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Pathogenic role of HIF-1α in prostate hyperplasia in the presence of chronic inflammation

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ABSTRACT

Benign prostatic hyperplasia (BPH) commonly occurs in older men with chronic prostatitis. Although BPH is frequently accompanied by inflammation, it is unclear whether inflammation underlies prostate enlargement. Recently, we reported that hypoxia-inducible factor 1α (HIF-1α), which is known to be induced by proinflammatory cytokines, is involved in testosterone-induced prostate hyperplasia. Therefore, we hypothesized that cytokines secreted from infiltrated macrophages under inflammatory conditions stimulate prostate enlargement by up-regulating HIF-1α. In the present study, we injected lipopolysaccharide (LPS) into rat prostates to mimic prostatitis and evaluated prostate hyperplasia 14 days later. Epithelial cells of LPS-treated prostates were found to be highly proliferative and HIF-1α levels in prostate tissues to be elevated. When prostate epithelial cells were incubated in conditioned medium from macrophages activated with LPS, they robustly expressed HIF-1α, and under these conditions IL-1β, IL-6, and TNF-α cytokines were found to mediate HIF-1α induction. In addition, HIF-1α was found to enhance the expression of Twist, which initiates epithelial–mesenchymal transition (EMT). Furthermore, profound EMT features were observed in LPS-treated rat prostates, and the natural HIF-1α inhibitors ascorbate and curcumin were found to attenuate EMT and prostate hyperplasia both *in vivo* and *in vitro*. Based on these results, we propose that HIF-1α mediates prostate enlargement under inflammatory conditions, and we suggest that HIF-1α be viewed as a promising target for blocking the transition from prostatitis to BPH.

1. Introduction

Benign prostatic hyperplasia (BPH) is one of the most common chronic diseases in the male population. Its incidence gradually increases with age, and about 50% of men aged over 50 suffer from BPH symptoms, such as, urinary urgency, and retention [1]. Histologically, BPH tissues are remodeled toward epithelial and stromal hyperplasia due to an imbalance between cell proliferation and death [2]. Because the prostate grows under the influence of androgen hormones, 5α-reductase inhibitors, which block activation process of testosterone, are widely used to treat BPH [3,4]. Other than the androgen inhibition, diverse therapeutic modalities are needed to realize the tailor-made therapy for BPH patients.

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor composed of HIF-1α and HIF-1β subunits, and is a key player in hypoxic response [5]. The expression and activity of HIF-1α, but not of HIF-1β, are tightly controlled under normoxic conditions, wherein HIF-1α is prolyl-hydroxylated, recruits the pVHL-containing E3 ubiquitin ligase complex, and then degraded *via* the ubiquitin–proteasome system. Accordingly, when this hydroxylation is inhibited under hypoxic conditions, HIF-1α is stabilized and activated [6–9]. Inflammation is another microenvironmental factor that can stimulate HIF-1 signaling. When tissues are inflamed, infiltrating immune cells and resident stromal cells secrete a variety of cytokines, and some of which enhance HIF-1α expression [10]. Subsequently, HIF-1α enters nuclei, where it in association with HIF-1β binds to the hypoxia response elements (HRE) of genes. Functionally, HIF-1α is a master factor that orchestrates the expressions of about 80 genes and determines cell fate under hypoxic or inflammatory conditions [11]. In addition, HIF-1α drives epithelial–mesenchymal transition (EMT) by inducing the expressions of EMT-promoting transcription factors like Twist and Snail, and participates in tissue remodeling by inducing enzymes degrading or modifying extracellular matrix [12].

Recently, HIF-1α was found to be induced in prostate epithelial cells by zinc ion and to help prostate cells survive in a zinc-rich

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environment of the prostate. Mechanistically, zinc ion inhibits the prolyl-hydroxylation process and consequently stabilizes HIF-1α [13], which facilitates the expression of survivin that promotes cell survival and proliferation [14]. HIF-1α has also been found to mediate testosterone-induced hyperplasia in the rat prostate [15]. In line with its malignant tumor promoting action, HIF-1α is involved in the pathogenesis of BPH, which exhibits features of benign tumors.

Androgens are known to enlarge the prostate and aggravate the symptoms of BPH, but the incidence of BPH is markedly greater in older men whose androgen levels decline with age [16,17]. This suggests that local factors other than circulating sex hormones contribute to the progress of BPH. Evidence accumulated over the last decade supports the notion that intraprostatic inflammation predisposes prostate to overgrow. In fact, inflammatory signs are usually detected in BPH specimens [18]. Furthermore, it has been suggested in several epidemiological studies that inflammatory reactions are involved in the pathogenesis of BPH and in the aggravation of its clinical symptoms. Bacterial and viral infections, autoimmune response against prostate antigens, hormonal imbalance, urinary reflux into the prostate, and other mechanisms have been proposed to trigger intraprostatic inflammation [19,20], and several proinflammatory cytokines, such as, interleukins, TNF-α, and interferon-γ have been suggested to mediate prostate enlargement under inflammatory conditions [21–23]. These cytokines are believed to be produced by stromal and immune cells, and to stimulate prostate cell proliferation in a paracrine manner. However, the identities of the key mediators of prostate cell proliferation under inflammatory conditions remain open, though it is evident that their identifications might provide new treatments for BPH.

In the present study, we explored the possible connection between inflammatory signaling and HIF signaling during BPH progression. More specifically, we investigated the validity of a hypothetical mechanism underlying inflammatory BPH, namely, macrophage → cytokines → HIF-1 → excessive prostate enlargement. In addition, we examined whether this mechanistic axis drives prostate tissue remodeling *via* EMT under inflammatory conditions. Accordingly, we injected LPS into rat prostates to induce the transition from prostatitis to BPH, and incubated normal prostate cells with conditioned media obtained from LPS-activated macrophages with the aim of identifying molecular entities associated with inflammation and hyperplasia. Furthermore, we treated with HIF-1 inhibitors both *in vivo* and *in vitro* to investigate the pathogenic role played by HIF-1 in inflammatory BPH.

2. Materials and methods

2.1. Materials

LPS, sodium ascorbate, curcumin, and anti-Vimentin Cy3 were purchased from Sigma-Aldrich (St. Louis, MO). Culture media, fetal bovine serum, Lipofectamine RNAimax, and Lipofectamine LTX-Plus were from Invitrogen (Carlsbad, CA). Anti-HIF-1α antiserum was generated in rabbits using a bacterially expressed fragment containing amino acids 418–698 of human HIF-1α [24]. Anti-VEGF and β-tubulin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-Vimentin, p-PKCα/β, p-mTOR, mTOR, p-JNK, JNK, p-ERK, ERK, p-AKT and AKT were from Cell Signaling Technology (Danvers, MA). Anti-E-cadherin, anti-IL-1β, anti-IL-6, and TNF-α were from BD (San Jose, CA), and anti-LYVE-1 was from Biotechnology (Billerica, MA).

2.2. The animal model

Male Sprague–Dawley rats (10 weeks age) were obtained from Orient Bio (Gueong-Gi Province, South Korea). All animal procedures were performed in accord with the Seoul National University Laboratory Animal Maintenance Manual. Animals were anesthetized with isoflurane, and the lower abdomen was incised to expose prostates. Briefly, PBS (100 μl) or LPS (100 μg/kg) was injected equally into the right and left prostate lobes in the control (C; n = 8) and LPS groups, respectively. LPS-treated rats were divided into three groups, and intraperitoneally injected with PBS (L group, n = 8), 100 mg/kg of ascorbate (L + Asc group, n = 8), or 120 mg/kg of curcumin (L + Cur group, n = 8). Rats were treated with LPS twice one week apart and with the indicated drugs three times a week for 2 weeks. On the 14th day after the first LPS injection, rat prostates were excised, weighed, and used for the experiments. The experimental protocol is illustrated in Fig. 1A.

2.3. Cell culture

PNT2 (a human prostate epithelium cell) and LNCaP (a human prostate cancer cell) were obtained from ECACC (London, UK). LNCap cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), HEPES (10 mM), penicillin and streptomycin; PNT2 cells were maintained in RPMI1640 medium containing 10% heat-inactivated fetal bovine serum in 5% CO_2 at 37 °C.

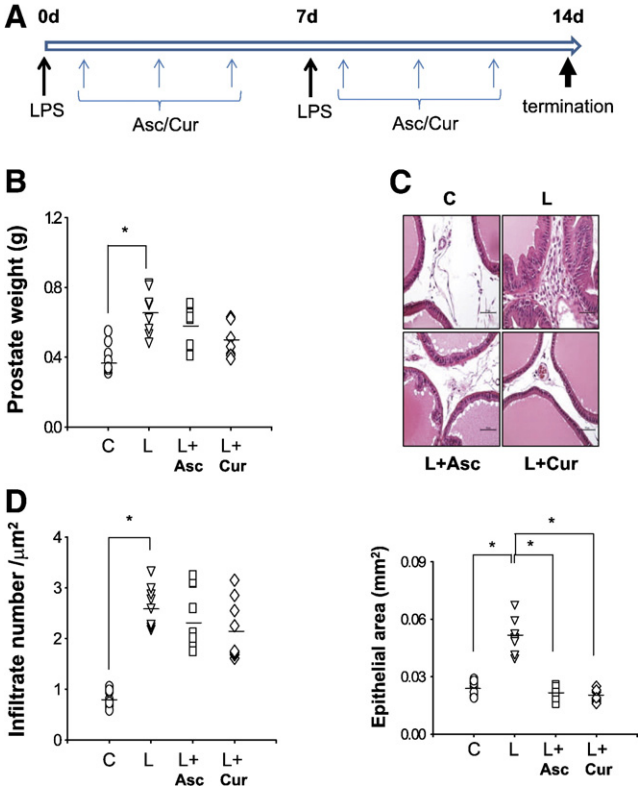

Fig. 1. LPS induces prostate hyperplasia, which is recovered by HIF-1 inhibition. (A) Experimental protocol. Rats were treated with LPS or HIF-1α inhibitors at the indicated time points. (B) On the 14th day after the first injection of LPS, prostates were excised and weighed. C, PBS control; L, LPS alone; L + Asc, LPS plus ascorbate; L + Cur, LPS plus curcumin. (C) Microscopic findings of prostates. Prostate tissues were fixed, embedded in paraffin, and sectioned at 4 μm. Sections were stained with hematoxylin and eosin, and examined at a 400X magnification (top). Epithelial cell layer areas in the slides were analyzed using ImageJ1.36b image analysis software (NIH, USA) (bottom). (D) In prostates excised on the 3rd day after LPS injection, macrophages were immunostained and their number was counted under a microscope. (E) PCNA was immunostained in prostates excised on the 14th day after LPS treatment. PCNA positivity was evaluated when the nuclear density *versus* the background density was more than 5 fold. The right panel presents PCNA-positive cell numbers per 100 cells. (F) Apoptotic cell death was analyzed by TUNEL staining (left). Results are presented as TUNEL-positive cell numbers per 100 cells (right). Arrows indicate representative cells with positive signal. Each horizontal bar represents the mean value of 8 samples, and * denotes $p<0.05$ between two groups.