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Platinum Priority – Prostate Cancer

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Next-generation Sequencing of Advanced Prostate Cancer Treated with Androgen-deprivation Therapy

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Abstract

Background: Androgen-deprivation therapy (ADT) is standard treatment for locally advanced or metastatic prostate cancer (PCa). Many patients develop castration resistance (castration-resistant PCa [CRPC]) after approximately 2–3 yr, with a poor prognosis. The molecular mechanisms underlying CRPC progression are unclear.
Objective: To undertake quantitative tumour transcriptome profiling prior to and following ADT to identify functionally important androgen-regulated pathways or genes that may be reactivated in CRPC.
Design, setting, and participants: RNA sequencing (RNA-seq) was performed on tumour-rich, targeted prostatic biopsies from seven patients with locally advanced or metastatic PCa before and approximately 22 wk after ADT initiation. Differentially regulated genes were identified in treatment pairs and further investigated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) on cell lines and immunohistochemistry on a separate CRPC patient cohort. Functional assays were used to determine the effect of pathway modulation on cell phenotypes.
Outcome measurements and statistical analysis: We searched for gene expression changes affecting key cell signalling pathways that may be targeted as proof of principle in a CRPC in vitro cell line model.
Results and limitations: We identified ADT-regulated signalling pathways, including the Wnt/β-catenin signalling pathway, and observed overexpression of β-catenin in a subset of CRPC by immunohistochemistry. We validated 6 of 12 (50%) pathway members by qRT-PCR on LNCaP/LNCaP-AI cell RNAs, of which 4 (67%) demonstrated expression changes consistent with RNA-seq data. We show that the tankyrase inhibitor XAV939 (which promotes β-catenin degradation) reduced androgen-independent LNCaP-AI cell line growth compared with androgen-responsive LNCaP cells via an accumulation of cell proportions in the G0/G1 phase and reduction in the S and G2/M phases. Our biopsy protocol did not account for tumour heterogeneity, and pathway inhibition was limited to pharmacologic approaches.
Conclusions: RNA-seq of paired PCa samples revealed ADT-regulated signalling pathways. Proof-of-principle inhibition of the Wnt/β-catenin signalling pathway specifically delays androgen-independent PCa cell cycle progression and proliferation and warrants further investigation as a potential target for therapy for CRPC.

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1. Introduction

At diagnosis, many men have "incurable" locally advanced or metastatic prostate cancer (PCa), the most common cancer in Europe [1]. PCa progression is initially driven by androgens acting via the cognate androgen receptor (AR) transcription factor. The initial treatment standard for patients with locally advanced or metastatic PCa is androgen-deprivation therapy (ADT), which inactivates the AR for a period of time. After approximately 2–3 yr, these patients can develop castration-resistant PCa (CRPC), for which the prognosis is poor despite newer second-line cytotoxic chemotherapy and endocrine therapies [2–4]. There is an urgent, unmet need for novel therapies for CRPC led by a better understanding of the biology underlying treatment resistance.

The mechanisms underlying CRPC are unclear and may be the result of cellular adaptation to or clonal selection by ADT [5]. AR signalling pathways and transcriptional activity may be reactivated [6], or cell growth may be supported by AR-independent outlaw cell signalling pathways [7]. Hence, a greater understanding of ADT-driven molecular changes may yield information on the mechanisms underlying progression to CRPC. Although previously published transcriptome-wide studies have successfully identified ADT-driven transcriptional events [8,9], these analyses have been limited by the inherent bias associated with microarrays [10].

In this study, we undertake quantitative transcriptome profiling of prostate tumours from patients prior to and following ADT using next-generation sequencing (RNA-seq) to identify functionally important novel androgen-regulated pathways and specific gene products that may be reactivated in CRPC as potential targets for therapy.

2. Materials and methods

2.1. Patient samples for RNA sequencing

Clinical samples for RNA-seq were prospectively collected as part of the GenTax study [11]. Illumina RNA-seq was performed with complementary DNA sample library normalisation using the Illumina duplex-specific nuclease protocol prior to cluster generation and library sequencing on the HiSeq 2000 sequencer (Illumina, San Diego, CA, USA) with a paired-end sequencing strategy. Further details are given in the Supplement.

2.2. Functional assays

All cells were grown at 37 °C in 5% carbon dioxide. LNCaP (CRL-1740, ATCC) cells were maintained in RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA; 31870-025) with 20 mM L-glutamine (Life Technologies, 25030-024) supplemented with 10% foetal bovine serum (PAA Laboratories, Yeovil Somerset, UK; A15-101). LNCaP-AI cells were derived from LNCaP parental cells and maintained as previously described [12]. Proliferation assays were carried out using the WST-1 reagent (Roche Diagnostics, Indianapolis, IN, USA; 05015944001) as per the manufacturer's instructions in medium containing either 10 μm XAV939 (Novartis Pharmaceuticals, Plantation, FL, USA) in 0.1% dimethyl sulfoxide (DMSO) [13] or vehicle. Cell cycle analysis was performed following treatment with 10 μm XAV939 in 0.1% DMSO or vehicle, as previously described [14]. Further details are given in the Supplement.

3. Results

3.1. The transcriptional landscape of androgen-deprivation therapy in clinical prostate cancer

RNA-seq was performed on 16 paired pre- and post-ADT samples from eight patients with locally advanced or metastatic PCa (Gleason score >7 [15]; Table 1). The post-ADT sample from patient 8 performed markedly worse on multiple quality control measures, and so both samples from this patient were excluded from further analysis (Supplemental Table 1). Recently, genomic rearrangements rendering ETS-family transcription factors under the control of androgen-responsive or other promoters have been hypothesised as a mechanism driving prostate carcinogenesis [16]. The *TMPRSS2/ERG* translocation yields the most common PCa-associated gene fusion product, reported in >50% cases [16]. Consistent with this, three of the seven (43%) patients expressed transcripts with sequences corresponding to this fusion event in the pre-ADT samples alone. We observed, on average, a sixfold downregulation of *ERG* expression following ADT, but expression of *TMPRSS2/ERG*

Table 1 – Patient demographics

Patient	Age, yr	KPS	GSS	TNM stage			iPSA, ng/ml	nPSA, ng/ml (% iPSA)	PFS, d
				T	N	M			
1	64.7	100	8	3b	0	0	370	3.1 (0.8)	942
2	65.4	90	9	3b	0	0	7.6	0.7 (0.1)	155
3	69.6	100	8	3b	1	0	5.9	0.7 (0.1)	223
4	64.6	90	8	3a	0	1	47.7	0.5 (1.6)	N/P
5	51.8	90	7	3a	0	0	158	0.4 (0.3)	N/P
6	58.6	100	7	3b	1	0	69	0.13 (0.2)	489
7	62.9	90	7	3b	1	0	32.7	<0.02 (0.06)	N/P
8*	54.9	90	7	3a	2	1	7.4	0.06 (0.8)	154

KPS = Karnofsky performance status; GSS = Gleason sum score; iPSA = initial prostate-specific antigen value at diagnosis; nPSA = nadir prostate-specific antigen value; PFS = progression-free survival; N/P = not yet progressed to date; RNA-Seq = RNA sequencing; ADT = androgen-deprivation therapy; TRUS = transrectal ultrasound.

* Patient excluded from RNA-seq analysis. All patients exhibited a response to ADT prior to second TRUS biopsy, as determined by a fall in levels of serum PSA. The mean time to second TRUS biopsy was 202 ± 143 d, and the mean time to biochemical or radiologic progression was 392 ± 336 d.