expression reinstallation in GAPDH-KO cells was also confirmed (Fig 1A). GAPDH Knockout in C4-2B cells severely reduced glucose uptake (Fig 1B), resulting in a significant reduction of glycolytic activity, including glucose consumption (Fig 2C) and L-lactate production (Fig 1D). Reinstallation of GAPDH expression in the KO subline cells produced an excellent recovery of glycolytic activity. AR antagonists (Enzalutamide and Abiraterone) significantly increased glucose consumption and lactate production in parental C4-2B cells, which were drastically reduced in GAPDH knockout subline cells (Fig 2). It was previously reported that GAPDH R234 methylation was involved in glucose metabolism [1]. We tested whether GAPDH mutants disrupt glycolytic activity, including the methylation-defect mutant (R234K) and methylation-mimetic (R234F). GAPDH-KO C4-2B subline cells were reinstalled with wild-type GAPDH, R234K, or R234F mutant constructs. As shown in Fig 3, GAPDH KO cells showed a significant reduction in glucose consumption and L-lactate production, which were rescued by either wild-type or mutant GAPDH constructs. To understand the global effect of GAPDH knockout on gene expression, we conducted a whole transcriptome RNA-seq analysis. Our data showed that GAPDH Knockout in C4-2B cells resulted in a more than 2-fold increase of 276 genes. Most of these increased genes are related to cholesterol biosynthesis and transcriptional repressors (Fig 4A & 4B). In addition, GAPDH knockout in C4-2B cells Results: in more than 2-fold downregulation of 389 genes. Most genes were related to central carbon metabolism and peptidase inhibitor activity (Table 1). We also conducted a metabolomic analysis using the GC-MS approach. As shown in Fig 5, principal component analysis (PCA) revealed a drastic difference between the parental and GAPDH-KO cells. The detailed study on individual metabolites showed that glycolytic metabolite D-Fructose and TCA cycle metabolite citric acid were significantly accumulated in GAPDH-KO cells. Five amino acids were reduced considerably after GAPDH knockout (Table 2). To elucidate the mechanism involved in AR antagonist-induced GAPDH upregulation, we utilized a GAPDH promoter-driven luciferase reporter assay. C4-2B cells were transfected with an hGAPDH-rLUC reporter plasmid (Renilla luciferase under the control of a 0.5 kb human GAPDH promoter [2]) and pretreated with pharmacological inhibitors, including GAPDH inhibitor Alternol (5 mM), BET inhibitor ABV-774 (10 mM) [3], pan-PI3K inhibitor BKM120 (5 mM) [4], JNK inhibitor SP600125 (10 mM) [5], GSK-3 inhibitors (TDZD8 [6] and Tideglusib [7]), AKT inhibitor ARQ-

092, PI3K/p110a inhibitor BYL-719 [8], PI3K/p110b inhibitor GSK2636771 [9], pan-PCK inhibitor LY333531 [10], NFkB inhibitor SN50 [11], PKCe peptide inhibitor. After Enzalutamide treatment overnight, cells were harvested for Renilla luciferase assay as described in our recent publication [12]. Our Results: showed that PI3K/JNK/GSK3 pathway inhibitors consistently blocked Enzalutamide-induced GAPDH promoter activity (Fig 8). We then examined the effect of GAPDH knockout on tumor growth in vivo. The GAPDH-KO C4-2B subline cells, GAPDH-KO with wild-type GAPDH gene reinstalled subline cells, and the parental C4-2B cells were used to establish the subcutaneous xenograft tumors in castrated nude mice. C4-2B xenograft tumors with GAPDH knockout exerted a significantly slowing growth curve compared to the parental and wild-type GAPDH reinstalled cells. Consistently, tumor wet weight at the end of observation in the GAPDH-KO group was also significantly lower than the parental and GAPDH reinstalled cells (Fig 7). We also examined the suppressing effect of a previously reported GAPDH inhibitor Koningic acid (KA) [13], on C4-2B xenograft tumor growth compared to AR antagonist Enzalutamide. Koningic acid was used at an intraperitoneal dose of 1 mg/kg, according to a recent publication [14]. Enzalutamide (30 mg/kg) had a moderate suppressing effect on xenograft tumor growth, while KA completely suppressed tumor growth (Fig 8). The combination of Enzalutamide and KA had no additive effect on KA-induced tumor suppression. However, KA treatment had no adverse impact on animal body weight.

Conclusion: GAPDH upregulation is critical in AR antagonist treatment-induced glycolysis modulated by multiple cell signal pathways. GAPDH-specific inhibitor Koningic Acid provided excellent support for targeting GAPDH to overcome AR antagonist resistance in prostate cancer.

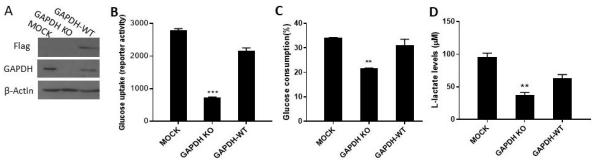

Fig 1. GAPDH knockout reduced glycolytic activity in prostate cancer cells. A GAPDH expression was knocked out using the CRISPR/Cas9 plus HDR system in C4-2B cells. Anti-GAPDH Western blot was conducted using the anti-GAPDH antibody obtained from Cell Signal Tech (Catalog #5174). GAPDH reinstallation was verified using the anti-Flag immunoblotting assay. Beta-actin blot served as a protein loading control. B Glucose uptake assay was conducted using the Glucose Uptake-Glo™ Assay kit obtained from Promega (catalog #J1341). C Glucose consumption was evaluated by measuring glucose levels in cell culture media during a 24-h period interval. A glucose assay was conducted using the GAGO20 kit obtained from Sigma-Aldrich. D L-lactate levels were determined using the Glycolysis Cell-Based Assay Kit from Cayman Chemical (Catalog #600450). All assay values were normalized using cell numbers. The asterisks indicated a significant difference compared to the MOCK control (Student t-test, ** p < 0.01, *** p < 0.001).

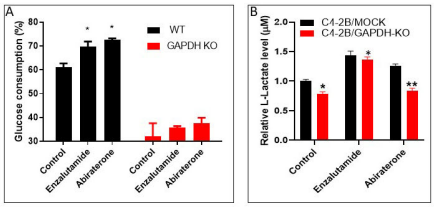

Fig 2. GAPDH knockout diminished AR antagonist-induced glycolytic activity. The parent and GAPDH-KO C4-2B cells were treated with Enzalutamide (10 μM) and Abiraterone (10 μM) for 24-h. Glucose and L-lactate assays were conducted as described earlier.

Table 1. Pathway enrichment for GAPDH-KO downregulated genes

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	Count	zscore
GO:0061134	**peptidase regulator activity**	9/177	219/17697	0.00036683	0.04044062	0.03919726	9	-3
GO:0004866	endopeptidase inhibitor activity	8/177	175/17697	0.00038435	0.04044062	0.03919726	8	-2.82842712
GO:0030414	peptidase inhibitor activity	8/177	182/17697	0.00049904	0.04044062	0.03919726	8	-2.82842712
GO:0061135	endopeptidase regulator activity	8/177	182/17697	0.00049904	0.04044062	0.03919726	8	-2.82842712
GO:0008191	metalloendopeptidase inhibitor activity	3/177	16/17697	0.0005005	0.04044062	0.03919726	3	-1.73205081
hsa05230	**Central carbon metabolism in cancer**	5/77	70/8076	0.00051585	0.08511481	0.08511481	5	-2.23606798

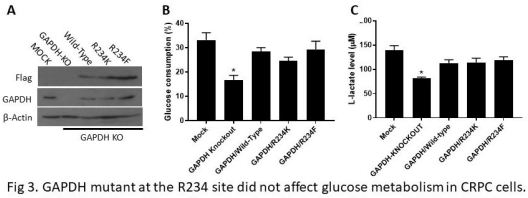

Fig 3. GAPDH mutant at the R234 site did not affect glucose metabolism in CRPC cells. A C4-2B/GAPDH-KO subline cells were reinstalled with wild-type, R234K, or R234F mutants [1] packaged in retroviral particles, as described earlier. B & C Glucose and L-Lactate levels were determined in cell culture media with the assay kits described before.

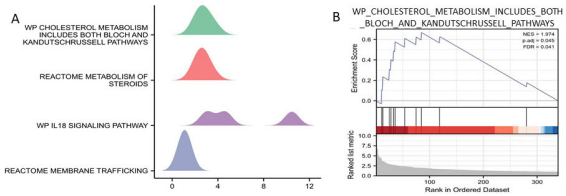

Fig 4. GAPDH knockout resulted in gene upregulation in the cholesterol biosynthesis pathway. Whole transcriptome RNA-seq analysis was conducted using C4-2B cells (parental and GAPDH-KO subline pair comparison). Gene ontology analysis (A) and network connection (B).

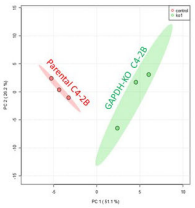

Table 2. Major alterations of GC-MS metabolite analysis

Compound	FC	log2 (FC)	raw.pval	LOG10(p)
D-Fructose	48.168	5.59	0.0020629	2.6855
Isocitric acid	12.575	3.6525	0.00070723	3.1504
Citric acid	8.5209	3.091	0.0010463	2.9803
Mannitol	5.9308	2.5682	0.0031555	2.5009
Pyroglutamic acid	3.4113	1.7703	0.0076453	2.1166
L-Threonine	0.531	-0.91322	0.031668	1.4994
Linoleic acid	0.49885	-1.0033	0.035881	1.4451
Ethanolamine	0.33799	-1.5649	0.016047	1.7946
L-Valine	0.28211	-1.8257	0.0030761	2.512
L-Methionine	0.27727	-1.8506	0.0075567	2.1217
L-Lysine	0.25895	-1.9493	0.0071204	2.1475
L-Phenylalanine	0.21466	-2.2199	0.0012258	2.9116
Adenosine	0.21264	-2.2335	0.019152	1.7178

Fig 5. GAPDH knockout induced a drastic change in cellular metabolism. C4-2B cells (parental and GAPDH-KO) were used for the extraction of metabolites, and the GC-MS approach was utilized for the metabolomic analysis. PCA was used to compare the parental and GAPDH-KO cells.

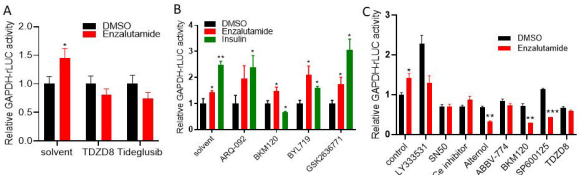

Fig 6. PI3K/JNK/GSK3beta pathways are involved in Enzalutamide-induced GAPDH expression. C4-2B cells were transfected with the hGAPDH-rLUC reporter plasmid and then pretreated with pathway inhibitors for 45 min. After being treated with Enzalutamide (10 μM) in 2% charcoal-stripped FBS (cFBS) overnight, cells were harvested for luciferase assay. The asterisk indicates a significant difference compared to DMSO control or Enzalutamide (* p < 0.05; ** p < 0.01).

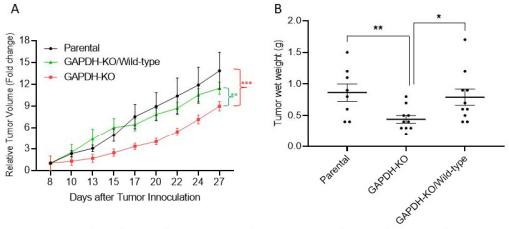

Fig 7. GAPDH knockout reduces xenograft tumor growth in nude mice. The parental C4-2B and its subline cells (5x10e-6) with GAPDH-KO and GAPDH reinstallation were used to establish subcutaneous xenograft tumors in nude mice (n = 10). The tumor growth rate was monitored using the caliper measurement three days a week. A relative tumor growth curve was created using the tumor volume size at the first measurement after one week of cell inoculation as the base volume (panel A). Xenograft tumors were harvested at the end of the monitoring period (4 weeks), and tumor wet weights were recorded (Panel B). The asterisks indicate a significant difference compared to the control. ANOVA test for panel A (** P < 0.01, *** p < 0.001) and Student t-test for panel B (* p < 0.05, ** p < 0.01).

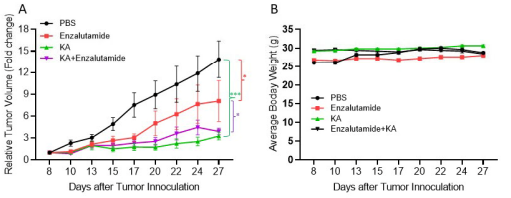

Fig 8. GAPDH inhibitor Koningic acid blocked xenograft tumor growth in vivo. C4-2B cells (6x10e-5 per inoculation) were used for establishing subcutaneous xenograft tumors in nude mice, as described earlier. Animals were randomly assigned to four groups to receive different treatments, as indicated in the figure. The asterisks indicated a significant difference from the control or other treatments (ANOVA test, * p < 0.05, *** p < 0.001).

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117

Spatial transcriptome of the cloaca orchestrates co-development of the urinary bladder and umbilical arteries

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Abstract

Spatial transcriptome of the cloaca orchestrates co-development of the urinary bladder and umbilical arteries

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Background: The urinary bladder is an evolutionary innovation of mammals, but its origin remains ill defined.

Methods: We analyzed spatial and morphological relationship of the developing bladder with the surrounding anatomic structures using a large developmental series of three-dimensional (3D) digitized mouse and human embryos generated with high-resolution episcopic microscope. Using Geo-seq technology, we have also profiled spatial transcriptome of the cloaca to identify the region and tissue-specific gene signatures that controls co-development of the bladder and umbilical arteries. Finally, we used gene knockout approach to evaluate functional significance of genes and pathways.

Results: Here, we show that both human and mouse urinary bladders emerge as a rostral outgrowth of the cloaca, a transient embryonic organ. The outgrowth is arrested in the absence of cloacal epithelial signal Shh.Shh-null mutants lack the bladder and, unexpectedly, exhibit severe umbilical artery defects, suggesting that those seemingly unrelated organs are mechanistically linked. Spatial transcriptome identifies region-specific gene signatures of the cloaca, including a bladder-specific mesenchymal progenitor cell marker Wnt2. Wnt2 expression is significantly downregulated in Shh mutants. Moreover, Wnt2-null mutants display small bladder and single umbilical artery phenotypes.

Conclusion: Collectively, these findings suggest that the Shh-Wnt2 molecular pathway orchestrates co-development of the bladder and umbilical arteries. Dependence of the cloacal signal in umbilical artery development further suggests that emergence of the bladder may offer a survival advantage of mammalian embryos.

118

IGFBP3 Promotes Resistance to Olaparib via Modulating EGFR Signaling in Advanced Prostate Cancer

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Abstract

Background: Castration-resistant prostate cancer (CRPC) is an incurable disease and a leading cause of cancer death in men worldwide. Olaparib (Lynparza) was among the first PARP inhibitors (PARPi) approved for the treatment of CRPC tumors harboring DNA repair defects. However, clinical resistance to PARPi's has been documented. The mechanisms underlying resistance to PARPi's remain elusive. To study acquired resistance, we developed olaparib-resistant LN-OlapR and 2B-OlapR cell lines generated through chronic olaparib treatment of the olaparib-sensitive cell lines LNCaP and C4-2B, respectively. RNA-seq revealed IGFBP3 is overexpressed in both OlapR cell lines. IGFBP3 overexpression is correlated with poor clinical outcome and is thought to participate in DNA repair pathways. IGFBP3 plays a key role in nonhomologous end joining (NHEJ) repair through a ternary complex with the epidermal growth factor receptor (EGFR) and DNA-PKcs. The IGFBP3/EGFR signaling axis is thought to modulate NHEJ repair and could have implications for PARPi sensitivity. We hypothesize that increased IGFBP3 expression promotes PARPi resistance by enhancing DNA repair capacity.

Methods: RNA-sequencing and gene set enrichment analysis were used to determine the expression profile changes in resistant cells compared to parental sensitive cells. Real time PCR (qPCR) and western blots confirmed the expression of DNA damage repair genes such as γH2AX, EGFR, and DNA-PKcs. RNA interference (RNAi) was used to inhibit IGFBP3 and EGFR expression. Gefitinib was used to inhibit EGFR activity. Cell viability and clonogenic assays were used to assess cell growth and survival.

Results: Transcriptomic profiling revealed that IGFBP3 is highly expressed in resistant models. We verified increased levels of IGFBP3 RNA and protein in both OlapR models. We found that RNAi inhibition of IGFBP3 increases γH2AX and cleaved-PARP protein levels in the resistant models, which suggests accumulation of DNA double strand breaks (DSBs) leading to genomic instability and cell death. We discovered increased phosphorylation of EGFR and DNA-PKcs in the resistant cells. Furthermore, silencing/inhibiting IGFBP3 and EGFR reduces OlapR cell viability and resensitizes resistant cells to treatment.

Conclusions: Our findings demonstrated that inhibiting IGFBP3 and EGFR aids in PARPi sensitivity in the resistant setting. Future work will utilize OlapR models to study how the IGFBP3/EGFR/DNA-PKcs protein complex promotes the development of resistance. Understanding the role of IGFBP3 in PARPi resistance will enhance our ability to re-sensitize resistant CRPC to PARPi therapeutics.

119

A novel mouse model for studying bladder cancer metastasis

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Abstract

Background: Experimental models of bladder cancer metastasis are needed for studying treatment response to immunotherapy in immunocompetent mice. We previously developed a murine triple knockout (Trp53-/-, Pten-/-, and Rb1-/-, TKO) model via ex vivo transduction with adenovirus (Ad5CMVCre). We sought to characterize the metastatic potential of the TKO cells in C57 BL/6J mice.

Methods: The TKO cells were labeled with a lentiviral luciferase and GFP double-expressing reporter (pFUGW-Pol2-ffLuc2-eGFP, Addgene #71394). TKO cells (0.5 or 1 million) were injected into tail veins of C57 BL/6J mice (n = 9). The mice were monitored for survival, In Vivo Imaging System (IVIS, Perkin Elmer) imaging, and analyses of tumor metastasis.

Results: EGFP protein expression was detected at the single-cell level of TKO cells, Luciferase expression was confirmed by a Luciferase Reporter Assay. Tumor bioluminescence was detected at day 15-17 after tail vein injection. All mice developed lung and/or bone metastases, including lung (88.9%, n = 8) and bone (77.8%, n = 7) metastases. The median survival was 31 days. Histology and immunostaining of lung and bone metastases revealed positive expression of urothelial markers (CK7, P63, GATA3, CK5) and negative expression of CK8 and Uroplakin 3. The molecular profile of metastatic sites was consistent with a basal subtype urothelial carcinoma.

Conclusion: The TKO metastatic model represents an ideal experimental model for studying tumor-cell dissemination, metastasis, and treatment response to immunotherapy and/or other novel systemic drugs.

120

A chronic NeMo model of de-obstruction shows altered ECM deposition and epigenetic machinery regulation.

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Abstract

Background: Partial bladder outlet obstruction (PBOO) causes severe urinary dysfunction, impaired voiding and changes in urethral pressure. After de-obstruction, symptoms can recur as continued pathology and altered function, which we term as Chronic bladder obstructive disease (COBD). Previously, RNAseq showed dysregulation of epigenetic and matrix-associated genes in acute obstruction. However, the changes of COBD on these gene products is unknown. Our objective was to investigate the pathology, and expression of matrix and epigenetic genes, during COBD. We hypothesized that COBD shows persistent changes in pathology and unique expression of genes.

Methods: Using the nerve-sparing model of obstruction, 8-12 week old adult female C57/Bl6 mice were obstructed by ligating above the urethral opening around the urethra plus a 25-gauge rod. The rod was removed leaving the suture in place. Control mice underwent a sham procedure, by passing a suture behind the urethra without securing it in place. At 8.5 weeks, micturition patterns were recorded. The ligature in obstructed mice was surgically removed at 9 weeks. Mice were followed for 4 more weeks, generating COBD and voiding measurements were taken. Following bladder harvest, residual volumes, bladder mass were measured. We assessed gene expression of candidates from previous work, including TET2, DNMTs and FN1 during obstruction in COBD versus sham samples by RT-qPCR and immunofluorescence on cryosections. The role of TET2 was further examined in human bladder SMC by siRNA downregulation, RT-PCR and immunostaining.

Results: COBD bladder mass was significantly increased compared to sham, $p<0.05$. Maximum voiding efficiency and mean bladder voided volumes were decreased in COBD vs. sham mice. FN1 deposition in the detrusor was significantly upregulated in endomysial spaces in COBD vs. sham. DNMT3A expression, localized to detrusor muscle, was upregulated during COBD. TET2 mRNA expression was significantly decreased between groups. In vitro, TET2 expression in human bSMC was required for normal SMC differentiation marker expression, including CNN1.

Conclusions: Long-term COBD in mice led to reduced voiding efficiency, gross bladder hypertrophy and altered ECM deposition, similar to human bladder obstruction pathology. TET2, which is downregulated during murine COBD, appears to play a role in bladder SMC phenotype. Further work to compare to reversible (early) de-obstruction and obstruction alone is ongoing.

121

Targeting lactate export resensitizes resistant prostate cancer cells to enzalutamide

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Abstract

Introduction: Prostate cancers (PCa) exhibit a unique metabolic profile with reliance on different forms of glucose metabolism at different stages of disease progression. Early stage PCa cells use the TCA cycle, while metastatic PCa cells switch to glycolysis (Warburg effect), leading to the accumulation of lactate. Lactate is exported out of the cells by monocarboxylate transporters (MCTs) to maintain redox balance. Such metabolic reprogramming can lead to gain-of-function mutations and affect drug sensitivity. Resistance to enzalutamide develops in most patients treated with enzalutamide within 9-15 months. Our preliminary data indicated that MCTs are expressed at higher levels in enzalutamide-resistant PCa cells. As Warburg effect is a hallmark of metastatic PCa, in this study, we hypothesized that lactate transport may mediate enzalutamide-resistance in PCa.

Methods: Cell survival, proliferation, clonogenicity, and tumorigenicity of parental and enzalutamide-resistant PCa cells treated with MCT inhibitors or enzalutamide alone or in combination were analyzed. Seahorse-based glycolytic rate assays were used to assess the metabolic capacity of the treated cells. CE-TOFMS/CE-QqQMS-based metabolomic analyses were performed to confirm lactate retention and to analyze the metabolic pathway alterations in the treated cells. Nanostring nCounter-based metabolic pathway gene expression analyses were performed to confirm the metabolic pathway alterations observed.

Results: We found that MCT antagonists resensitized enzalutamide resistant C4-2B and 22Rv1 cells to treatment with enzalutamide. Combining MCT inhibitors with enzalutamide significantly reduced glycolysis in the enzalutamide-resistant PCa cells. Treatment with MCT antagonists either singly or in combination with enzalutamide suppressed the growth of parental or enzalutamide-resistant C4-2B cell xenografts. Metabolomics revealed that higher levels of lactate were trapped in the treated enzalutamide-resistant cells, indicating that enzalutamide resistance includes higher reliance on glycolysis. Several metabolic pathways were differentially regulated in the treated cells, suggesting that novel pathway alterations may be exploited to overcome resistance.

Conclusions: The above findings demonstrated that targeting MCTs may be an attractive strategy to overcome enzalutamide resistance in PCa.

122

Phosphoserine Phosphatase (PSPH) is overexpressed in African American prostate cancer and stroma and regulates cell proliferation.

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Abstract

Phosphoserine Phosphatase (PSPH) is overexpressed in African American prostate cancer and stroma and regulates cell proliferation.

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Background:

Metabolic reprogramming is one of the key characteristics of cancer and tumor microenvironment for fueling the rapid and self-sufficient growth of cancer cells. L-3-phosphoserine phosphatase (PSPH) is one of the five rate-limiting enzymes in the biosynthesis of serine from glucose, which generate nucleotides to support cell proliferation. Here, we aim to understand a role of PSPH expression and its regulation in prostate cancer and its relation to prostate cancer disparity.

Methods:

Patient-derived carcinoma associated fibroblasts (CAFs) and matched benign associated fibroblasts (BAFs) from the contralateral tumor-free portion of the prostate have been derived from fresh radical prostatectomy specimens of African American (AA) and European American (EA) men. Ancestries of all AA and EA cells were quantitatively assessed by a large single-nucleotide polymorphism (SNP) profile. We also used the Cancer Genome Atlas (TCGA) data to analyze the differential expression of PSPH mRNA in prostate cancer tissues of AA vs. EA men and its association with patient's survival. Prostate cancer cell lines, including MDAPCa2b cells derived from an AA prostate cancer patient were knockdown by short-hairpin RNA to study the functional role of PSPH in prostate cancer cell growth and invasion.

Results:

PSPH protein is overexpressed in CAFs, and prostate cancer cell lines compared to BAFs and normal prostate epithelial cells (PrEC) and benign prostate epithelial cells (BPH-1). Notably, MDAPCa2b cells and AA CAFs have even higher levels of PSPH protein. The mRNA levels of PSPH are also significantly higher in prostate cancer tissues in AA men compared to EA men and predict poor survival of prostate cancer patients. Knock-down of PSPH expression significantly inhibits the growth and colony formation of prostate cancer cell lines. The mRNA expression of PSPH is inversely related to that of Integrin Subunit Alpha 5 (ITGA5) in prostate cancer tissues. Inhibition of ITGA5 expression by siRNA increases the expression of PSPH and TGF-beta stimulation decreases its expression in BPH-1 cells, but not in prostate myofibroblasts WPMY-1 cells. Analysis of publicly available PCa RNA seq data bases revealed that PSPH amplification or over expression was associated with activation of oxidative phosphorylation, EIF2, estrogen and androgen biosynthesis signaling.

Conclusions

Our Results: suggest that prostate cancer cells and their tumor microenvironment express higher levels of PSPH to provide nutritional support to tumor growth. TGFß1 may play a differential role in regulation of PSPH expression between benign prostate epithelial cells and tumor microenvironment.

123

Cell-type specific roles of miR-21 in the prostate cancer tumor microenvironment

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Abstract

Background: The regulation of prostate cancer (PCa) gene expression by microRNAs (miRNA) remains incompletely understood. In particular, miR-21 is robustly expressed in human PCa, and associated with Gleason grade and disease recurrence. Microdissection and in situ hybridization experiments have revealed that miR-21 expression is elevated in both epithelial and stromal compartments of prostate tumors, implicating potential roles for miR-21 in PCa cells and within the prostate tumor microenvironment (TME). Here, we characterize the cell-type specific role of miR-21 in the prostate TME using a MYC-driven transgenic mouse model of PCa with miR-21 knocked out (Hi-Myc/miR-21^{KO} mice).

Methods: To generate Hi-Myc/miR-21^{KO} mice, C57BL/6 miR-21^{KO} mice were backcrossed with FVB mice, and the resulting congenic progeny were cross-bred with FVB Hi-Myc mice. The Hi-Myc/miR-21^{KO} mice and their control counterparts (FVB, miR-21^{KO}, Hi-Myc) were aged until sizable tumors formed in the Hi-Myc mice (9 months). The dorsal and lateral lobes of each genotype were dissected and dissociated (N = 2 per genotype). The 10X Genomics platform and 3' DGE cell partitioning and library prep reagents were used to generate scRNA-seq libraries and sequenced to a depth of >50,000 reads per cell for ~5,000 cells from each sample. Cells were analyzed by dimensionality reduction and clustering analysis using the R-based package Seurat and subsequently annotated by marker genes. Transcriptional differences between genotypes within each cell type were examined using the computational packages RAISIN for differential gene expression (DGE) analysis, and Presto and Fgsea for gene set enrichment analysis (GSEA).

Results: PCa progression was significantly attenuated in Hi-Myc/miR-21^{KO} mice. Cluster analysis of scRNA-seq samples revealed cell types and subtypes that formed unique clusters within uniform manifold approximation and projection (UMAP) dimensionality reduction plots. DGE analysis and GSEA confirmed elevated expression of miR-21 target genes across multiple cell types of Hi-Myc/miR-21^{KO} tumor tissue, including epithelial, cancer, immune, and stromal cell types. Additionally, GSEA using the Hallmark collection revealed an upregulation of MYC targets V1 and V2, TNF-α signaling via NFκB, and the p53 pathway in Hi-MYC/miR-21^{KO} tissue, thus confirming a role for miR-21 in regulating oncogenic and immunologic signaling pathways in prostate cancer.

Conclusions: Collectively, these Results: demonstrate that miR-21 contributes to PCa progression and broadly regulates gene expression across multiple cell types within the prostate TME.

124

miR-21 contributes to the prostate tumor immune microenvironment

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Abstract

Background: Prostate Cancer (PCa) develops an immune-suppressed tumor microenvironment (TME) that ultimately limits the efficacy of cancer immunotherapy, particularly immune checkpoint blockade. The molecular underpinnings of this immunosuppressed TME, and its relationship with therapeutic resistance, remain incompletely understood. Our laboratory previously reported that an oncogenic microRNA, miR-21, is highly expressed in human PCa, especially within the TME. Macrophage and dendritic cells are among the highest miR-21 expressing cell types. Here we apply a miR-21 gene knockout (KO) mouse model and syngeneic tumors to investigate the role of miR-21 in the tumor immune microenvironment.

Methods: To study effects of miR-21 in the prostate TME, we developed a syngeneic murine PCa model where miR-21+/+ Myc-CaP PCa cells were implanted subcutaneously in Wild Type (WT) FVB mice or germline knockouts (KO) of Mir21a (the mouse homolog of miR-21). In this way, miR-21 was only ablated in host cells. We studied tumor development and growth by caliper measurements, immune cell populations using flow cytometry, and immune checkpoint inhibition response using PD-1 antibody therapy. In a separate series of experiments, we implanted an immunogenic Her2-overexpressing murine breast cancer cells (NT2.5) in female miR-21-WT and -KO mice.

Results: The loss of miR-21 from the PCa TME significantly reduced tumor take and attenuated the growth of miR-21+/+ prostate and breast tumors. Subcutaneous Myc-CaP tumors, grown in miR-21-KO hosts, presented with altered tumor immune microenvironment with elevated percentages of NK cells, CD4+ T cells, Tregs and MHCII-high macrophages, as well as increased PD-1 immune checkpoint expression. Anti-PD-1 immune checkpoint therapy did not significantly affect tumor growth in miR-21 WT or KO animals. Subcutaneous NT2.5 tumors displayed similar developmental delays, when grown in miR-21-KO hosts. Notably, NT2.5 tumor growth peaked approximately one-month post-implantation, then gradually regressed, and subsequently recurred to develop much larger tumors. The majority of tumors in miR-21 KO mice regressed without recurrence, while the majority of tumors in miR-21 WT mice recurred and grew to volumes significantly larger than the original tumor.

Conclusions: MiR-21 expression contributes to the development and maintenance of the prostate tumor immune microenvironment, cytokine signaling, and immunotherapeutic resistance. These studies support miR-21 as a potential target for PCa immunotherapy.

125

Determination of HULLK oncogenicity in PCa; identification of DMPK as a HULLK binding partner

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Abstract

Title: Determination of HULLK oncogenicity in PCa; identification of DMPK as a HULLK binding partner

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Abstract:

Background: Treatment of high-grade prostate cancer (PCa) with androgen deprivation therapy leads to the development of castration resistant prostate cancer (CRPC) in the majority of patients. There is a great need for new targets to treat CRPC. Recently we discovered the lncRNA HULLK (hormone-upregulated lncRNA within LCK) that is upregulated upon androgen stimulation and correlates positively with Gleason score in PCa patients. HULLK is necessary and sufficient for PCa cell proliferation, however the specific mechanism for how HULLK functions as an oncogene is not yet known. The aim of this project is to identify HULLK binding partners to reveal the mechanism of HULLK oncogenicity in PCa.

Methods: In order to identify potential RNA binding partners of HULLK, HULLK RNA was tagged with the MS2 RNA element and associated RNAs were enriched by pull-down of an HA-tagged MS2-interacting protein (the MS2 trap method). The efficiency of pulldown was evaluated by RT-qPCR, and enriched material from LNCaP and CWR22Rv1 PCa cell lines were subjected to RNAseq. SHAPE-MaP chemical probing and mutational profiling revealed the structure of HULLK RNA, and IntaRNA predicted HULLK interactions with candidate mRNAs. Alterations in the expression of candidate mRNA binding partners upon knock-down and over-expression of HULLK were evaluated by RT-qPCR. Endogenous and exogenous HULLK pull-downs were used to validate interactions with candidate binding partners.

Results: The RNA-seq revealed transcripts enriched in HULLK-MS2-tag compared to MS2-tag alone in the Rv1 PCa cell line. The top 10 most enriched transcripts in Rv1 and the 29 common transcripts enriched in Rv1 and LNCaP cell lines were used to generate a list of candidate mRNA binding partners to HULLK. Transcripts were prioritized based on expression in prostate and PCa, by correlation with HULLK (LCK) expression in patient cohorts, and predicted interaction from SHAPE-MaP and IntaRNA analysis. The mRNA encoding DMPK (DM1 Protein Kinase) has emerged as the lead HULLK binding partner. The DMPK protein is a non-receptor serine-threonine kinase closely related to kinases that interact with small GTPase Rho family members. Knock-down of HULLK increases DMPK mRNA levels.

Conclusions: HULLK interacts with DMPK mRNA, downregulating DMPK expression, leading to the hypotheses that HULLK's negative regulation of DMPK is necessary for the oncogenic role of HULLK in PCa.

126

A Defense Against Heme in the Urinary Bladder

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Abstract

Background: The urine normally contains ~3,000,000 RBC per day ("Addis Count") each cell containing a billion iron atoms bound to heme. Heme-iron is not only toxic to urothelial cells but, in addition, can provide nutrient iron for bacterial growth and invasion of the bladder since urinary bacteria express receptors for both heme and elemental iron. These data imply that the urothelium expresses defensive mechanisms to deactivate heme and sequester iron from bacteria. We previously identified a protein called Lipocalin-2 or NGAL that captures ferric iron-siderophores and serves as a critical component of the bladder's antimicrobial defense. Yet NGAL is structurally constrained and captures only a small subset of siderophores implicating additional mechanisms that control nutrient iron. We have identified a heme deactivation pathway in the urothelium.

Methods: To identify acute transcripts, we created a method to specifically isolate nascent RNA from Upk2+-urothelium at specific time points after UPEC infection. In addition, we developed a technique to physically isolate the urothelial layer from the bladder for transcriptomics. We utilized a series of cellular knockouts HMOX (gift of A. Agarwal) and HRG1 (gift of I. Hamza) to probe the heme pathway. To identify the activity of heme deactivation system we created a novel Nile Red-Palladium based fluorescent probe that measures Carbon Monoxide.

Results: Nascent transcripts of heme transporting genes (Hp, HMOX1, Blvrb,Slc48a1) and heme regulatory genes (NPAS2, BMAL) were expressed by the urothelium within 4-6 hours of infection and by collecting ducts of the kidney by 12 hours. To determine if heme metabolism was ongoing after UPEC infection we imaged the release of CO gas from bladder and kidney in real time by IVIS. The heme pathway was critical for defense of the urothelium because 1. CO gas inhibited bacterial growth, 2. Mice with Hrg-Hmox knockouts reduced the death and shedding of urothelial cells from the bladder and increased cytokine-interleukin expression levels by urothelial cells. In addition, 3. heme transport defective ChuA, HMA bacteria were not competitive with wild type bacteria.

Conclusions: We identified a new function of urothelium; the detoxification of heme presented in urine. The heme regulatory system controls urothelial gene expression and ultimately death of bladder cells.

127

Alternative Polyadenylation as a Therapeutic Vulnerability in Prostate Cancer

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Abstract

Background:

Prostate cancer is the second leading cause of male cancer death in the United States. While localized disease can be cured by radiation or surgery, metastatic prostate cancer presents a clinical challenge. Metastatic prostate cancer can initially be controlled by endocrine therapies that target the androgen receptor (AR), however, these tumors will inevitably develop resistance. This stage of the disease, termed castration-resistant prostate cancer (CRPC), is responsible for practically all prostate cancer-specific deaths. Truncated AR variant (AR-V) proteins are expressed in CRPC cells, and can function as ligand-independent, constitutively active transcription factors that support the growth of CRPC cells. The mechanisms governing expression of AR-Vs are not well-understood, but our work shows that alternative polyadenylation plays a critical role.

Methods:

To define the mechanism and biological significance of AR polyadenylation in CRPC we conducted siRNA and shRNA targeting of candidate trans-acting factors in CRPC cell lines and performed poly(A)-ClickSeq (PAC-Seq), and RNA-Seq global analysis techniques. To inhibit cis-sequence elements of AR mRNA we employed antisense oligomer targeting.

Results:

We have identified a targetable polyadenylation (poly(A)) site in the cryptic exon 3 (CE3) of AR that regulates the expression of AR-Vs and full-length (FL) AR. We have determined the cleavage and polyadenylation stimulating factor (CPSF) component, CPSF1, regulates usage of this alternative poly(A) site to promote expression of AR-Vs in place of FL-AR and found that knockdown of CPSF1 inhibits growth of CRPC cells. To define how CPSF1 regulates alternative polyadenylation of AR and to determine novel pathways that are manipulated by CPSF1 in prostate cancer, we identified CPSF1-dependent gene expression using RNA-seq and CPSF1-dependent poly(A) site usage using PAC-seq. PAC-seq data revealed global poly(A) site usage beyond the 3’ untranslated region of transcripts upon CPSF1 knockdown and gene set enrichment analysis of RNA-seq data revealed the Glycolysis Hallmark gene set was positively regulated by CPSF1, demonstrating AR independent mechanisms regulated by CPSF1 in prostate cancer.

Conclusions:

We have identified a targetable alternative poly(A) site in exon CE3 of AR that promotes expression of AR-Vs in CRPC cells and have determined CPSF1 as a regulator of the poly(A) site. Our Results: also highlight AR independent pathways that are regulated by CPSF1 that could be exploited for novel therapies for CRPC.

128

The galectin-1 inhibitor LLS80 suppresses neuroendocrine differentiation in prostate cancer by suppressing G-protein coupled receptor activity

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Abstract

Background: We previously reported the development of LLS80, an inhibitor of galectin-1, a member of a family of beta-galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions. Treatment of the castration resistant prostate cancer cell line 22Rv1 downregulates markers of neuroendocrine differentiation (NED); hence we investigated whether LLS80 affects neuroendocrine prostate cancer (NEPC), an aggressive variant of prostate cancer that may arise in patients treated with androgen receptor (AR) signaling inhibitors.

Methods: NEPC cell lines, H660 and LASCPC (ATCC) were treated with different doses of LLS80 and IC50 calculated based on MTT assay. H660 cells treated with LLS80 or DMSO (vehicle) were subjected to RNA-Sequencing analysis. Differential expression was identified using DeSeq2 v.1.20. (adjpvalue<0.05 and log2FC ≤ -1, or log2FC ≥ 1) and analyzed by TOPPFUN (TOPPGENE Suites, OH). Gene Ontology (GO) enrichment analysis defined biological processes (bp), cellular components (cc), and molecular functions (mf) that were enriched after LLS80 treatment (adjp value <0.05). Cluster profiler v.3.8.1 was used for Gene Ontology (GO) and KEGG enrichment analyses.

Results: NEPC cell lines H660 and LASCPC were treated with increasing doses of LLS80 and MTT assay conducted to estimate viability, showing an IC50 of about 2.5 µM for both cell lines. To determine changes in gene expression caused by LLS80 in NEPC cells, H660 cells were subjected to 2.5 µM LLS80 for 11 days, after which the cells were collected, RNA extracted and analyzed by RNA-Seq. Of 58,736 genes analyzed, 3022 were differentially expressed after LLS80 treatment, and of the latter, 1658 genes fit our criteria above (487 upregulated including 403 known genes, 1171 downregulated including 1004 known). The most significant upregulated mf included transcription factor activity (49 genes) and signaling receptor ligands (50 genes) while the most prominent downregulated mf included signaling receptors (119 genes). BP upregulated included morphogenesis (67) and processes related to gene transcription (66) whereas those downregulated were specific to neural development (155 genes). Pathways that were upregulated included cytokine signaling (32) while the most prominent pathways downregulated were those related to the extracellular matrix (ECM) (107) and G-protein coupled receptors (GPCR) (47).

Conclusion: The above analysis demonstrates that LLS80 treatment upregulated genes transcribing cytokines but suppressed those related to signaling receptors causing NED including GPCRs and the ECM. These functions and in line with suppression of the known roles of galectin-1, such as ECM regulation and indicate that LLS80 may have a significant role in future trials of NEPC, which currently has no standard lines of treatment.

129

Effects of Long-Term Adaptation to Androgen in Prostate Cancer Cells

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Abstract

Background:

African American men are twice as likely to be diagnosed with prostate cancer, have almost twice the mortality rates as compared to other populations, are often diagnosed at a younger age and with aggressive/metastatic disease. This is not just a trend seen in the US, however, as black race is a risk factor for prostate cancer globally. The exact cause of this disparity is unknown, although research points towards factors such as socioeconomic status, cultural and educational factors as well as differences in access to healthcare. Yet, when these variables are controlled for, the disparity persists, suggesting a biological role. Our lab recently demonstrated that African Americans have higher concentrations of prostatic dihydrotestosterone, the active androgen within the prostate. Considering that androgen receptor transcriptional activity is necessary for development, growth, repair and homeostasis of the prostate, it is thought that perturbation of the androgen axis plays a role in the prostate cancer disparity of African American men. To examine this notion, we have adapted three prostate cancer cell lines to two concentrations of androgen over 6 months.

Methods:

Three different prostate cancer cell lines LAPC4, LNCaP, and MDA-PCa-2b, were grown in 0nM, 1nM and 10nM of R1881 over 6 months. Adapted and control cells were analyzed by in vitro proliferation, RT-qPCR, RNASeq, AR- CUT&RUN (Epicypher), and grown as xenografts in NUDE mice.

Results:

The 1nM/10nM adapted LN-CaP and MDA-PCA-2b cells grew slower in vitro than controls cells. However, in vivo, the 1nM R1881 adapted MDA-PCa-2B and LNCaP cells grew significantly faster than both naïve and 10nM as xenografts. LAPC4 1/10nM adapted cells grew faster than controls in vitro. Ongoing work includes analyses of the LAPC4 xenografts and the transcriptional landscape with RNASeq and AR-CUT&RUN.

Conclusions:

LNCaP, and MDA-PCa-2b prostate cancer cells were adapted to varying levels of androgen. In vivo growth as xenografts resulted in 1nM treated cells producing tumors at a faster rate than control and 10nM treated cells. In vitro, however, 1nM and 10nM cells grew slower than the control. Understanding why changes in androgen concentration affect in vitro and in vivo growth differently and how changes in prostatic androgen impact proliferation, gene transcription and the AR transcriptional landscape may help reveal the biological mechanisms which contribute to the disparity in African American men. By revealing such biological mechanisms, future work may uncover better prevention and treatment options for this cohort.

130

SOX2 as a metabolic regulator in prostate cancers

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Abstract

Background: The bioenergetic paradigm of prostate cancers is unique amongst the solid tumors where oxidative phosphorylation (OXPHOS) is promoted by the transformation events/principles favoring the cancer growth and metastasis. One of the major signaling pathways found to be altered in these cells is the androgen receptor (AR) signaling which is responsible for the metabolic shift towards OXPHOS coupled with the deficiency in zinc transporter-mediated zinc accumulation leading to the TCA cycle restoration. We previously reported SOX2-mediated metabolic reprogramming in the CWR-R1 prostate cancer cells, which is androgen receptor-independent, indicating this protein's significant role in metabolic rewiring.

Hypothesis: We hypothesize that SOX2 in prostate cancer functions as a metabolic regulator affecting glycolytic and mitochondrial respiratory pathways and rate-limiting enzymes to uphold oxidative metabolism, a hallmark of prostate cancers.

Methods: SOX2-specific gene knockouts and rescue experiments were carried out in prostate cancer cell line CWR-R1 by CRISPR/Cas9 and lentiviral transfections respectively to observe the effects of this protein on cellular metabolism. Oxygen consumption rates and metabolic potential were evaluated by the Seahorse XF analyzer. RNA sequence database analysis and subsequent qPCR analysis were performed to study prioritized SOX2 gene targets.

Results: Genetic manipulation of the SOX2 expression was correlated with the cellular metabolic potential as measured by the Seahorse XF analyzer. SOX2 knockdown reduced mitochondrial gene transcription regulators TFAM and PGC1α, and the glycolytic regulators phosphofructokinase-P and hexokinase 2 isoform indicating a prospective role of SOX2 in cellular bioenergetics.

Conclusion: Our findings suggested SOX2 is a potent metabolic regulator and a key molecule participating in the metabolic reprogramming events during prostate carcinogenesis.

131

Loss of Thrombospondin-1 in Macrophages Promotes Benign Prostatic Hyperplasia

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Abstract

Background: Benign prostatic hyperplasia (BPH) affects the vast majority of aging men and is associated with chronic inflammation. Recent work indicates that systemic targeting of inflammatory pathways reduces hyperplasia in the prostate, but a mechanistic understanding of how inflammation contributes to BPH is lacking. Thrombospondin-1 (TSP1) is a secreted glycoprotein with anti-angiogenic, anti-inflammatory, and extracellular matrix remodeling functions. TSP1 induces TGFβ activation and expression decreases in BPH versus normal tissues. These studies were conducted to determine if immune cells contribute to TSP1 loss in BPH, resulting in increased proliferation.

Methods: Single-cell mRNA-sequencing studies of CD45+ immune cells were conducted on the transition zone of 10 small (<40 grams) and 10 large (>90 grams) human prostate. Analysis of differentially expressed genes between groups and evaluation of primary cells were completed. TSP1 expression was evaluated with immunofluorescence (IF) and ELISA, and either TSP1 siRNA or LSKL peptides were used to decrease expression or inhibit TGFβ activation function of this protein, respectively.

Results: A subpopulation of macrophages (Mφs) identified as HLADR+CD16+ accumulate in large versus small prostate tissues and their abundance positively correlates with patient symptoms. TSP1 is significantly downregulated between leukocytes from large versus small prostates. IF staining indicates that TSP1 is primarily secreted by BPH Mφs, and HLADR+CD16+ Mφs express lower TSP1 than HLADR+CD16- Mφs. In vitro analysis of TSP1 function in THP-1 cells revealed that pro-inflammatory M1 Mφs secrete less TSP1 than anti-inflammatory M2 Mφs. Additionally, inhibition of TSP1-mediated activation of TGFβ using LSKL peptides induced higher secretion of pro-inflammatory cytokines IL1b and IL6 versus control peptides.

Conclusions: TSP1 is secreted by BPH Mφs but downregulated during prostate expansion. A subpopulation of Mφs with low TSP1 expression accumulates as prostate size increases and symptoms worsen, which may exacerbate inflammation and result in the loss of TGFβ-mediated regulation of stromal/epithelial proliferation. Ongoing work is pursuing novel therapeutic strategies that target chronic inflammation in BPH.

132

Evaluating the role of MEIS1/HOXB13-mediated proteoglycan regulation in prostate cancer.

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Abstract

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

Methods: CWR22Rv1 and LAPC4 cells were modified to express ectopic MEIS1 (LV-MEIS1); HOXB13 was deleted using CRISPR-Cas9 targeting. MEIS1 ChIP-Seq and RNA-Seq were conducted and bioinformatics analyses were done as previously described (VanOpstall et al., eLife 2021). CWR22Rv1 cells harboring heterozygous HOXB13 mutants were a generously shared from Dr. Sandor Spisak, Ph.D. and Matthew Freedman, M.D.. Western blotting and qPCR for DCN and LUM were conducted.

Results: Analyses of paired RNAseq and ChIP-seq of MEIS1 in the presence or absence of HOXB13 prioritized proteoglycan signaling, and in particular direct regulation of DCN and LUM by MEIS and HOXB13. DCN and LUM expression was validated using western blotting and qPCR. Induction of DCN and LUM expression in cells harboring HOXB13(G84E) mutations was decreased compared to wild-type HOXB13.

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

133

Single Cell Proteomics as a Method to Analyze Circulating Tumor Cells in Prostate Cancer Patients

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Abstract

Background: The analysis of circulating tumor cells (CTCs) has long been promised as the next big leap for cancer diagnostics. Despite their numerous advantages, the analysis of CTCs has not migrated into clinical use due to difficulty of purification and their minute numbers. Combined, this Results: in only miniscule amounts of sample, if any, for analysis. In the lab, research has primarily focused on genomics and transcriptomics. Only select papers look at the proteome, often analyzing a handful of targets at most. Recent developments in single cell proteomics by liquid chromatography tandem mass spectrometry (LC-MS/MS) promise to solve this issue and increase the depth of proteome coverage.

Methods: To analyze CTCs we took previously developed single cell methodologies and modified them to be amenable for the analysis of CTCs. Our novel method uses a separately prepared carrier channel as opposed to traditional Methods: which rely on a large pool of the single cells of interest to act as carriers. This is necessary as collected CTC's will not have a large pool of similar cells to draw upon to form a carrier, and thus an alternative method must be developed. Additionally, as the carrier is prepared in bulk it allows for improved quality control measurements to be implemented. This is critical as for clinical use these metrics will be vital for ensuring repeatability and reliability.

Results: Using our developed protocol we are able to analyze hundreds of proteins in simulated CTCs while maintaining or improving throughput rates compared to previously published Results:. Additionally, we demonstrate that a separately prepared carrier composed of cells from a different lineage still permits the identification of a wide array of proteins within single cells as well as allows for better quality control over the digestion and TMT labeling efficiencies.

Conclusions: Our protocol is capable of detecting and quantifying hundreds of proteins from single cells. We show that a carrier does not need to be composed of cells from the same lineage as the single cells being analyzed for proteome coverage. With further development to LC-MS/MS, combined with our approach, we can meet all the requirements needed to analyze CTCs. This method could guide individualized cancer treatment in the clinic.

134

Androgen Receptor Variants mRNA Absolute Quantification in Prostate Cancer Cell Models

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Abstract

Background: Patients treated with Androgen Deprivation Therapy (ADT) have been shown to increase the expression of constitutively active Androgen Receptor (AR) splice variants (AR-Vs) which lack the ligand binding domain at the C-terminus of AR, required for binding to antiandrogen therapies. AR-V7 has also been correlated to the development and progression of castration resistant prostate cancer (CRPC). As not all Circulating Tumor Cells (CTCs) contain AR-V7, we hypothesized that other AR-Vs are involved in the mechanism of resistance in CRPC. To test this, we developed an mRNA quantitative assay targeting AR-Vs and then correlated our Results: with a targeted mass spectrometry (Targeted MS) based protein assay. This work will provide a feasible quantification of AR-Vs and provide a possible stratification for future patient's predicted response to treatment which could be useful to clinicians.

Methods: The absolute copy numbers of total AR, AR-V2, AR-V7, AR-V12, and AR-V23 were determined in eight different PCa cell lines and 48 patient derived xenographs (PDXs) using qRT-PCR assays. We performed siRNA targeting of these variants to evaluate primer specificity by evaluating knockdown efficiencies and these Results: will be correlated to targeted mass spectrometry (MS) quantification of AR-Vs.

Results: We found that the most highly expressed variant in 22Rv1s was AR-V7, AR-V12 in LNCaPs, and AR-V2 in VCaP-16 cells. Densiometric measurements of western blots targeting AR Total and AR-V7 were correlated to mRNA copy number for each cell line. Knockdown of AR-Vs using siRNAs was shown to be specific through testing using AD-1 (which only expresses the full length AR transcript) and R1-D567 (which only expresses AR-V567es, an AR-V12-like variant) cell lines.

Conclusions: We were able to determine the landscape of AR-Vs mRNA expression in 8 PCa cell lines and in 48 PDX tumor samples. This data will be correlated to protein expression currently being analyzed using a targeted MS method developed in our lab. Our work will be useful to help clinicians to stratify PCa patients based on the AR-Vs expression profiles. This will establish a prognostic biomarker program that measures AR-Vs proteins in real time from clinical biopsy tissues, circulating tumor cells, or exosomes and informs the clinician on which course of treatment may be effective for each patient. Establishing a CRPC biomarker other than AR-V7 that can help explain the AR-V7 negative CRPC patients could eventually improve clinical application and predictive treatment outcomes.

135

Single-cell Analysis of Cribriform Prostate Cancer Reveals Aggressive Disease Mediated by both Tumor Cell Intrinsic and Tumor Microenvironmental Pathways

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Abstract

Background: Cribriform prostate cancer, found in both invasive cribriform carcinoma (ICC) and intraductal carcinoma (IDC), is an aggressive histological subtype of prostate cancer that is associated with progression to lethal disease. Prior studies have begun to define genetic and molecular alterations associated with ICC/IDC, but not at the single cell level. Furthermore, little has been reported about the ICC/IDC tumor microenvironment (TME).

Methods: To delineate the molecular and cellular underpinnings of ICC/IDC aggressiveness, this study examined paired ICC/IDC and benign prostate tissue obtained from radical prostatectomy by single-cell RNA-sequencing, TCR sequencing, and histology. Key findings were validated by RNAscope or IHC in a larger cohort.

Results: ICC/IDC cancer cells expressed genes associated with metastasis and targets with potential for therapeutic intervention. Consistent with prior studies, *SCHLAP1*, a long non-coding RNA associated with metastatic progression, was increased in ICC/IDC. ICC/IDC cancer cells also expressed potential therapeutic targets including *FOLH1* and *CD276*. Hallmark pathway analyses and ligand/receptor expression modeled cellular interactions between ICC/IDC and the TME including JAG1/NOTCH and FGF13/FGFR1 signaling. The ICC/IDC TME was characterized by increased angiogenesis and immunosuppressive fibroblasts (*CTHRC1+ASPN+FAP+ENG+*) along with fewer T cells, elevated T cell dysfunction, and increased *C1QB+TREM2+APOE+* macrophages.

Conclusions: These findings support that cancer cell intrinsic pathways and a complex TME contribute to the aggressive phenotype of ICC/IDC. These data highlight potential therapeutic opportunities for patients with ICC/IDC that may improve outcomes.

136

CRISPRi genome-wide library screening identified novel lncRNAs conferring lineage plasticity and AR therapy resistance

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Abstract

Background: Recently, lineage plasticity has been authenticated as a widely utilized avenue to escape AR dependence and to promote AR targeted therapy resistance in prostate cancer (PCa), which carries various genomic and transcriptomic aberrations. However, only a limited number of patients carry the known lesions mediating lineage plasticity, underlying the necessity for identifying other "long-tail" modifiers conferring resistance via lineage plasticity. Emerging evidences has revealed the important function of long non-coding RNA (lncRNA) in cell fate decision, cell differentiation and therapy resistance. However, the roles of lncRNAs in regulating lineage plasticity and AR resistance in PCa have yet to be characterized.

Methods: By constructing a CRISPRi based library targeting over 10,000 lncRNAs in human genome, we performed library screening for lncRNAs conferring lineage plasticity and AR therapy resistance. Through the integrated analysis of CRISPRi library screening Results: and SU2C patients data, we identified 16 novel lncRNAs as potential regulators of lineage plasticity and therapy resistance. Using FACS-based competition assays, we validated that 5 lncRNAs could confer significant resistance to enzalutamide treatment.

Results: We successfully conducted the genome-wide library screening and identified 5 lncRNAs as novel regulator of linage plasticity and AR therapy resistance. We have further validated those lncRNAs in various *in vitro* and *in vivo* PCa models. Currently, we are examing the molecular mechanism through which those lncRNAs regulate lineage plasticity and AR therapy resistance.

Conclusions: Leveraging the CRISPRi-based genomic library screening approach, we have identified 5 lncRNAs as novel regulator of lineage plasticity and AR therapy resistance in PCa. These new findings not only expand our understanding of how cancer cells acquire lineage plasticity and resistance, but also suggest that lncRNAs may be used as potential biomarker or druggable targets.

137

Examining the Role of NDUFS3 in Benign Prostatic Hyperplasia

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Abstract

Examining the Role of NDUFS3 in Benign Prostatic Hyperplasia

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Background: Benign prostatic hyperplasia (BPH) is an aging condition of the prostate, associated with lower urinary tract symptoms (LUTS) such as increased urinary retention and urgency. Prostatic fibrosis has been associated with the progression and subsequent treatment failure in BPH, but there are no current therapies to target fibrosis. We have implicated mitochondrial complex I dysfunction as a potential mediator of fibrosis in BPH. NDUFS3 is a mitochondrial protein critical in complex I and is a marker for mitochondrial function. In both humans and mice, NDUFS3 expression is shown to decrease with age in the prostate coincident with an increase in LUTS. This study aims to interrogate the importance of complex I disruption in the development of fibrosis and LUTS.

Methods: IHC of NDUFS3 was performed in human and mouse models of disease. Human stromal (BHPrS1) and epithelial (BPH1) prostate cell lines were used to examine the effect of NDUFS3 disruption on downstream collagen production. Prostate cells were treated with 25nM rotenone to inhibit mitochondrial complex I. Collagen gene expression was assessed in BHPrS1 cells by RT-qPCR. To assess the contribution of complex I dysfunction to fibrosis, CRISPR/Cas9 was used to target the NDUFS3 gene. Cas9 was stably expressed in both BHPrS1 and BPH1 cells under blasticidin selection. A pool of 3 sgRNAs that target NDUFS3 was transfected along with a transient puromycin plasmid for selection.

Results: IHC of human and mouse samples showed a significant decrease in NDUFS3 in both stromal and epithelial compartments of the prostate. BHPrS1 cells treated with rotenone showed a significant increase in Col1a1 and Col3a1 gene expression. BHPrS1 cells were also treated with conditioned media from rotenone treated BPH1 cells. Because of the residual rotenone in the conditioned media, the paracrine effect of epithelial disruption of complex I by rotenone on BHPrS1 cells is more difficult to interpret. Using CRISPR/Cas9 technology, we are disrupting NDUFS3 specifically in both cell types.

Future Directions In addition to the effect of NDUFS3 disruption on collagen and fibrosis in stromal cells, we will examine the effects of paracrine signaling between stromal and epithelial cells. Additionally, with NDUFS3-floxed mice, we can examine the effects in vivo of complex I disruption on the initiation and progression of lower urinary tract dysfunction using our well described rodent models of disease.

138

Lineage plasticity is associated with an altered MAX cistrome through super-enhancer remodeling

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Abstract

Background: Roughly 20% of late stage prostate cancer patients display a lineage conversion from typical androgen receptor (AR) driven castrate resistant prostate cancer (CRPC) to a particularly lethal neuroendocrine prostate cancer (NEPC) phenotype as a result of AR pathway inhibition (ARPI). Notably, the mutational landscape of CRPC and NEPC is quite similar, suggesting that epigenetic changes may guide this lineage conversion. Our objective was to explore changes in active epigenetic marks with a particular focus on super-enhancers (SEs) and identify factors that regulate these active regions during lineage plasticity.

Methods: We performed ChIP-seq for H3K27ac, H3K4me3 and various TFs in our CRPC and ENZ resistant (ENZR) NEPC-like cell models. We leveraged publicly available H3K27ac data in cell lines as well as PDX models to validate our findings. RNA-seq was integrated with our ChIP-seq Results: to create a signature which we applied to patient data sets. GIGGLE analysis was used to identify candidate factors at SEs.

Results: CRPC and NEPC showed similar active marks at promoters however, enhancer elements were significantly remodeled. We observed extensive remodeling of SEs in our ENZR model compared to CRPC and these unique regions showed greater H3K27ac signals in other ENZR models such as NCI-H660. SE linked genes are associated with stemness pathways and are strongly upregulated as a result of AR pathway inhibition in vitro, and in vivo. Factor enrichment analysis of these regions showed enrichment for MAX and MYC family binding similar to small cell lung cancer cell lines. ChIP-seq for MYC and MAX in our cell models revealed an expansion of MAX binding and contraction of MYC binding in our ENZR model compared to CRPC. In particular, we saw increased binding of MAX at plasticity SEs without MYC suggesting an interaction with MYCL or other factors since MYCN is not expressed in our models.

Conclusions: This work improves our understanding of the epigenetic changes that occur during lineage plasticity and ARPI resistance. SE analysis in our models suggests that MAX may be activating these regions with MYCL or independently of the MYC family. MYCL expression is upregulated in NEPC and has higher expression than MYCN suggesting a potential role during lineage plasticity.

139

Pre-Clinical Studies to Advance Zilovertamab-Based, Anti-ROR1 CAR-T cell Therapy for Metastatic Prostate Cancer.

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Abstract

Background: Alterations in WNT signaling are frequently associated with tumorigenesis and metastasis in many cancers including prostate cancer (PCa). The Wnt ligand, WNT5A, is required for normal prostate gland development and is increased in bone metastatic, castration resistant PCa (CRPC) patients. WNT5A signaling is mediated in part through ROR1, a non-canonical Wnt receptor and fetal oncoprotein for which the therapeutic inhibitory antibody, Zilovertamab, has been developed. Its safety has been clinically proven in trials for chronic lymphocytic leukemia (CLL) and metastatic breast cancer. We sought to investigate Zilovertamab-based anti-ROR1 therapies for metastatic prostate cancer.

Our Hypothesis is that WNT5A may activate a stem-cell-like program via ROR1 which leads to therapy resistance in metastatic prostate cancer. The anti-ROR1 biologic, Zilovertamab, may inhibit this mechanism of resistance and sensitize metastatic prostate cancer to standard of care therapies such as docetaxel. The expression of ROR1 on CRPC and NEPC tumors and its lack of expression on normal adult tissues also makes it a promising CART cell target.

Methods: We used the patient-derived xenograft (PDX), PCSD13, small cell bone metastatic prostate cancer model and the neuroendocrine PCa cell lines, PC3 and DU145, to test the effect of Zilovertamab and Zilovertamab-CART cells in vitro using the real time cell viability, proliferation, and cell cycle tracking assays in an Incucyte S3. We used in vivo bioluminescence and tumor caliper measurements to monitor effects in vivo. Tumor tissues were collected for analyses of ROR1 expression and signaling.

Results: Studies using RNASeq, qRT-PCR, FACS and Westerns showed high expression of ROR1 in PC3, DU145, and in PCSD13. CRISPR-Cas9 Knock out of ROR1 in PC3 and DU145 cells showed increased inhibition of proliferation at lower docetaxel concentrations. Treatment of PCSD13 PDX in vivo with Zilovertamab increased docetaxel-mediated tumor growth inhibition. Mice bearing PC3 xenografts injected intravenously with Zilovertamab anti-ROR1 CAR-T cells showed durable, tumor ablation in 67% of mice compared to 22% of mice injected with activated T cells from the same donor and 0% of untreated mice. Survival of mice at Day 70 injected with CART was 78% compared to 0% of mice injected with control donor T cells and 0% of untreated mice. Zilovertamab antibody could inhibit Zilovertamab CART cell tumor cell killing.

Conclusions: ROR1 was expressed at high levels on castration resistant small cell PCa and neuroendocrine PCa cell lines and PDX models. Zilovertamab synergized with docetaxel to inhibit tumor growth in patient derived xenograft in vivo and in organoid cultures. Zilovertamab-based CART cells durably eradicated ROR1+ prostate cancer xenograft tumors. These studies supported the recently launched Phase 1b clinical trial of Zilovertamab plus docetaxel in metastatic CRPC patients. Clinical

development of GMP Zilovertamab CART cells for a clinical trial in CLL is in progress and may lead to rapid progression to a clinical trial for metastatic CRPC and NEPC.

140

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

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Abstract

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

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

Methods:—EAP was induced by subcutaneous injection of rat prostate antigen and adjuvant, as previously described (1). Pain responses were assessed by tactile allodynia using Von Frey filament behavioral testing every seven days. Animals were sacrificed on day 28 following injection, and prostates were collected. For ex vivo experiments, the DRGs (dorsal root ganglion) were collected at the level of L_4-S_2 from B6 mice and co-cultured for 24 hours with SE-LTA treatment. Intracellular live calcium imaging was performed on adult DRG neurons culture using the fura-2 ratiometric dye. Images were taken on multi-color confocal microscopy and analyzed by NIH Image J. Statistical analyses were performed using Microsoft Excel and GraphPad Prism software.

Results:— Following SE-LTA instillation, PD-1 immunoreactivity of EAP-induced prostate tissue on day 28 was increased and localized to βIII-tubulin-labeled nerve fibers in the stroma and nerve endings surrounding epithelial cells. Elevated PD-1 immunoreactivity was similarly observed in L_4-S_2 DRG by SE-LTA treatment ex vivo. Functionally, SE-LTA inhibited ATP-induced Ca^{2+} influx in cultured DRG neurons. Both a TLR2 antagonist and neutralizing antibodies to PD-1 reversed the inhibitory effect of SEL-TA in the cultured DRG neurons. Finally, inhibitory cytoplasmic residues of PD-1 and downstream signaling adapters were shown to be specifically phosphorylated in cultured DRG neurons following SE-LTA treatment.

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

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

141

Identifying novel regulators of macrophage tumor cell metabolic interactions in prostate cancer of African American men

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Abstract

BACKGROUND: African American men (AA) are more than twice as likely to die of prostate cancer (PCa) compared to European American men (EA). Among many contributing factors, unraveling the metabolic derangements in PCa of AA holds a promise in reducing health disparity.

METHODS: We examined publicly available prostate cancer datasets for race dependent differential gene expression. We prospectively examined the differential gene expression of PCa from clinically matched AA and EA in the VANDAAM clinical trial. The VANDAAM study is a validation study of DecipherTM genomic testing in 240 men with localized PCa. We then combined computational and experimental approaches to explore the molecular mechanisms of top candidate genes.

RESULTS: We identified aminopeptidase N (ANPEP) as one of the top candidate genes to be overexpressed in AA. Aminopeptidase N is involved in endocytosis, cholesterol, amino acid transport and peptide hydrolysis. Unbiased computational analyses of the VANDAAM revealed that ANPEP correlates with signatures of cholesterol transport, estrogen and androgen receptor (AR) signaling. Based on our recent study demonstrating dominance of these signatures in macrophage-rich PCa, we reasoned that ANPEP expression may be driven in part by high macrophage infiltration in AA. Thus, we compared immune cell repertoire in patients with high ANPEP and low ANPEP by deconvoluting immune cell content using the in silico approach, CIBERSORT. These analyses illustrated that only AA patients with high ANPEP expression significantly accumulated high content of M1 inflammatory macrophages. Immune phenotyping of prostate tumors demonstrated that ANPEP indeed represents a marker of M1 inflammatory macrophages. Future work will focus on unraveling role of ANPEP in regulating cholesterol and amino acid metabolism using Liquid chromatography-high resolution mass spectrometry (LC-HRMS) and explants derived from AA and EA prostate cancer patients.

CONCLUSION: ANPEP represents a macrophage related protein in PCa with a potential role in cholesterol transport and / or androgen signaling.

142

Stromal Androgen Signaling Regulates IGFBP3 to Function as Tumor Niche in Prostate Oncogenesis

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Abstract

Stromal Androgen Signaling Regulates IGFBP3 to Function as Tumor Niche in Prostate Oncogenesis

Background: The androgen receptor (AR) signaling pathways is essential for prostate tumorigenesis. In the past decades, significant effort has been devoted to understand the promotional role of AR action in prostate tumor cells. However, the role of stromal AR in prostate tumorigenesis remains elusive. In this study, we directly examined AR in stromal Shh-responsive Gli1 lineage cells in prostate oncogenesis and tumor development.

Methods: In vivo tissue recombination assays were used to define the role of stromal AR in Gli1 lineage cells in prostate epithelial oncogenesis. Newly generated mouse models were utilized to examine the deletion of stromal AR in Gli1 lineage cells during prepubescent and adult age in supporting prostate epithelial tumor development. Single cell RNA sequencing on prostate tissues with endogenous or deleted AR in Gli1 lineage cells was performed to discover molecular pathways in both stroma and epithelium. Organoid culture systems uncover the mechanism for stromal AR as a tumor niche in supporting epithelial tumor growth.

Results: We demonstrate that AR loss in stromal Gli1-lineage cells reduces prostate epithelial oncogenesis in xenograft models, combining transformed UGE by loss of Pten or expression of stabilized b-catenin with AR-deficient or control UGM. Impairment of prostate epithelial tumor development was then observed in Myc-induced tumor mice with conditional deletion of AR in Gli1 lineage cells during prepubescent and adult age. Increased expression of insulin-like growth factor binding protein 3 (IGFBP3) was identified in AR deleted Gli1 lineage cells. Basal epithelial cells show reduced IGF1 activating Wnt signaling, caused by the increased IGFBP3 expression, inhibiting prostatic epithelial oncogenic growth. AR deletion directly alleviates the suppression of Sp1 regulating transcription of IGFBP3 inhibiting IGF1-induced Wnt signaling and oncogenic growth of tumor epithelium in a paracrine manner. In prostatic epithelial organoid cultures, addition of IGFBP3 and conditional media from AR deleted stromal cells blocked tumor cell growth.

Conclusions: These data implicate the role of stromal AR in Gli1 lineage cells as a tumor niche to support prostate epithelial tumorigenesis. Dysregulation of stromal AR on IGFBP3-IGF1 signaling suggest an underlying mechanism for hormone refractoriness. Therefore, co-targeting reciprocal interactions of AR and IGF1 pathways between epithelial tumor cells and surrounding tumor niches may improve clinical outcomes for advanced prostate cancer.

143

Oncogenic Role of Androgen Signaling in Prostatic Basal Epithelial Progenitors to Initiate Prostate Oncogenesis and Promote Tumor Development

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Abstract

Background: Androgen signaling mediated through binding to the androgen receptor (AR) is essential for prostate tumorigenesis. However, the molecular mechanisms by which the AR functions as a tumor promoter and induces prostate oncogenesis, tumor development, and progression still remain unclear. One of the major reasons for this fallback is due to the lack of biologically relevant *in vivo* models to characterize AR action in prostate cancer development.

Methods: Using newly generated genetically engineered mouse models (GEMMs), which conditionally express mTmG reporter gene driven by odd skipped-related 1 (*Osr1*)-*Cre*, we traced prostatic *Osr1*-lineage cells during prostate embryonic and pubertal development. The compound transgenic mice, in which both human AR transgene (*hARtg*) and mTmG reporter are controlled by *Osr1-Cre*, were used to assess the effect of *hARtg* on the prostate tumorigenesis and identify tumor initiating cells. Single cell RNA sequencing and other approaches were applied to demonstrate cellular and molecular changes induced by *hARtg* expression in prostatic *Osr1*-lineage cells. Prostatic epithelial organoid cultures were used to validate the promotional roles of AR, IGF, and Wnt signaling in prostate tumor cell growth.

Results: *In vivo* cell tracing experiments showed prostatic *Osr1*-lineage cells possess prostatic basal epithelial progenitor properties and differentiate to prostate luminal cells during early and pubertal prostate development. Conditional expression of *hARtg* in *Osr1*-lineage cells induces high-grade prostatic intraepithelial neoplasia and tumor development. Upregulation of IGF1 signaling was identified in a subpopulation of *Osr1*-lineage basal epithelial cells. Activated IGF1 signaling further promotes canonical Wnt signaling activation by increasing stabilized β-catenin. Inhibition of AR and Wnt signaling pathways significantly represses prostate cancer cell growth in prostate epithelial organoid culture and kidney capsule transplantation. Analyses of human prostate cancer samples further validate correlation among altered androgen, IGF1, and WNT signaling.

Conclusions: Our data uncover an underlying mechanism by which AR acts as a tumor promoter to induce prostate oncogenesis and tumor development. Identifying AR activation elevating IGF1 and Wnt signaling pathways in prostate cancer cells provides new and clinically relevant evidence to design alternative therapeutic strategies by co-targeting AR and Wnt/β-catenin signaling pathways for the treatment of advanced prostate cancer.

144

Organized Microarchitecture of Prostatic Peptidergic Sensory Nerves.

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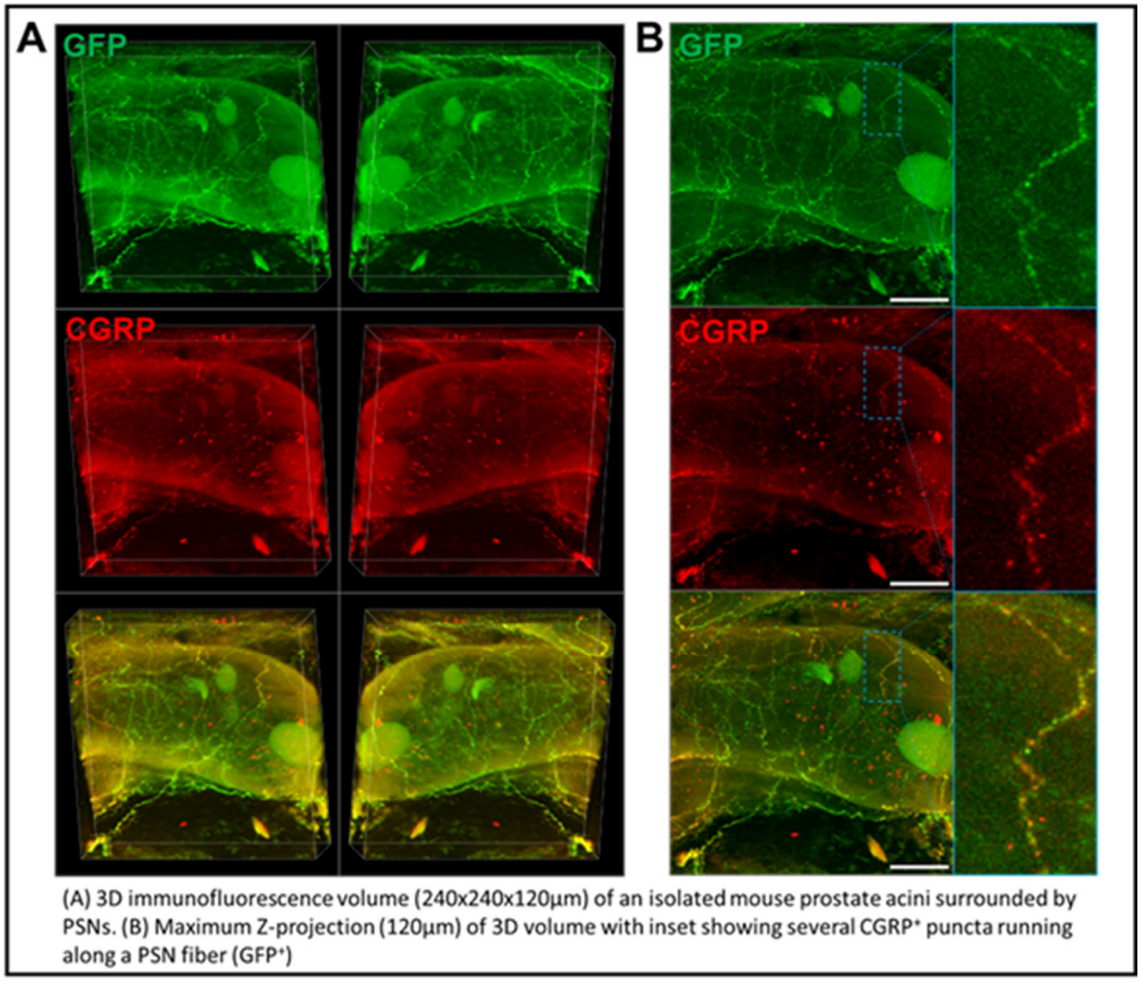
Abstract

Background:
The prostate is densely innervated. Both sympathetic and parasympathetic nerves regulate smooth muscle tone and manipulations of these nerve populations play a role in prostate pathologies. Sensory nerve-associated neuropeptides, such as tachykinins and calcitonin gene-related peptide (CGRP) are present in the prostate, however, peptidergic sensory nerve (PSN) fibers are reported to be sparse in the prostate. This may be due to suboptimal imaging.

Neuropeptides released by PSNs are known to mediate inflammation. PSNs are critical for a myriad of physiological processes other than nociception, including immune cell regulation, vascular patterning, stem cell regulation, bone homeostasis, and tissue repair and recovery during epidermal wound healing. However, no studies have selectively examined the functional role of sensory nerves in the prostate and in prostate pathologies.

Methods:
Prostates were harvested from transgenic mice that expresses EGFP driven by the CGRP promoter (*Calca-fEGFP*) and fixed with 4% paraformaldehyde. Tissues were immunolabeled and processed through a modified ethyl cinnamate-based optical tissue clearing protocol before being imaged by confocal microscopy (Leica SP8).

Results:
Continuous, tortuous, GFP^+/$CGRP^+$ nerves fibers are seen in 50–100µm thick volumes in immunolabeled, cleared, prostates. Punctate CGRP signals are dispersed along continuous GFP^+ fibers indicative of large, peptidergic dense core vesicles in PSNs.



(A) 3D immunofluorescence volume (240x240x120µm) of an isolated mouse prostate acini surrounded by PSNs. (B) Maximum Z-projection (120µm) of 3D volume with inset showing several CGRP⁺ puncta running along a PSN fiber (GFP⁺)

Conclusions

Immunofluorescence labeling with ethyl cinnamate-based tissue clearing has enabled us to clearly delineate the microarchitecture of PSN fibers interwoven around prostatic acini. The highly organized structure of innervating PSNs proximal to epithelial glands suggests that PSNs play a role in normal prostatic function. In accordance with previous findings in 2D tissue sections, PSNs are also in the stroma between acini, however, the abundance and organization of PSN fibers was a surprising finding. Future studies will be aimed at determining PSN activity in the prostate and in prostate pathologies.

145

TNFα suppresses AR expression in prostate cancer stroma through NF-κB binding at the AR promoter to suppress expression of epithelial differentiation factors

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Abstract

TNFα suppresses AR expression in prostate cancer stroma through NF-κB binding at the AR promoter to suppress expression of epithelial differentiation factors

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Background: Prostate stromal AR expression is a critical determinant of normal prostate gland differentiation, mediated through the induction and secretion of epithelial differentiation factors. Loss of stromal AR is associated with increasing Gleason grade and development of aggressive prostate cancer. The mechanisms that lead to stromal AR loss are unknown. We found that AR expression (protein and mRNA) in benign immortalized prostate stroma cells (BHPrs1), is significantly downregulated by treatment with tumor necrosis factor TNFα, which correlated with NF-κB activation. In addition, shRNA mediated knockdown, as well as inhibitors of NF-κB, can block the ability of TNFα to downregulate AR protein and mRNA. Furthermore, a query of the promoter of the AR gene, identified six putative NF-κB binding elements. Therefore, we hypothesize that NF-κB could negatively regulate AR promoter activity which in turn leads to downregulation of important epithelial differentiation factors.

Methods: Using cytokine array profiling, we identified TNFα as common factors secreted by two different PCa cell lines, C4-2 and 22RV1. RT-qPCR, immunoblotting, pharmacological inhibitors, and shRNA knock-down were utilized to identify the TNFα mediated AR downregulation. We cloned a 1.5 kb region (-1040 to +499) of the human AR promoter upstream of a luciferase reporter. We also created several mutant constructs by deleting different NF-κB-binding regions within this AR promoter region. To determine the consequence of AR loss in the stroma, we identified Wnt16 and FGF10 as epithelial differentiation factors whose mRNA upregulated by qPCR following androgen treatment.

Results: We demonstrate that TNFα treatment decreases AR-promoter driven luciferase activity. We predict that deletion of at least one of the predicted NF-κB binding sites will abolish TNFα-mediated repression of AR expression. Treatment with TNFα caused loss of expression of both Wnt16 and FGF10 expression, but not KGF. Blocking NF-κB activity prevented TNFα-induced downregulation of Wnt16 and FGF10.

Conclusion: Cancer secreted TNFα is responsible for the loss of stromal AR expression seen in prostate cancer stroma and may contribute to oncogenesis by removing pro-differentiation factors that would normally maintain prostate gland homeostasis.

146

Therapeutic efficacy of AURKA and PARP co-targeting in Prostate Cancer

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Abstract

Background: Prostate cancer (PC) is the most commonly diagnosed non-skin cancer among males in the US. The primary reason of PC mortalities is metastatic castration-resistant PC (mCRPC). mCRPC accumulates genomic mutations in homologous recombination (HR) genes such as BRCA1/2, causing deficiency in proper DNA double-stranded breaks (DSBs) repair. Poly (ADP-ribose) polymerase (PARP) is an enzyme that regulates DNA repair, and PARP inhibition (PARPi) causes lethal DSBs that are not repaired in HR-deficient tumors. However, only 19.3% of mCRPC patients are HR mutants and benefit from PARPi.

Aurora Kinase A (AURKA) is a kinase that is upregulated in mCRPC and associated with poor prognosis. AURKA regulates mitotic entry during cell division. When overexpressed, it is able to override cell cycle arrest upon genomic mutations caused by HR deficiency and/or PARPi. Moreover, AURKA has been shown to regulate protein stability of MYC oncogene, which inter alia induces HR gene expression. This suggests that AURKA inhibitors (AURKAi) might be useful for both HR-deficient and -proficient PC and may synergize with PARPi.

Methods: We evaluated viability of HR-deficient and HR-proficient PC cell lines with cell titer-glo assay. We used Incucyte live-cell imaging to assay the therapeutic effect of AURKAi and PARPi Olaparib, either alone or in combination. We used immunoblotting to survey DSB, DNA damage response, and apoptosis. Whether AURKAi impairs HR was assayed by pLCN DSB reporter system. Finally, in vivo efficacy of AURKAi, PARPi was evaluated on CRPC xenograft model.

Results: Here we tested a new-generation AURKAi with high selectivity towards AURKA and favorable safety profiles as shown in early clinical trials. We found that AURKAi remarkably reduced AURKA activation in PC cells at concentration as low as 10nM. By contrast, AURKB and AURKC remained unaffected. Critically, as a single agent AURKAi inhibited the growth of both HR-deficient cells such as LNCaP, C4-2B, and 22Rv1, and HR-proficient VCaP cells with IC50 less than 0.35uM. Mechanistically, we observed that AURKAi induced DSBs, DNA damage response, and apoptosis, along with reduced MYC protein levels that may be associated with HR suppression. As controls, the HR-deficient cells showed high sensitivity to PARPi Olaparib, whereas VCaP is unaffected by RAPR inhibition. However, we observed as strong synergistic effect with the combination of AURKAi and PARPi in both HR-proficient and -deficient cells, further suggesting that AURKAi might lead to HR deficiency de novo.

Conclusion: We report that a new-generation high-selective and safe AURKAi creates de novo HR deficiency, is effective in suppressing the growth of a wide-range of PCa cells and shows synergy with Olaparib. Our data suggest that AURKAi might allow PC patients with both HR-proficient and -deficient tumors to benefit from PARP inhibitors.

147

Global Proteomics and Phosphoproteomics Analysis of Castration Resistance

Prostate Cancer Patient Derived Xenografts Tumors from the LuCaP series

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Abstract

Castration Resistant Prostate Cancer (CRPC) is a form of prostate cancer (PCa) that is resistant to androgen deprivation therapies. Resistance to these therapies leads to metastatic CRPC of adenocarcinoma (AD) origin and can transform to emergent aggressive variant prostate cancer (AVPC) which has genetic aberration features similar to neuroendocrine (NE) phenotypes. Understanding and identifying the underlying mechanisms of resistance and genetic transformations leading to protein alterations will enable us to develop novel therapeutic Methods: to counteract this resistance and improve outcomes for men with lethal metastatic prostate cancer. To this end, we used patient-derived xenografts (PDXs), an in-vivo clinically relevant model from advanced prostate cancer (PC) patients, to investigate the biology, identify novel protein targets and evaluate new treatment modalities. We aimed to identify known and novel protein kinase targets that might be phospho-regulated unique between CRPC, in AD and AVPC in NE.

To pursue this, we applied a mass spectrometry base phosphoproteomics analysis. We processed 48 PDX samples, which included 15 non-castrated (NCR), 18 castration resistant (CR), which are the AD samples and 15 AVPC, the NE tumors. To enrich for global phosphorylated residues, we used sequential metal oxide affinity chromatography. We sequenced peptides from each sample using liquid chromatography in tandem with Orbitrap Eclipse Tribid MS and FAIMS technology for 2 hour gradients. To normalize and calibrate the LC method and to compare across all 48 runs, we spiked-in 400 femtomol of internal standards (iRT) to each sample. For peptide sequence and search analysis, we used Maxquant search algorithm. Using a > 0.75 probability phospho-residue cut-off and 5% FDR, we identified more than 9,700 phospho-residues.

We identified RET and ASCL1 in NEPC and Androgen Receptor (AR) in the adenocarcinoma PDX LuCap tumor samples, which confirms that our approach detected the signature proteins already previously established. We subsequently performed a kinase enrichment analysis and identified that GSK-3, ERK1, ERK2, CDK5 substrate motif was significantly enriched among the top 10 hits from NE and ATM kinase PKC alpha and beta from the AD PDX tumor samples. Hierarchical clustering analysis across NE versus AD tumor samples indicated that samples within each group clustered uniquely which indicates that analysis should be personalized when studying aggressive cancer such metastatic CRPC and AVPC. We generated pathway enrichment and GSEA analysis compendium and identified novel targets for therapeutic treatments.

In conclusion, we have performed a global mass spectrometry based proteomic analysis and created an important first global phosphoproteomics and proteomics database that provides insights into the differentially regulated protein and phosphoproteins between AD and NE PDX tumors.