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Review

Differential expression and regulation of vitamin D hydroxylases and inflammatory genes in prostate stroma and epithelium by 1,25-dihydroxyvitamin D in men with prostate cancer and an in vitro model

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ABSTRACT

Previous work on vitamin D in the prostate has focused on the prostatic epithelium, from which prostate cancer arises. Prostatic epithelial cells are surrounded by stroma, which has well-established regulatory control over epithelial proliferation, differentiation, and the inflammatory response. Here we examined the regulation of vitamin D-related genes and inflammatory genes by 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2$D) in laser-capture microdissected prostate tissue from a vitamin D_3 clinical trial and in an in vitro model that facilitates stromal–epithelial crosstalk. Analysis of the trial tissues showed that VDR was present in both cell types, whereas expression of the hydroxylases was the highest in the epithelium. Examination of gene expression by prostatic (1,25$(OH)_2$D) concentrations showed that VDR was significantly lower in prostate tissues with the highest concentration of 1,25$(OH)_2$D, and down-regulation of VDR by 1,25$(OH)_2$D was confirmed in the primary cell cultures. Analysis of inflammatory genes in the patient tissues revealed that IL-6 expression was the highest in the prostate stroma while PTGS2 (COX2) levels were lowest in the prostate cancer tissues from men in the highest tertile of prostatic 1,25$(OH)_2$D. In vitro, TNF-α, IL-6 and IL-8 were suppressed by 1,25 $(OH)_2$D in the primary epithelial cells, whereas TNF-α and PTGS2 were suppressed by 1,25$(OH)_2$D in the stromal cells. Importantly, the ability of 1,25$(OH)_2$D to alter pro-inflammatory-induced changes in epithelial cell growth were dependent on the presence of the stromal cells. In summary, whereas both stromal and epithelial cells of the prostate express VDR and can presumably respond to 1,25$(OH)_2$D, the prostatic epithelium appears to be the main producer of 1,25$(OH)_2$D. Further, while the prostate epithelium was more responsive to the anti-inflammatory activity of 1,25 $(OH)_2$D than stromal cells, stroma–epithelial crosstalk enhanced the phenotypic effects of 1,25$(OH)_2$D and the inflammatory process in the prostate gland.

Contents

Abbreviations: 1α,25$(OH)_2$D, 1α,25-dihydroxyvitamin D_3; PCa, prostate cancer; VDR, vitamin D receptor; CYP, cytochrome P450; LCM, laser capture microdissection; RT, reverse transcription; qPCR, quantitative polymerase chain reaction; PTGS2 or COX2, prostaglandin synthase 2; IL, interleukin; TNF, tumor necrosis factor; PrE, primary prostatic epithelial cells; PrS, primary prostatic stromal cells.

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1. Introduction

The potential chemopreventive effects of the pro-hormone vitamin D on prostate tissue and cells have been shown in vitro cell culture and in vivo animal studies as well as in epidemiologic studies and clinical trials [1]. In addition to its classical function in calcium homeostasis and bone health, 1α,25-dihydroxyvitamin D (1,25(OH)$_2$D) has tumor suppressive functions in cells and tissues including the prostate. In prostate cells, 1,25(OH)$_2$D, the active metabolite of vitamin D, is anti-proliferative [2,3], pro-apoptotic [4], pro-differentiating [5], and anti-inflammatory [5,6]. CYP2R1 and CYP27A1 are the enzymes proposed to convert cutaneously-synthesized or dietary vitamin D$_3$ into circulating 25 (OH)D [1] . CYP27B1 further hydroxylates 25(OH)D to the active hormone 1,25(OH)$_2$ D [1]. CYP24A1 hydroxylates and inactivates 1,25(OH)$_2$D into 1,24,25(OH) $_3$D [1].

The anti-inflammatory activities of 1,25(OH)$_2$D involve inhibition of PTGS2 (COX-2), IL-6 and IL-8 in human prostate cells [5,6]. *In vivo*, the 1,25(OH)$_2$D analog, elocalcitol, decreased inflammatory infiltrate in the prostate, nitric oxide signaling, and cytokine production in a mouse model of prostatitis [7].

Prostate glandular epithelium is surrounded by stroma which contains smooth muscle cells, fibroblasts, myofibroblasts and resident macrophages [8]. Although prostate cancer (PCa) emerges from epithelial cells, there is evidence that a permissive stromal environment influences epithelial tumor growth. The stroma microenvironment can become "reactive" stroma around prostatic intraepithelial neoplasia (PIN) and PCa, which further evolves as carcinoma progresses [9,10]. This interplay may be especially important during the early stages of cancer when immune cells may be tumor suppressive or tumor promoting depending on the existing immune environment [11,12]. Tumor growth promoting effects of the stroma have been shown in several studies in which co-inoculation of fibroblasts with PCa cells increased xenograft growth [13–15].

In general, inflammation is a risk factor for many cancers including PCa [16,17]. A meta-analysis showed an increased risk of PCa incidence in men with a history of prostatitis (odd ratio 1.65; 95% confidence interval (CI): 1.32–2.06) or syphilis (odds ratio 1.5; 95% CI: 0.6–3.5) [18]. Proliferative inflammatory atrophy (PIA) lesions, which are associated with chronic inflammation, have similar gene expression changes (NKX3.1, PTEN, and p27) as PIN and PCa and thus may be precursors to carcinogenesis [19–21].

The expression of VDR and vitamin D metabolism genes has not previously been reported according to cell type in prostate tissue. Considering the importance of the stroma–epithelial interaction in the prostate and that both these cell types express the vitamin D receptor (VDR) [22–24], we hypothesized that stromal and epithelial cells have unique functions in mediating vitamin D actions within the prostate gland. To address the possibility, the present study examined the gene expression of vitamin D-related and inflammatory genes in laser capture-microdissected tissue from a clinical trial of men who received oral vitamin D$_3$ prior to radical prostatectomy. We further dissected the differential responses of prostate epithelial and stromal cells to 1,25(OH)$_2$D in a 3-D co-culture model of inflammation. Our findings establish a role for stromal–epithelial crosstalk for the therapeutic response to vitamin D in patients.

2. Materials and methods

2.1. Clinical trial specimens and serum samples

As previously described [25], paraffin blocks of prostatectomy specimens and serum from 45 patients (15 randomly selected patients from each treatment group) enrolled in a phase II clinical trial were obtained from University Health Network in Toronto, Canada. The randomized trial, registered with www.clinicaltrials.gov (NCT00741364), involved 66 patients given oral vitamin D$_3$ (cholecalciferol) at doses 400, 10,000, or 40,000 IU/day for 3–8 weeks prior to prostatectomy at which time fresh tissue was collected and cryostored. The benign and PCa areas of the blinded specimens were demarcated by a pathologist for laser-capture microdissection (LCM). LCM was used to separately collect benign epithelium, PCa epithelium and benign stroma via a procedure previously described for these samples [25]. Serum samples were obtained at entry and at the final visit before as previously described [26].

2.2. Cell culture

Primary human epithelial and stromal cells were isolated from radical prostatectomy tissue from PCa patients at the University of

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