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Expression of human β–defensin–2 in the prostate

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OBJECTIVE

To investigate the expression and regulation of human β-defensin-2 (HBD-2) in the prostate.

PATIENTS AND METHODS

Normal human prostate epithelial cell line (RWPE-1), human prostate cancer cell lines (DU-145, PC-3), and paraffin-embedded prostate tissue from patients with benign prostatic hyperplasia (BPH) were analysed by RT-PCR and immunohistochemical staining. HBD-2 expression was also analysed by RT-PCR and ELISA in RWPE-1 cells treated with lipopolysaccharide (LPS). Nuclear factor-κB (NF-κB) activation was assessed by IκBα immunoblotting and electrophoretic mobility shift assay (EMSA).

RESULTS

BPH tissue and all of the tested prostate cell lines other than PC-3 constitutively express HBD-2 mRNA. HBD-2 protein was strongly detected in prostate gland tissue surrounded by inflammatory cells including macrophages. Exposure to LPS induced HBD-2 upregulation and NF-κB activation, as assessed by IκBα phosphorylation and degradation in RWPE-1 cells. Bay11-7082, an NF-κB inhibitor prevented LPS-induced HBD-2 production in RWPE-1 cells.

CONCLUSIONS

Prostate epithelial cells may constitutively express HBD-2, and its expression was upregulated by LPS. Our data indicate that HBD-2 may be an important immunomodulatory factor in prostate function. Expression of HBD-2 in normal prostates and the potential role of HBD-2 in prostatitis and BPH should be addressed in the future.

What's known on the subject? and What does the study add?

We found the expression of human β-defensin-2 (HBD-2) in the prostate for the first time and LPS, a gram negative bacterial component, upregulated HBD-2 in prostate epithelial cells.

We are looking for other antimicrobial peptides expressed in the prostate besides human β-defensin-2.

Also, we are studying the relationship between antimicrobial peptides and the development or progression of prostate diseases.

KEYWORDS

prostate, human beta-defensin-2, lipopolysaccharide, NF-κB activation

INTRODUCTION

Antimicrobial peptides are important components of the innate host-defence system against bacteria, fungi, parasites and viruses. In humans, these peptides include α- and β-defensins, and cathelicidins [1]. Unlike α-defensins, which are produced mainly by neutrophils, β-defensins are produced directly by epithelial cells, and combat infection both through direct microbicidal action and by modulation of cell-mediated immunity [2]. So far, four human β-defensins (HBD-1 to -4) have been identified and characterized [2]. Among them, HBD-1 is constitutively produced by various epithelial tissues such as urogenital and respiratory tracts, and skin [3], whereas the expressions of HBD-2, HBD-3 and HBD-4 are inducible [1].

Human β-defensin-2 is a cysteine-rich cationic low-molecular-weight antimicrobial peptide that was discovered in psoriatic lesional skin and is suggested to be involved in cutaneous defence and inflammation [4]. HBD-2 exhibits a stronger potential antimicrobial activity against Gram-negative organisms, such as *Escherichia coli* and *Pseudomonas aeruginosa* and the yeast *Candida albicans*, than against gram-positive organisms such as *Staphylococcus aureus* [4]. HBD-2 is poorly expressed in normal epithelial cells, but is expressed by epithelial cells stimulated with microorganisms or cytokines such as TNF-α and IL-β [5,6]. However, expression of HBD-2 in the prostate has never been studied.

In addition to their microbicidal abilities, defensins have additional functions related to host defence, such as induction of histamine

release by mast cells and chemoattraction of various cells of the immune system including neutrophils and T cells [7]. Moreover, they seem to be involved in carcinogenesis [8–10].

Prostate epithelium has a powerful innate immune system that protects male reproductive organs from infections. The stratified columnar epithelium of the prostate presents a physical barrier to pathogenic microorganisms which is further supported by an indigenous microflora and an acidic environment, which provides antagonistic activity against a variety of bacteria related to prostate infections [11]. Zinc and lactoferrin are known to have defensin-like functions in the prostate. Zinc accumulates in the prostate, and this gland typically contains the highest concentration of zinc in the body [12]. Prostate cancer is linked to the inability of prostate cells to accumulate zinc [13], which indicates that zinc has a very important role in the prostate. Lactoferrin has been reported to kill bacteria, play an immunomodulatory role, and participate in prostatic inflammatory responses [14]. Lactoferrin and zinc can thus combat bacteria when the prostate is infected.

The role of antimicrobial peptides from the defensin family in mediating protective responses has also been established in other tissues [15–17]. However, whether or not β-defensin expression is induced in prostate epithelial cells in response to bacterial infection has not been examined.

Nuclear factor-κB (NF-κB) is an important intracellular signal of both innate and acquired immunity, which induce the activation of antimicrobial peptides. The transcription factors of the NF-κB family remain in a quiescent state, complexed with inhibitory IκB proteins, in the cytosol of virtually all vertebrate cells [18]. Upon activation, IκB proteins are phosphorylated and its subsequent proteosomal degradation by the 26S proteasome allows NF-κB nuclear translocation and activation of NF-κB-dependent transcriptional activity [19]. To examine whether lipopolysaccharide (LPS) induces NF-κB activation in RWPE-1, phosphorylation of IκBα and IκBα degradation were determined.

The major aim of this study was to determine if prostate epithelial cells constitutively express HBD-2, and/or if bacterial infection induces HBD-2 expression in prostate epithelial cells. To analyse the effects of bacterial infection on prostate epithelial cells, we used LPS and RWPE-1 cells in a model system, because LPS recognition is one of the best studied systems, and in addition, Gram-negative bacteria (*Escherichia coli*, *Klebsiella*, *Enterobacteria*, *Proteus* and *Serratia species*) are the predominant pathogens affecting this organ [20].

In this study, we investigated HBD-2 gene expression in BPH tissue and immortalized prostate epithelial and cancer cell lines (RWPE-1, DU-145 and PC-3). This is the first report on the expression of HBD-2 in prostate epithelial cells and its up-regulation by LPS.

PATIENTS AND METHODS

Lipopolysaccharide was purchased from Sigma-Aldrich (Seoul, Korea). Bay11-7082 was purchased from Calbiochem (La Jolla, CA, USA). The human β-defensin-2 (HBD-2) ELISA kit was obtained from KOMA Biotech Inc. (Seoul, Korea). Anti-IκBα and anti-phospho-IκBα antibodies were purchased from Cell Signaling Technology Inc (Danvers, MA, USA).

Prostate tissue samples were obtained from three patients with BPH. All patients consented to the use of their prostate tissues for the present investigation, and the study was approved by the Institutional Review Board of Chung-Ang University College of Medicine. The patients had been treated by transurethral resection of the prostate at Chung-Ang University Hospital, during May 2009. After surgery, each prostate specimen was divided into two, one piece was immediately frozen in liquid nitrogen and the other was routinely fixed with 10% formalin and paraffin-embedded until use.

The immortalized human prostate epithelial cell line (RWPE-1) and human prostate cancer cell lines (PC-3 and DU-145) were obtained from the American Type Culture Collection (Manassas, VA, USA). RWPE-1 cells were cultured in Keratinocyte-SFM (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 0.05 mg/mL bovine pituitary extract (BPE) and 5 ng/mL epidermal growth factor (EGF) in a humidified atmosphere containing 5% CO_2. PC-3 and DU-145 cells were cultured in growth medium containing RPMI 1640 (WelGENE, Seoul, Korea), 10% heat-inactivated fetal bovine serum (Invitrogen Corp.) and 1% penicillin-streptomycin (WelGENE). The cells were routinely passaged at 80–90% confluence.

Expression of HBD-2 in BPH tissue was studied using the Ultravision LP Detection System (LabVision Corp., Fremont, CA, USA). Briefly, slides with BPH tissue sections were dewaxed in xylene for more than 20 min and then were sequentially rehydrated in 100%, 95%, 90%, and 80% ethanol solutions. After a 5-min rinse in water, the slides were pretreated with 0.01 M sodium citrate buffer and autoclaved for 1 min at 121 °C to retrieve the antigen. After the slides were rinsed, endogenous peroxidase activity was blocked by treatment with 3% H_2O_2 for 30 min. Primary mouse polyclonal antibody to human β-defensin-2 (1:50, Abcam, MA, USA) was applied to the sections on the slides, which were incubated for 2 h in a moist chamber at room temperature. A negative control was prepared by substituting non-immune serum for primary antibody. After the slides were rinsed with PBS, they were incubated with secondary antibody for 10 min at room temperature and then rinsed in PBS. The slides were incubated in tertiary horseradish peroxidase (HRP-) conjugated antibody for 10 min, rinsed in PBS, and incubated with diaminobenzidine for 10 min. After the slides were counterstained using Mayer's hematoxylin, they were dehydrated and coverslipped.

RNA was isolated using an RNeasy kit (Qiagen, Seoul, Korea) and 2 μg of total RNA were reverse-transcribed using Accupower® RT/PCR Premix (Bioneer, Daejeon, Korea). cDNA was amplified using the following primers synthesized by Genotech (Daejeon, Korea): sense 5′-CCAGCCATCAGCCATGAGGGT-3′ and antisense 5′-GGAGCCCTTTCTGAATCCGCA-3′ (255 amplicon). PCR conditions comprised denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s. In all experiments there were two negative controls consisting of no reverse transcription or no RNA. PCR products including the internal RT-PCR control glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were subjected to electrophoresis on 1.5 % agarose gels after they were stained with SYBR® Green (Roche Applied Science, Indianapolis, IN, USA) for 15 min in the dark. Gels were photographed and analysed by the Bio-Rad Molecular Imager Gel Doc XR+ System (Bio-Rad Laboratories, Inc. Hercules, CA, USA).

Concentrations of human β-defensin-2 in the supernatants of RWPE-1 cells stimulated with LPS were measured using the HBD-2 ELISA kit (KOMA Biotech Inc, Seoul, Korea) following the manufacturer's instructions. The minimum detectable concentrations were approximately 8 pg/mL of the HBD-2 recombinant peptide. Serial dilutions of recombinant peptide provided by the kit were used for creating standard curves. The optical density of the wells was determined using a microplate reader (TECAN Systems Inc, San Jose, CA, USA) set to 450 nm.

Total protein extracts from RWPE-1 cells were prepared in a lysis buffer of 1.5% SDS, 62.5 mM Tris–HCl (pH 6.8), 5 mM EDTA, 1% 2-mercaptoethanol (2-ME), 1 μg/mL antipain dihydrochloride, 1 μg/mL chymostatin, and 1 μg/mL leupeptin (all from Sigma-Aldrich, Seoul, Korea). Lysates were cleared by centrifugation and supernatants were stored at −80 °C. The constituent proteins of the epithelial cell lysates were separated by SDS/PAGE on a 10% separating gel and then transferred to a nitrocellulose membrane (Bio-Rad Laboratories). In order to verify the equivalent loadings of proteins in the wells, the gel and the nitrocellulose membrane were stained using Coomassie Brilliant Blue and Ponceau S (all from Sigma), respectively. Membranes were blocked by incubation in Tris-buffered saline (150 mM NaCl, 25 mM Tris (pH 7.4)), containing 0.05% Tween 20 (Sigma) and 3% non-fat dry milk (Sigma), for 2 h at room temperature and subsequently incubated overnight at 4 °C with 1:1000 dilutions of primary antibodies (rabbit anti-phospho-IκBα, rabbit anti-IκBα and rabbit anti-β-actin) (Cell Signaling Technology, Inc.). HRP-conjugated goat anti-rabbit IgG (Cell Signaling Technology Inc.) was used as a secondary antibody, diluted 1:10 000 in blocking buffer, and membranes were incubated for 1 h at room temperature. Blots were developed using enhanced chemiiluminescence (GE Healthcare, Milwaukee, WI, **USA**) as a substrate.

RWPE-1 cells, unstimulated or stimulated with LPS (10 μg/mL), were centrifuged and washed once in cold PBS. Cells were collected and cell nuclear proteins were extracted by Nuclear Extract Kit (Active Motif, Carlsbad, CA, USA). Protein was quantified by the DC Protein Assay kit (Bio-Rad Laboratories). Biotin 3′ end-labelled oligonucleotide containing the NF-κB sequence (5′-AGTTGAGGGGACTTTCCCAGG-3′) was used as

a DNA probe after annealing. Binding reactions were performed at 25°C for 15 min in the binding buffer (10 mM Tris–HCl [pH 7.5], 50 mM KCl, 50 mM NaCl, 1 mM MgCl$_2$, 1 mM EDTA, 5 mM DTT, and 5% glycerol) with 5 μg of nuclear protein and labelled probe to a total volume of 20 μL. For the competitive assay, an additional 200-fold molar excess of the unlabelled wild-type probe was added to the binding reaction. The DNA–protein complex was separated in a 4% non-denaturing polyacrylamide gel by electrophoresis in 0.5X Tris-borate-EDTA buffer at room temperature. After electrophoresis, the gel was transferred to Biodyne® B Pre-cut Modified Nylon Membranes (Thermo Fisher Scientific Inc, Rockford, IL, USA) and analysed by Chemiluminescent Nucleic Acid Detection Module (Thermo Scientific).

All data are presented as mean±standard deviation. The data were analysed with the use of one-way ANOVA followed by the Scheffe test for multiple comparisons. A *P* value <0.05 was considered statistically significant.

RESULTS

Expression of HBD-2 mRNA was detected in all of the tested samples other than PC-3: normal prostate epithelial cell line RWPE-1, prostate cancer cell line DU-145 and three BPH patient samples (Fig. 1). HBD-2 protein was also detected in prostate tissue samples from BPH patients by immunohistochemical analysis (Fig. 2A,B). HBD-2 expression was more strongly detected in prostate gland surrounded by inflammatory cells or with intraluminal inflammatory cells than in areas with no inflammatory cell infiltration (Fig. 2A). Also, inflammatory cells including macrophages displayed enhanced expression of HBD-2. HBD-2-expressing macrophages are shown scantily infiltrating non-inflammatory stromal BPH tissue (Fig. 2B).

The levels of HBD-2 mRNA were significantly elevated in RWPE-1 cells challenged for 3 to 24 h with LPS (Fig. 3A). To confirm that increased HBD-2 mRNA was associated with an increase in protein translation, ELISA for HBD-2 protein was performed. Concomitant with HBD-2 mRNA expression (Fig. 3A), HBD-2 protein concentrations in the culture medium of RWPE-1 cells stimulated with LPS was also significantly increased in a time-dependent manner (Fig. 3B).

FIG. 1. RT-PCR detection of human β-defensin-2 (HBD-2) gene expression in prostate tissues and cell lines. Lane 1: RWPE-1 cells; lane 2: PC-3 cells; lane 3: DU-145 cells; Lanes 4–6: BPH samples; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): internal control.
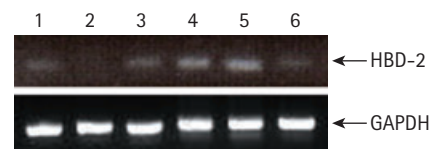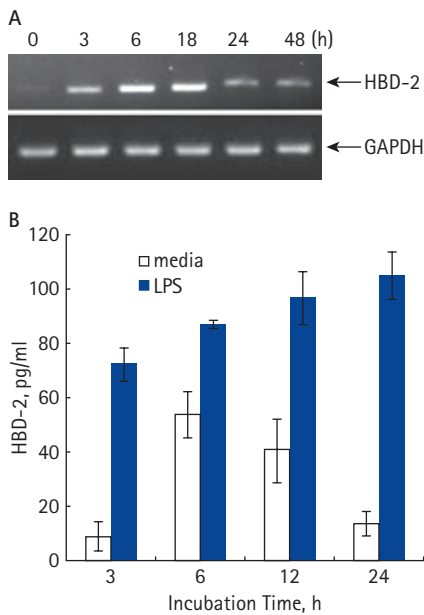

*FIG. 2. Immunohistochemical staining of human β-defensin-2 (HBD-2) in prostate tissue samples from BPH patients. (**A**) HBD-2 expression is more strongly detected in areas of the prostate gland surrounded by inflammatory cells or with intraluminal inflammatory cells (open curved arrows) than in areas with no inflammatory cell infiltration (open straight arrow). Also, inflammatory cells, including macrophages, show enhanced expression of HBD-2 (closed straight arrow) (×400). (**B**) Macrophages expressing HBD-2 are scantily infiltrating non-inflammatory stroma of BPH tissue (×400).*
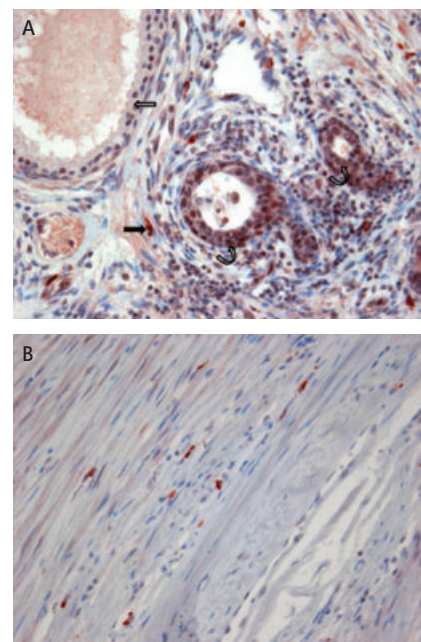
As shown in Fig. 4A, LPS stimulated an increase in IκBα phosphorylation. Consistently, IκBα degradation was observed within the same exposure periods (Fig. 4B). These data suggest that LPS exposure induced NF-κB activation in RWPE-1 cells. NF-κB activation was confirmed
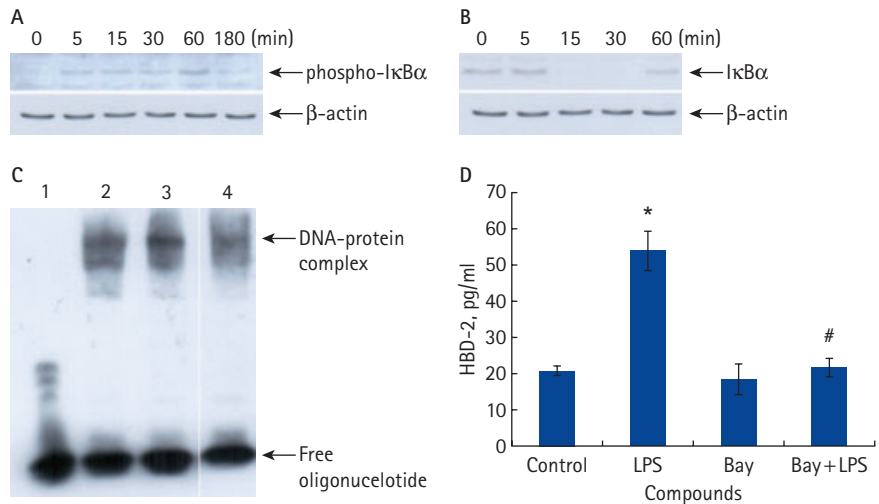
FIG. 3. (A) Lipopolysaccharide (LPS) induces human β defensin-2 (HBD-2) mRNA expression in prostate epithelial cells. RT-PCR analysis of HBD-2 mRNA expression in RWPE-1 cells after treatment with LPS (10 μg/mL). GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (B) LPS induces HBD-2 protein production in prostate epithelial cells. Concentrations of HBD-2 were measured by ELISA in the supernatants obtained from cultured RWPE-1 cells treated with LPS (10 μg/mL). Concentrations are shown as picograms of protein per mL of supernatant and are representative of three distinct experiments.

FIG. 4. Nuclear factor-κB (NF-κB) activation is required for lipopolysaccharide (LPS)-induced HBD-2 expression in RWPE-1 cells. (A) IκBα phosphorylation determined by immunoblotting in RWPE-1 cells following LPS stimulation (10 μg/mL). (B) Kinetics of IκBα degradation in LPS-activated RWPE-1 cells. (C) Electrophoretic mobility shift assay (EMSA) of NF-κB in RWPE-1 cells stimulated for 1 h with LPS (10 μg/mL), lane 3. Lane 1, no nuclear extract added; lane 2, control cells; lane 4, cold oligonucleotide competition. (D) Effect of NF-κB inhibition using Bay11-7082 on LPS induction of HBD-2 in RWPE-1 cells. Bay: Bay11-7082. *: P < 0.05, compared with control; #: P < 0.05, compared with LPS-treated group.

by electrophoretic mobility shift assay (EMSA). A low level of NF-κB consensus probe-binding complex was present in untreated nuclei. The DNA-protein complex was increased by stimulation with LPS (Fig. 4C). A 200-fold molar excess of unlabelled NF-κB probe caused a decrease in the level of DNA-protein complex, suggesting that the consensus sequence for NF-κB was critical for formation of the DNA-protein complex (Fig. 4C).

To further demonstrate whether NF-κB activation is required for LPS-induced HBD-2 production, the effect of Bay11-7082, an NF-κB inhibitor, on LPS-induced HBD-2 production was examined. Bay11-7082 prevents the activation of NF-κB by inhibiting IκB phosphorylation [21]. Bay11-7082 prevented LPS-induced HBD-2 production (Fig. 4D), suggesting the involvement of NF-κB in LPS-induced HBD-2 production.

DISCUSSION

In this study, we demonstrated that HBD-2 is constitutively expressed in prostate epithelial cells (normal epithelial cell line RWPE-1, prostate cancer cell line DU-145, and BPH tissue) and that its expression is upregulated by the Gram-negative bacterial component LPS through the activation of the NF-κB pathway. These results suggest that prostate epithelium can trigger a host defence reaction in response to microbial infection. HBD-2 expression was more strongly detected in the prostate gland tissue that was surrounded by inflammatory cells and with intraluminal inflammatory cells than in areas with no inflammatory cell infiltration (Fig. 2A).

In humans, defensins are cationic antimicrobial peptides that are rich in cysteine residues, and they contribute to host defence against bacterial, fungal and viral infections [22]. The role of β-defensins in protecting the prostate from microbial infection has not been directly documented. In this study, we showed that immortalized prostate epithelial and cancer cell line constitutively express HBD-2, and that LPS enhanced HBD-2 expression and production in prostate epithelial cells. Our report of expression of HBD-2 in prostate epithelial

cells is the first finding, although Gombart et al. reported on the induction of CAMP by 1,25(OH)$_2$D$_3$ in the prostate [23]. In this study, we didn't examine HBD-2 expression in normal prostates, but in prostate tissue from BPH patients. Although many studies have demonstrated that HBD-2 is induced in response to several stimuli, our results showed that HBD-2 is constitutively expressed in human prostate epithelial cells. In addition to our findings, there are some reports that HBD-2 is constitutively expressed in human gingival epithelial cells [24] and normal human skin [25].

In the present study, macrophages expressing HBD-2-expressed macrophages were more frequently observed on BPH slides in microscopic fields that were densely infiltrated by inflammatory cells than in other fields on the slide. This phenomenon may be associated with the defensin's chemotactic activity or with BPH progression. Since defensins are stimulatory molecules for epithelial cells and fibroblasts, HBD-2 may play a role in prostate inflammation, which could be a key component in prostate enlargement and BPH progression. HBD-2-expressing macrophages were seen scantily infiltrating non-inflammatory stromal tissue in BPH samples (Fig. 2B). HBD-2 was also

expressed in prostate epithelial cells and infiltrating macrophages in BPH tissue (Fig. 2A). The significance of these observations will be elucidated in the future.

Until now regulation of HBD-2 expression in prostate epithelial cells has not been documented. Our IκBα immunoblotting and EMSA data demonstrated that LPS induced NF-κB activation, and the latter's requirement for LPS-induced HBD-2 expression was confirmed by our inhibitor experiment. Pretreatment of RWPE-1 cells with the NF-κB inhibitor Bay11-7082 almost blocked HBD-2 production caused by LPS (Fig. 4D), suggesting that NF-κB is required for LPS-induced HBD-2 expression. This finding is consistent with previous studies in which inhibitor experiments have demonstrated the necessity of NF-κB for HBD-2 induction, and by the identification of an NF-κB consensus sequence in the proximal promoter region of the HBD-2 gene. The sequence appears to be necessary for optimal HBD-2 gene expression in other cell lines [26]. In addition to the NF-κB pathway, several other mechanisms such as AP-1 pathways, protein kinase C, phosphatidylinositol-3-kinase, and c-Jun N-terminal kinase have been identified as modulators of HBD-2 signalling in other tissues. We suggest that multiple signalling pathways may be involved in the upregulation of this antimicrobial peptide in the prostate. We are studying other signalling pathways modulating HBD-2 signalling in prostate epithelial cells.

There are few studies on the relationship between prostate cancer and antimicrobial peptides. Donald *et al.* performed immunohistochemical analysis of the human β-defensin-1 (DEFB1) protein in clinical specimens of both renal cell carcinoma and prostate cancer, and demonstrated the cancer-specific down-regulation of DEFB1 in a large sample of prostate cancer and renal carcinomas [27]. Unlike β-defensin-1 whose loss may enhance or promote prostate tumour formation, it has been reported that HBD-2 stimulates proliferation of human umbilical vein endothelial cells [28], epidermal keratinocytes [29] and transformed cells of human cervix [30]. However, the role of HBD-2 in BPH or prostate cancer remains unknown.

Our results demonstrated that HBD-2 was constitutively expressed in prostate epithelial cells and macrophages. HBD-2 expression was

more strongly detected in the prostate gland regions surrounded by inflammatory cells, and its expression was increased by LPS, suggesting that innate immune responses have important roles in combating infections in prostate epithelium. Taken together, our findings suggest that HBD-2 may be one of the most important immunomodulatory factors in the prostate. Expression of HBD-2 in normal prostates and the potential role of HBD-2 in prostatitis and BPH should be addressed in the future.

CONFLICT OF INTEREST

None declared.

REFERENCES

1 **Chen X, Niyonsaba F, Ushio H** *et al.* Antimicrobial peptides human b-defensin (hBD)-3 and hBD-4 activate mast cells and increase skin vascular permeability. *Eur J Immunol* 2007; **37**: 434–44

2 **MacRedmond R, Greene C, Taggart CC, McElvaney N, O'Neill S.** Respiratory epithelial cells require Toll-like receptor 4 for induction of human β-defensin 2 by lipopolysaccharide. *Respir Res* 2005; **6**: 116–26

3 **Valore EV, Park CH, Quayle AJ, Wiles KR, McCray JPB, Ganz T.** Human β-defensin-1: an antimicrobial peptide of urogenital tissues. *J Clin Invest* 1998; **101**: 1633–42

4 **Ganz T.** Defensins: antimicrobial peptides of innate immunity. *Nat Rew Immunol* 2003; **3**: 710–20

5 **Harder J, Meyer–Hoffert U, Teran LM** *et al.* Mucoid Pseudomonas aeruginosa, TNF-α and IL-1β but not IL-6, induce human β-defensin-2 in respiratory epithelia. *Am J Respir Cell Mol Biol* 2000; **22**: 714–21

6 **Singh PK, Jia HP, Wiles K** *et al.* Production of β-defensins by human airway epithelia. *Proc Natl Acad Sci USA* 1998; **95**: 14961–6

7 **Schwab M, Reynders V, Loitsch S, Steinhilber D, Schroder O, Stein J.** The dietary histone deacetylase inhibitor sulforaphane induces human β-defensin-2 in intestinal epithelial cells. *Immunology* 2008; **125**: 241–51

8 **Papo N, Shai Y.** Host defense peptides as new weapons in cancer treatment. *Cell Mol Life Sci* 2005; **62**: 784–90

9 **Markeeva N, Lisovskiy I, Lyzogubov V** *et al.* Expression of β-defensin-2 in human gastric tumors: a pilot study. *Exp Oncol* 2005; **27**: 130–5

10 **Bullard RS, Gibson W, Bose SK** *et al.* Functional analysis of the host defense peptide Human Beta Defensin-1: new insight into its potential role in cancer. *Mol Immunol* 2008; **45**: 839–48

11 **Takeyama K, Mitsuzawa H, Shimizu T** *et al.* Prostate cell lines secrete IL-8 in response to *Mycoplasma hominis* through Toll-like receptor 2-mediated mechanism. *Prostate* 2006; **66**: 386–91

12 **Feng P, Li TL, Guan ZX, Franklin RB, Costello LC.** Effect of zinc on prostatic tumorigenicity in nude mice. *Ann NY Acad Sci* 2003; **1010**: 316–20

13 **Wong PF, AbuBakar S.** LNCaP prostate cancer cells are insentitive to zinc-induced senescence. *J Trace Elem Med Biol* 2008; **22**: 242–7

14 **Reese JH, McNeal JE, Golenberg SL, Redwine EA, Sellers RG.** Distribution of lactoferrin in the normal and inflamed human prostate: an immunohistochemical study. *Prostate* 1992; **20**: 73–85

15 **Wehkamp J, Stange EF.** Paneth cells and the innate immune response. *Curr Opin Gastroenterol* 2006; **22**: 644–50

16 **Sansonetti PJ.** War and peace at mucosal surfaces. *Nat Rev Immunol* 2003; **3**: 710–20

17 **Zasloff M.** Antimicrobial peptides in health and disease. *N Engl J Med* 2002; **347**: 1199–200

18 **Zhang G, Ghosh S.** Toll-like receptor-mediated NF-kappaB activation: a phylogenetically conserved paradigm in innate immunity. *J Clin Invest* 2001; **107**: 13–9

19 **Wang T, Zhang X, Li JJ.** The role of NF-kappaB in the regulation of cell stress responses. *Int Immunopharmacol* 2002; **2**: 1509–20

20 **Domingue SGJ, Hellstrom WJ.** Prostatitis. *Clin Microbiol Rev* 1998; **11**: 604–13

21 **Mori N, Yamada Y, Ikeda S** *et al.* Bay11-7082 inhibits transcription factor NF-κB and induces apoptosis of HTLV-1-infected T-cell lines and primary adult T-cell leukemia cells. *Blood* 2002; **100**: 1828–34

22 **Kumar A, Zhang J, Yu F–S.** Toll-like receptor 2-mediated expression of β-defensin-2 in human corneal epithelial cells. *Microbes Infect* 2006; **8**: 380–9

23 **Gombart AF, O'Kelly J, Saito T, Koeffler HP.** Regulation of the CAMP gene by 1,25(OH)$_2$D$_3$ in various tissues. *J Steroid Biochem Mol Biol* 2007; **103**: 552–7

24 **Matsuzaka K, Sato D, Ishihara K** *et al.* Age-related differences in localization of beta-defensin-2 in human gingival epithelia. *Bull Tokyo Dent Coll* 2006; **47**: 167–70

25 **Ali RS, Falconer A, Ikram M, Bissett CE, Cerio R, Quinn AG.** Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. *J Invest Dermatol* 2001; **117**: 106–11

26 **Wehkamp J, Harder J, Wehkamp K** *et al.* NF-KappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. *Infect Immun* 2004; **72**: 5750–8

27 **Donald CD, Sun CQ, Lim SD** *et al.* Cancer-specific loss of beta-defensin 1 in renal and prostatic carcinomas. *Lab Invest* 2003; **83**: 501–5

28 **Baroni A, Donnarumma G, Paoletti I** *et al.* Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. *Peptides* 2009; **30**: 267–72

29 **Niyonsaba F, Ushio H, Nakano N** *et al.* Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol* 2007; **127**: 594–604

30 **Markeeva N, Lysovskiy I, Zhuravel E** *et al.* Involvement of human beta-defensin-2 in proliferation of transformed cells of human cervix. *Exp Oncol* 2005; **27**: 308–13

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Abbreviations: **HBD–2**, human β-defensin-2; **LPS**, lipopolysaccharide; **NF–κB**, nuclear factor-κB; **EMSA**, electrophoretic mobility shift assay; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase.