Heliyon 6 (2020) e04267

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Botanical preparation HX109 inhibits macrophage-mediated activation of prostate epithelial cells through the CCL4-STAT3 pathway: implication for the mechanism underlying HX109 suppression of prostate hyperplasia

Seonung Lim [a], Hyun-keun Kim [a], Wonwoo Lee [b], Sunyoung Kim [b,*]

[a] School of Biological Sciences, Seoul National University, Seoul, 151-742, Republic of Korea
[b] Helixmith Co. Ltd., 21, Magokjungang 8-ro 7-gil, Gangseo-gu, Seoul, 07794, Republic of Korea

ARTICLE INFO

Keywords:
Cell biology
Cell culture
Botany
Bioactive plant product
Inflammation
Natural product
Biomolecules
HX109
Macrophage
Prostate epithelial cell
CCL4
STAT3
NF-κB

ABSTRACT

Benign prostatic hyperplasia (BPH) is one of the most frequently observed diseases in the elderly male population worldwide. A variety of factors such as aging, hormonal imbalance, chronic inflammation, and oxidative stress play an important role in its pathogenesis. We have previously shown that HX109, an ethanol extract prepared from 3 plants (*Taraxacum officinale*, *Cuscuta australis*, and *Nelumbo nucifera*), alleviates prostate hyperplasia in the BPH rat model and suppresses AR signaling by upregulating Ca^{2+}/CAMKKβ and ATF3. In this study, we used macrophage cell lines to examine the effects of HX109 on inflammation, which is considered an important causative factor in BPH pathogenesis. In the co-culture system involving macrophage-prostate epithelial cells, HX109 inhibited macrophage-induced cell proliferation, migration and epithelial-mesenchymal transition (EMT) by inhibiting the expression of CCL4 and the phosphorylation of STAT3. Furthermore, HX109 inhibited the expression of inflammatory cytokines and the phosphorylation of p65 NF-κB in a concentration dependent manner. Taken together, our results suggested that HX109 could regulate macrophage activation and its crosstalk with prostate cells, thereby inhibiting BPH.

1. Introduction

BPH is the most common chronic diseases in the elderly male population around the world. It is reported that 50% of men over the age of 50 have enlarged prostates, with the incidence increasing with age [1, 2]. BPH is characterized by prostate enlargement and induces lower urinary tract symptoms (LUTS) such as nocturia, dysuria, and bladder obstruction [3, 4].

Despite the worldwide prevalence of BPH, the pathogenesis of the disorder is unclear, although several factors, such as aging, hormonal imbalance, chronic inflammation, and oxidative stress have been postulated [5]. Many recent studies show that prostatic inflammation is an important causative factor in BPH pathogenesis [6, 7, 8, 9]. In BPH patients, infiltrated lymphocytes and macrophages were commonly found in prostate tissue [10]; and the number of inflammatory infiltrates, mostly macrophages and T cells, is higher in aged mouse prostates [11].

Studies using co-cultures between macrophages and prostate cells have elucidated how these two cell types communicate with each other. Co-culture with macrophage increases cell proliferation of the prostate epithelial cells and stromal cells [12, 13]. In addition, macrophages play an important role in BPH development and progression by promoting the migration of prostate epithelial cells and EMT, a highly conserved cellular process [14, 15]. In the crosstalk process between the two cells, a variety of cytokines and chemokines are reported to be involved [16, 17, 18]. Macrophage activation in prostate tissue secretes a large number of different inflammatory cytokines to induce the inflammatory response as well as crosstalk with prostate cells. Since these pro-inflammatory cytokines enhance the proliferation of prostate epithelial cells and stromal cells [19, 20, 21], targeting the activated macrophage of prostate tissue may be a strategy for developing therapeutic agents for BPH.

HX109 is an ethanol extract prepared from three plants: *Taraxacum officinale*, *Cuscuta australis*, and *Nelumbo nucifera*. We previously reported on the development of standardization and quality control methods for HX109 by using HPLC and MS analyses, and the capability of this botanical extract inhibiting BPH in *in vivo* studies, probably by suppressing AR signaling through the upregulation of Ca2+/CAMKKβ and

* Corresponding author.
E-mail address: sk@helixmith.com (S. Kim).

https://doi.org/10.1016/j.heliyon.2020.e04267
Received 22 January 2020; Received in revised form 19 April 2020; Accepted 17 June 2020

Table 1. Sequences of primers used for quantitative real-time polymerase chain reaction (qRT-PCR).

Target gene	Primer sequence (5'->3')	
	Forward	Reverse
hE-cadherin	CGAGAGCTACAC GTTCACGG	GTGTCGAGGGAAAAATAGGCTG
hN-cadherin	GTTTGATGGAGGTCTCCTAACAC	ACGTTTAACACGTTGGAAATGTG
Snail	GAGGCGGTGGCAGACTAGAGT	CGGGCCCCCAGAATAGTTC
hCCL3	AGTTCTCTGCATCACTTGCTG	CGGCTTCGCTTGGTTAGGAA
hCCL4	CTGTGCTGATGATCCCAGTGAATC	TCAGTTCAGTTCCAGGTCATACA
hCCL5	ATCCTCATTGCACTGCCCTC	GCCACTGGTGTAGAAATACTCC
hIL-6	CGGGAACGAAAGAGAAGCTCTA	CGCTTGTGGAGAAGGAGTTCA
hTNFα	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
hGAPDH	CCCCTTCATTGACCTCAACT	ATGACCTTGCCCACAGCCTT
mIL-6	CCTCTGGTCTTCTGGAGTACC	ACTCCTTCTGTGACTCCAGC
mTNFα	ATGAGCACAGAAAGCATGA	AGTAGACAGAAGAGCGTGGT
mGAPDH	CTGGAAAGCTGTGGCGTGAT	CCAGGCGGCACGTCAGATCC

CCL: CC-motif ligand; IL, interleukin; TNF, tumor necrosis factor

ATF expression [22]. AR has been known to play an important role in this cell-to-cell communication [23]. As such, this study, hypothesizes that HX109 might regulate crosstalk between macrophages and prostate epithelial cells. In addition, since *Taraxacum officinale* has been reported to contain anti-inflammatory activities [24], it was hypothesized that HX109 might also inhibit macrophage activation.

Here, we demonstrate that HX109 could inhibit macrophage-induced proliferation, migration, and EMT of prostate cell and macrophage activation. These results suggest that HX109 might be used to inhibit infiltrated macrophage-mediated prostate hypertrophy.

2. Materials & methods

2.1. Cell culture & reagents

RWPE-1 cells (human prostate epithelial cell line) and THP-1 cells (human acute monocytic leukemia cell line) were purchased from ATCC (Manassas, VA). RWPE-1 cells were cultured in Keratinocyte-serum free medium(KSFM) supplemented with bovine pituitary extract (BPE) and EGF in a humidified 5% CO2 atmosphere at 37 °C. THP-1 cells were maintained in RPMI1640 containing 10% heat-inactivated fetal bovine serum, HEPES (10 mM), penicillin and streptomycin in a humidified 5% CO2 atmosphere at 37 °C. THP-1 cells were differentiated into macrophages (THP-1 macrophages) with 60 ng/ml phorbol myristate acetate (PMA) for 48 h. Then the plates were washed with PBS and incubated with normal RPMI1640 with 10% FBS medium for 48 h, and later used for further experiments. Lipopolysaccharides from *Escherichia coli* O111:B4 (Sigma-Aldrich, MA) were used at 100 ng/ml for macrophage activation. HX109 was prepared, and its batch-to-batch consistency was controlled as previously described [22]. Briefly, combination of three plants—*Taraxacum officinale*, *Cuscuta australis*, and *Nelumbo nucifera*—was extracted in 25% EtOH at 20 °C for 8 h, followed by filtration, concentration, and lyophilization.

2.2. Co-culture experiments

Co-culture experiments were performed using 24-well transwell inserts (0.4 μm pore; Corning, NY, USA). RWPE-1 cells (10^4 cells/well) were seeded in 24-well transwell plates and insert wells including THP-1 macrophages (10^4 cells/well) were put into each cell seeded-well and cultured with or without HX109 treatment. Cells were harvested for 48 h and WST-1 assay or RNA isolation were performed. For protein preparation, cells were harvested for 24 h and total proteins were prepared from RWPE-1 cells.

2.3. Cell migration assay

Cell migration assay was performed using 24-well transwell inserts (8 μm pore; Corning, NY, USA) according to the manufacturer's instructions. RWPE-1 cells (10^5 cells/well) were seeded in the upper chamber of

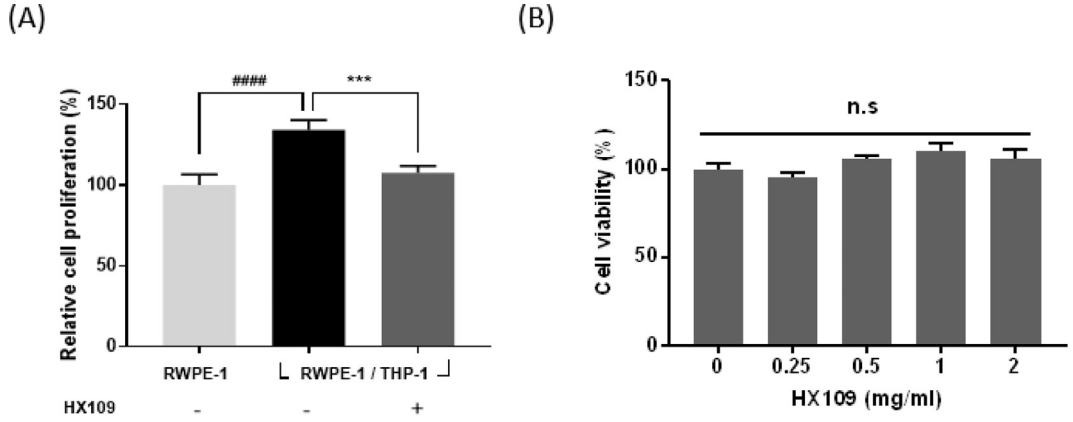

Figure 1. Effects of HX109 on macrophage-induced prostate epithelial cells. (A) Effects on cell proliferation. RWPE-1 cells were plated in culture media, and insert wells including THP-1 macrophages or control media were placed into each cell-seeded well, and incubated with or without 2 mg/ml HX109 for 48 h. Cell proliferation was measured by WST-1 assay. (B) Effects on cell viability. RWPE-1 cells were cultured in the presence of various concentrations of HX109 for 48 h. Cell viability was measured by WST-1 assay. $^{####}p < 0.0001$ (one-way ANOVA) compared with control, $^{***}p < 0.001$ (one-way ANOVA) compared with RWPE-1/THP-1 co-culture. n.s, not significant. Values are normalized to control. All Data are shown as mean ± S.E.M of three independent experiments.

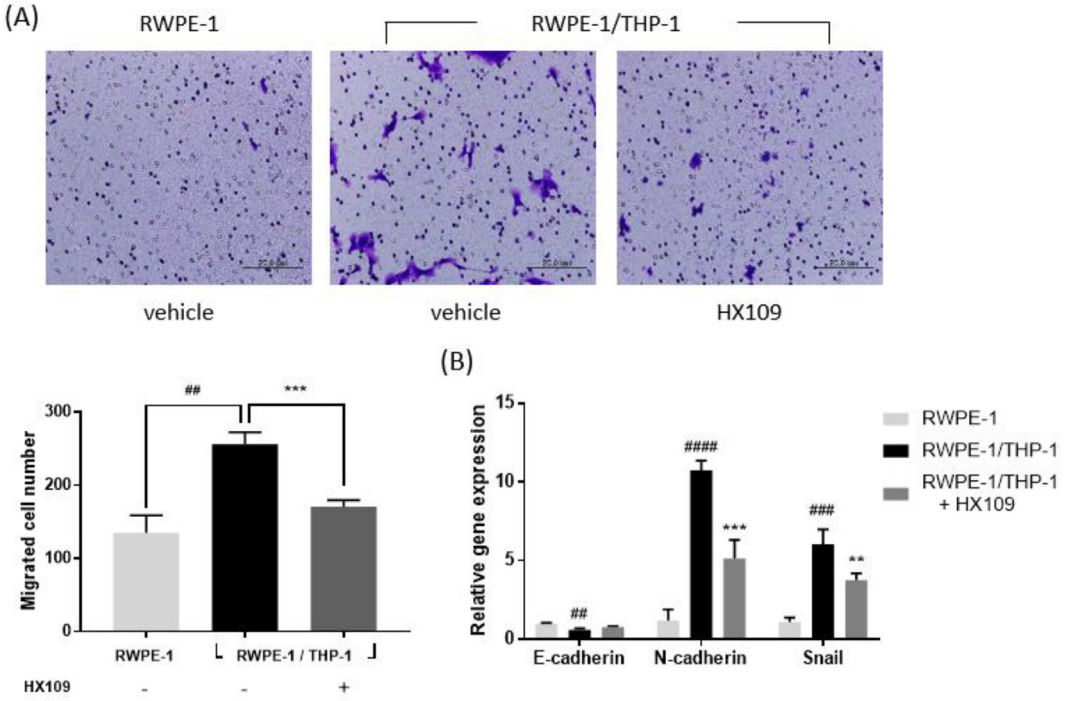

Figure 2. Effects of HX109 on epithelial cell migration and EMT in macrophage-prostate epithelial co-culture. (A) Effects on cell migration. RWPE-1 cells were seeded in the upper chamber of 8 μm transwell plates and THP-1 macrophage or control medium was added to the lower chamber. Cells were incubated with or without 2 mg/ml HX109 for 24 h. The cells migrated through pores were stained with 0.2% crystal violet and counted in 6 random fields. In the graph, the number of migrated cells is expressed as the average number of cells per field. (B) Effect on the RNA levels of EMT-related genes. The RNA levels of RWPE-1 cells 48 h after co-culture with THP-1 macrophages in the presence of 2 mg/ml HX109 were analyzed by qRT-PCR. Values of qRT-PCR were normalized to GAPDH. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ (one-way ANOVA) compared with control, $^{**}p < 0.01$, $^{***}p < 0.001$ (one-way ANOVA) compared with RWPE-1/THP-1 co-culture. All Data are shown as mean ± S.E.M of three independent experiments.

transwell plates and THP-1 macrophages (10^5 cells /well) or control medium was added to the lower chamber. Cells were incubated for 24 h with or without HX109 treatment. The cells migrated to the lower part of the membrane were stained with 0.2% crystal violet and counted in six random fields.

2.4. RNA isolation and qRT-PCR

Total RNA was prepared from RWPE-1cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. One microgram of RNA was converted to cDNA using oligo dT primers (QIAGEN, Hilden, Germany) and Reverse Transcriptase XL (avian myeloblastosis virus [AMV]) (Takara, Kusatsu, Japan). Real-time quantitative RT-PCR was performed with SYBR Premix (Takara, Kusatsu, Japan) and Thermal Cycler Dice Real Time System TP800 (Takara, Kusatsu, Japan). PCR conditions were denaturation at 95 °C for 5 s, followed by annealing and extension at 60 °C for 30 s. The sequences of synthesized PCR primer sets (Bioneer Co.Ltd., Seoul, Korea) are listed in Table 1. Single amplicons were verified for each set of primers.

2.5. Western blot

RWPE-1 cells or THP-1 macrophages were washed with cold PBS lysed with RIPA lysis buffer (Sigma-Aldrich, MO) containing a protease inhibitor (Roche, Basel, Switzerland) and a phosphatase inhibitor (Roche, Basel, Switzerland). Equal amounts of protein were then separated by 10% SDS-polyacrylamide gel and electrophoretically transferred to PVDF membranes (Millipore, MA, USA). The membranes were blocked with 5% BSA (Gibco, MA) in TBST (1 M Tris-HCl [pH 7.4], 0.9% NaCl, and 0.1% Tween 20) for 1 h and incubated with primary antibodies diluted in a 3% BSA blocking solution overnight at 4 °C. Membranes were then treated with HRP-conjugated anti-mouse or anti-rabbit IgG (1: 100,000; Sigma-Aldrich, MO) for 1 h, and protein bands were visualized with an ECL (Millipore, MA, USA) and X-Omat film (Kodak, Rochester, NY).

2.6. Enzyme-linked immunosorbent assay (ELISA)

Human TNFα, IL-6, mouse TNFα, IL-6 (R&D systems, Minneaplis, MN) in cell culture supernatants were measured using commercially available ELISA kits according to the manufacturer's instructions.

3. Results

3.1. HX109 inhibits macrophage induced prostate epithelial cell proliferation

To investigate the effects of HX109 on macrophage induced prostate epithelial cell proliferation, co-culture of macrophages and prostate epithelial cell lines were prepared and treated with 2 mg/ml of HX109. The presence of THP-1 macrophages increased proliferation of prostate epithelial cell line RWPE-1 cells by 34% compared to the control group, which was inhibited when treated with 2 mg/ml HX109 (Figure 1A). To be certain that these inhibitory effects were not due to cytotoxicity, cells were treated with various concentrations of HX109. HX109 did not show cytotoxic effects in any of the concentrations (Figure 1B). These data indicate that HX109 inhibited the macrophage-mediated proliferation of prostate cells.

3.2. HX109 suppresses prostate epithelial cell migration and EMT in macrophage-prostate epithelial cell co-culture

It has been reported that prostate cell migration and EMT markers are increased in the co-culture of macrophages with prostate cells, resulting in the promotion of the development of prostatic hyperplasia [23]. To investigate the effect of HX109 on the macrophage-induced migration of

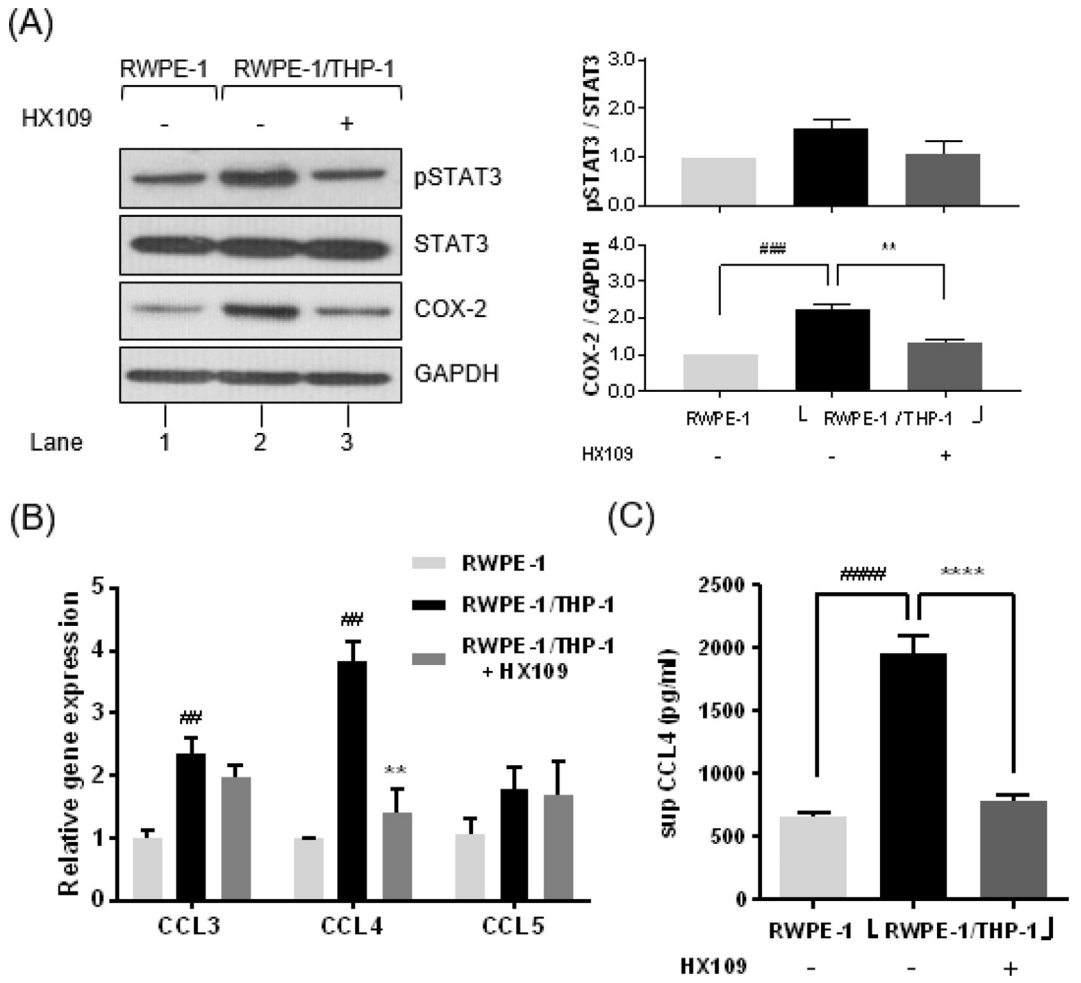

Figure 3. Effects of HX109 in macrophage-epithelial cell co-culture are mediated by the CCL4/STAT3 dependent pathway. RWPE-1 cells were plated in culture media and insert wells including THP-1 macrophage or control media were put into each cell-seeded well, and incubated with or without 2 mg/ml HX109. (A) Effects on STAT3 pathway. Total proteins were prepared after 24 h co-culture and analyzed for the protein levels of pSTAT3, STAT3, COX-2 and GAPDH proteins used as loading control. For Western blot, three independent experiments were performed, and one representative result is shown here. (B) Effects on chemokine expression levels. The RNA levels of RWPE-1 cells 24 h after co-culture with THP-1 macrophages in the presence of 2 mg/ml HX109 were analyzed by qRT-PCR. Values of qRT-PCR were normalized to GAPDH. (C) Effects on protein level of CCL4. The protein level of CCL4 in co-culture supernatant was determined by ELISA, 48 h after co-culture in the presence of 2 mg/ml HX109. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ (one-way ANOVA) compared with control, $^{**}p < 0.01$, $^{****}p < 0.0001$ (one-way ANOVA) compared with RWPE-1/THP-1 co-culture. All Data are shown as mean ± S.E.M of three independent experiments.

prostate cells, THP-1 macrophages and RWPE-1 cells were co-cultured and a migration assay was performed. The migration ability of RWPE-1 cells, when co-cultured with THP-1 macrophages, was increased by 89% compared to the control group, which was highly suppressed by treatment with 2 mg/ml of HX109 (Figure 2A).

Co-culture of RWPE-1 cells with THP-1 macrophages has been known to induce the expression of various EMT-related genes in these epithelial cells [25]. Total RNA was prepared for 48 h of co-culture followed by quantitative RT-PCR. The RNA levels of N-cadherin and snail were increased after co-culture, but were significantly reduced when treated with 2 mg/ml of HX109 (Figure 2B). These results suggest that HX109 could inhibit macrophage-induced prostate epithelial cell migration and EMT gene expression.

3.3. HX109 regulates macrophage-prostate epithelial cell crosstalk by inhibiting the CCL4-STAT3 pathway

It has been shown that constitutively active forms of STAT3 promote EMT and the migration of prostate epithelial cells [26], so we examined the effect of HX109 on the STAT3 signaling pathway. RWPE-1 cells were co-cultured with THP-1 macrophages for 24 h, and total proteins were prepared followed by Western hybridization analysis. As shown in Figure 3A, co-culture of the two cell types increased the level of phosphorylated STAT3 and its downstream gene, COX-2 (Figure 3A). When treated with HX109 2 mg/ml, however, the levels of pSTAT3 and COX2 which had been increased by co-culture was reduced.

It was reported that chemokines such as CCL2, CCL3 and CCL4 are involved in the regulation of STAT3 signaling in co-cultures between macrophages and prostate epithelial cells [25, 27, 28]. To determine which mediators are involved in the HX109-mediated regulation of STAT3 signaling, we measured the RNA levels of these chemokines. Among the three chemokines whose levels were increased in the co-culture, only CCL4's expression was inhibited by HX109 (Figure 3B). These results suggest that HX109 might inhibit STAT3 signaling through the regulation of CCL4 levels during macrophage-prostate epithelial cell crosstalk.

3.4. HX109 suppresses macrophage activation by inhibiting TAK1-IKK-Iκbα–NF–κB pathway

Next, we investigated the effects of HX109 on macrophage activation at the molecular level, using two macrophage cell lines, THP-1

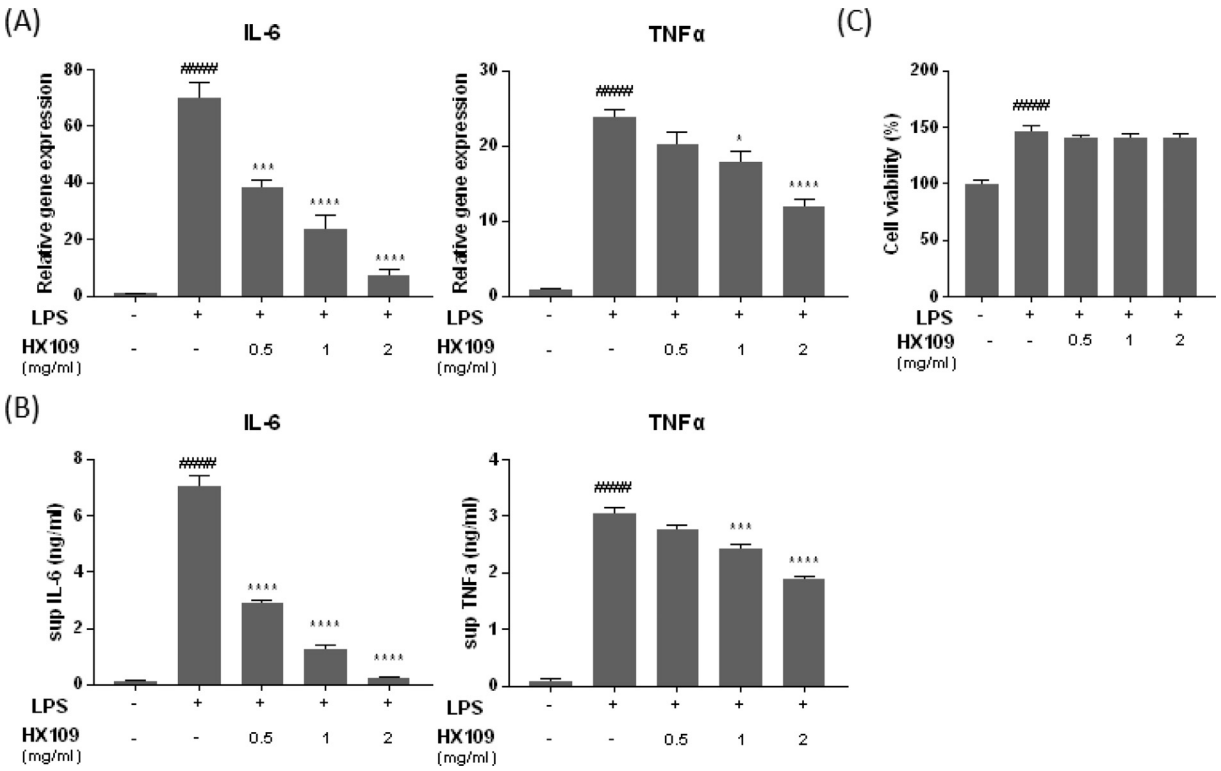

Figure 4. Effects of HX109 on activated macrophages. THP-1 macrophages were treated with or without 100 ng/ml LPS and cultured in the presence of various concentrations of HX109. (A) Effects on the RNA levels of inflammatory cytokines. The RNA levels of THP-1 macrophages after 3 h of LPS and HX109 treatment were analyzed by qRT-PCR. Values of qRT-PCR were normalized to GAPDH. (B) Effects on the protein levels of inflammatory cytokines. The protein levels of inflammatory cytokines were analyzed by ELISA after 24 h. (C) Cytotoxicity effects of HX109. THP-1 macrophages were treated with or without 100 ng/ml LPS, and cultured with various concentrations of HX109 for 24 h. Cell viability was measured by WST-1 assay. $^{\#\#\#\#}p < 0.0001$ (one-way ANOVA) compared with control, $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (one-way ANOVA) compared with LPS only. All Data are shown as mean ± S.E.M of three independent experiments.

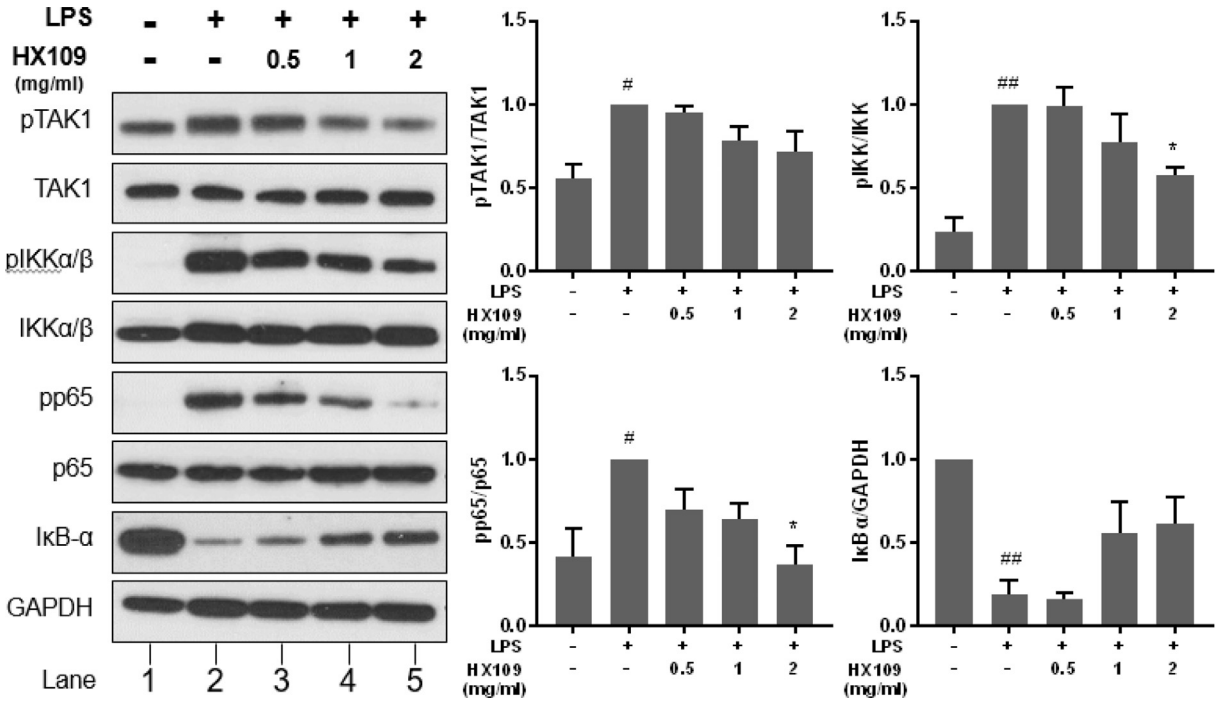

Figure 5. Effect of HX109 on p65 NF-κB signaling pathway. THP-1 macrophages were treated with or without 100 ng/ml LPS and cultured in the presence of various concentrations of HX109. Total proteins of THP-1 macrophage were prepared after 30 min of LPS and HX109 treatment and analyzed by Western blot. GAPDH proteins were used as loading control. Three independent experiments were performed, and one representative result is shown here. The level of each phosphorylated protein was normalized to that of respective total protein, except that GAPDH was used for IκBα. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (one-way ANOVA) compared with control, $^{*}p < 0.05$ (one-way ANOVA) compared with LPS only. All Data are shown as mean ± S.E.M of three independent experiments.

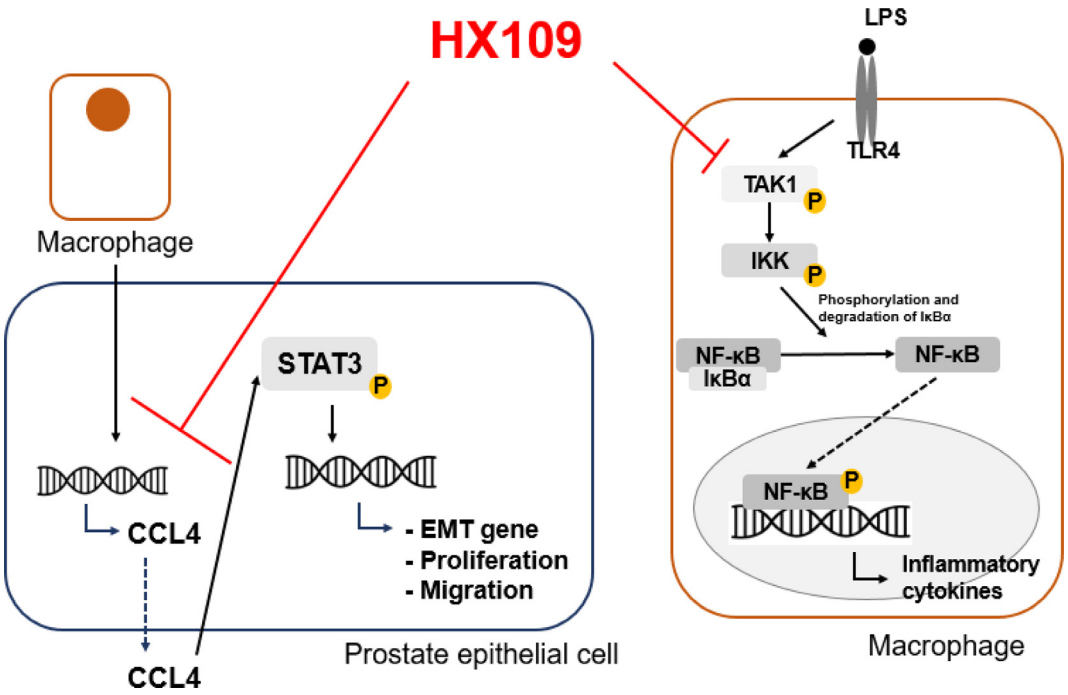

Figure 6. Role of HX109 in prostate epithelial-macrophage crosstalk and inflammatory response of macrophages. HX109 suppresses macrophage-mediated CCL4 expression and STAT3 phosphorylation, thereby inhibiting macrophage-mediated EMT, proliferation, and migration. HX109 also downregulates the expression of inflammatory cytokines in activated macrophages by controlling TAK1-IKK–NF–κB signaling.

macrophages and Raw264.7. In LPS-stimulated THP-1 macrophages, both RNA and protein levels of IL-6 and TNFα increased radically (Figures 4A and 4B), but treatment with HX109 lowered the levels of both inflammatory cytokines in a concentration-dependent manner. Similar results were also obtained when Raw264.7 cells were used (Supplement Figure 1).

Regulation of NF-κB phosphorylation via Toll-Like-Receptor 4 (TLR4) is a key feature in the LPS-induced inflammatory response in macrophages [29]. To test whether HX109 controls NF-κB, THP-1 macrophages were treated with LPS and HX109 for 30 min, and the levels of NF-κB signaling proteins were measured by Western blot hybridization. LPS stimulation increased the levels of phosphorylated TGF-β activated kinase 1 (TAK1), IκB kinase (IKK) and p65, but co-treatment with HX109 lowered of these proteins in a concentration dependent manner (Figure 5). On the other hand, IκBα levels, which had dropped after LPS treatment, were recovered after HX109 treatment in a concentration-dependent manner (Figure 5) These results showed that HX109 could suppress macrophage activation and the induction of inflammatory cytokines by inhibiting the TAK1-IKK-IκBα–NF–κB pathway. The role of HX109 in prostate epithelial-macrophage crosstalk and inflammatory response of macrophage is shown in schematic diagram (Figure 6).

4. Discussion

In this study, we show that the botanical extract HX109 regulates crosstalk between macrophages and prostate cells and macrophage-mediated inflammation. In co-culture experiments, HX109 controlled macrophage-induced proliferation and the migration of prostate epithelial cells, and also inhibited EMT. Treatment of THP-1 macrophage and RWPE-1 cultures with HX109 reduced the level of pSTAT3 and COX2 while lowering the expression level of CCL4 specifically, indicating that CCL4-STAT3 signaling might be the major target of HX109. In addition, HX109 suppressed the expression of inflammatory cytokines in activated macrophages by controlling NF-κB signaling.

It is well established that inflammation plays important roles during the pathogenesis of prostatic hyperplasia [30, 31, 32]. For example, it promotes the infiltration of immune cells like macrophage, and induces the expression of a variety of cytokines and chemokines that influence the proliferation of prostate cells [17, 20, 33]. Our data reveals that HX109 could exert multiple effects to regulate the pathogenies of BPH. Firstly, HX109 could inhibit the effects of macrophages on prostate epithelial cells at the cellular as well as gene expression levels. It appears that in epithelial cells, CCL4 and STAT3 signaling are the main target of HX109. Secondly, HX1019 seems to directly target macrophages to suppress their inflammatory effects as demonstrated by the effective reduction of the protein levels of phosphorylated TAK1, IKK, and p65 NF-κB and increase in that of IκBα. In summary, HX109 simultaneously targets two key cell types involved in BPH pathogenesis, indicating that it might be an ideal starting point for developing safe and effective therapeutic agents.

In BPH pathogenesis, crosstalk between prostate and immune cells, particularly macrophages, has been reported to be important. In this process, AR is shown to play a key role by controlling the expression of several chemokines that mediate crosstalk and increase prostate hypertrophy [12]. Indeed, knock-out of AR resulted in decrease of macrophage infiltration [28]. Therefore, the regulation of the CCL4-STAT3 pathway by HX109 may have resulted from the inhibition of AR signaling by HX109. The understanding of how HX109 does this would be important in developing agents that control crosstalk between the two major cell types involved in BPH pathogenesis.

The *in vitro* co-culture systems used in this study mimic crosstalk between infiltrated macrophage and prostate epithelial cell. In this context, it is worth noting that the most commonly used prostatic hypertrophy models are induced by excessive androgen, which do not cause inflammatory responses or the infiltration of immune cells in the prostate [34]. To study crosstalk in vivo, it would be necessary to use a mouse model showing macrophage infiltration to the prostate, such as the recently developed prolactin-induced BPH model [35].

We have yet to identify the active compounds responsible for the effects of HX109. Indeed, the major challenge associated with developing

botanical therapeutics as medicines is that they contain multiple compounds, making it difficult to pinpoint the biologically responsible compound(s). There are two ways to overcome this obstacle. One is to identify all the compounds present in an extract and match respective chemicals to certain biological activities. Although no one has shown convincing results with this approach, the recent development of massive, high throughput analytical devices, together with advances in informatics technology may make this approach more viable than before. The other approach, which we have taken, was to accept the complex nature of a "mixture" as it is, and use cell-based bioassays to ensure the consistency of the extract. in the context of bioactivities. In our previous report, we described the development of cell-based bioassays for HX109, using human PSA as a biomarker whenever different batches of the extract were prepared [22].

Our data indicate that HX109, previously shown to have significant therapeutic effects in the rat prostate hyperplasia model, controls multiple targets. Examples include: modulating the crosstalk between macrophage and prostate cells; inhibiting increased levels of proliferation, migration and EMT gene expression through the downregulation of the CCL4-STAT3 pathway in epithelial cells; and controlling the production of inflammatory cytokines in macrophages by suppressing NF-κB signaling. Given the high unmet medical needs in BPH treatment, further molecular and clinical studies of HX109 are warranted to unravel the detailed mechanisms and determine the safety and efficacy for patients with BPH.

Declarations

Author contribution statement

S. Lim: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

H. Kim and S. Kim: Analyzed and interpreted the data; Wrote the paper. W. Lee: Performed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by the Basic Science Research Programs through the National Research Foundation of Korea (NRF) (No. 2014R1A1A2055890) funded by the Ministry of Education and Viromed Co. Ltd.

Competing interest statement

The authors declare the following conflict of interests: W. Lee, and S. Kim are the employees of Helixmith Co. Ltd. S.Kim owns stocks of this company.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04267.

References

[1] K.T. McVary, BPH: epidemiology and comorbidities, Am. J. Manag. Care 12 (2006) S122–128.
[2] J.K. Parsons, J. Bergstrom, J. Silberstein, E. Barrett-Connor, Prevalence and characteristics of lower urinary tract symptoms in men aged > or = 80 years, Urology 72 (2008) 318–321.
[3] M.J. Barry, F.J. Fowler Jr., M.P. O'Leary, R.C. Bruskewitz, H.L. Holtgrewe, W.K. Mebust, Measuring disease-specific health status in men with benign prostatic hyperplasia. Measurement Committee of the American Urological Association, Med Care 33 (1995) AS145–155.
[4] A.V. Sarma, J.T. Wei, Clinical practice. Benign prostatic hyperplasia and lower urinary tract symptoms, N. Engl. J. Med. 367 (2012) 248–257.
[5] C.G. Roehrborn, Pathology of benign prostatic hyperplasia, Int. J. Impot. Res. 20 (Suppl 3) (2008) S11–18.
[6] J.C. Nickel, C.G. Roehrborn, R. Castro-Santamaria, S.J. Freedland, D.M. Moreira, Chronic prostate inflammation is associated with severity and progression of benign prostatic hyperplasia, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.
[7] G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Gessl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign hyperplastic prostate tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2002) 43–58.
[8] G. Kramer, D. Mitteregger, M. Marberger, Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? Eur. Urol. 51 (2007) 1202–1216.
[9] J.C. Nickel, Inflammation and benign prostatic hyperplasia, Urol. Clin. 35 (2008) 109–115, vii.
[10] G. Robert, A. Descazeaud, N. Nicolaiew, S. Terry, N. Sirab, F. Vacherot, P. Maille, Y. Allory, A. de la Taille, Inflammation in benign prostatic hyperplasia: a 282 patients' immunohistochemical analysis, Prostate 69 (2009) 1774–1780.
[11] D. Bianchi-Frias, F. Vakar-Lopez, I.M. Coleman, S.R. Plymate, M.J. Reed, P.S. Nelson, The effects of aging on the molecular and cellular composition of the prostate microenvironment, PloS One 5 (2010).
[12] D. Xu, X. Wang, C. Jiang, Y. Ruan, S. Xia, X. Wang, The androgen receptor plays different roles in macrophage-induced proliferation in prostate stromal cells between transitional and peripheral zones of benign prostatic hypertrophy, EXCLI J 16 (2017) 939–948.
[13] T.H. Lin, K. Izumi, S.O. Lee, W.J. Lin, S. Yeh, C. Chang, Anti-androgen receptor ASC-J9 versus anti-androgens MDV3100 (Enzalutamide) or Casodex (Bicalutamide) leads to opposite effects on prostate cancer metastasis via differential modulation of macrophage infiltration and STAT3-CCL2 signaling, Cell Death Dis. 4 (2013) e764.
[14] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.
[15] P. Alonso-Magdalena, C. Brossner, A. Reiner, G. Cheng, N. Sugiyama, M. Warner, J.A. Gustafsson, A role for epithelial-mesenchymal transition in the etiology of benign prostatic hyperplasia, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 2859–2863.
[16] K.P. Lai, S. Yamashita, C.K. Huang, S. Yeh, C. Chang, Loss of stromal androgen receptor leads to suppressed prostate tumourigenesis via modulation of pro-inflammatory cytokines/chemokines, EMBO Mol. Med. 4 (2012) 791–807.
[17] D.G. Alleva, C.J. Burger, K.D. Elgert, Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production. Role of tumor-derived IL-10, TGF-beta, and prostaglandin E2, J. Immunol. 153 (1994) 1674–1686.
[18] P.E. Gleason, J.A. Jones, J.S. Regan, D.B. Salvas, J.N. Eble, W.W. Lamph, C.J. Vlahos, W.L. Huang, J.F. Falcone, K.S. Hirsch, Platelet derived growth factor (PDGF), androgens and inflammation: possible etiologic factors in the development of prostatic hyperplasia, J. Urol. 149 (1993) 1586–1592.
[19] D. Giri, M. Ittmann, Interleukin-8 is a paracrine inducer of fibroblast growth factor 2, a stromal and epithelial growth factor in benign prostatic hyperplasia, Am. J. Pathol. 159 (2001) 139–147.
[20] G.E. Steiner, M.E. Newman, D. Paikl, U. Stix, N. Memaran-Dagda, C. Lee, M.J. Marberger, Expression and function of pro-inflammatory interleukin IL-17 and IL-17 receptor in normal, benign hyperplastic, and malignant prostate, Prostate 56 (2003) 171–182.
[21] L.A. Begley, S. Kasina, J. MacDonald, J.A. Macoska, The inflammatory microenvironment of the aging prostate facilitates cellular proliferation and hypertrophy, Cytokine 43 (2008) 194–199.
[22] S. Lim, W. Lee, D.S. Lee, I.J. Nam, N. Yun, Y. Jeong, T. Rho, S. Kim, Botanical formulation HX109 ameliorates TP-induced benign prostate hyperplasia in rat model and inhibits androgen receptor signaling by upregulating Ca(2+)/CaMKKbeta and ATF3 in LNCaP cells, Nutrients 10 (2018).
[23] T. Lu, W.J. Lin, K. Izumi, X. Wang, D. Xu, L.Y. Fang, L. Li, Q. Jiang, J. Jin, C. Chang, Targeting androgen receptor to suppress macrophage-induced EMT and benign prostatic hyperplasia (BPH) development, Mol. Endocrinol. 26 (2012) 1707–1715.
[24] H.J. Jeon, H.J. Kang, H.J. Jung, Y.S. Kang, C.J. Lim, Y.M. Kim, E.H. Park, Anti-inflammatory activity of Taraxacum officinale, J. Ethnopharmacol. 115 (2008) 82–88.
[25] L.Y. Fang, K. Izumi, K.P. Lai, L. Liang, L. Li, H. Miyamoto, W.J. Lin, C. Chang, Infiltrating macrophages promote prostate tumorigenesis via modulating androgen receptor-mediated CCL4-STAT3 signaling, Cancer Res 73 (2013) 5633–5646.
[26] J. Azare, K. Leslie, H. Al-Ahmadie, W. Gerald, P.H. Weinreb, S.M. Violette, J. Bromberg, Constitutively activated Stat3 induces tumorigenesis and enhances cell motility of prostate epithelial cells through integrin beta 6, Mol. Cell Biol. 27 (2007) 4444–4453.
[27] X. Wang, W.J. Lin, K. Izumi, Q. Jiang, K.P. Lai, D. Xu, L.Y. Fang, T. Lu, L. Li, S. Xia, C. Chang, Increased infiltrated macrophages in benign prostatic hyperplasia (BPH): role of stromal androgen receptor in macrophage-induced prostate stromal cell proliferation, J. Biol. Chem. 287 (2012) 18376–18385.
[28] K. Izumi, L.Y. Fang, A. Mizokami, M. Namiki, L. Li, W.J. Lin, C. Chang, Targeting the androgen receptor with siRNA promotes prostate cancer metastasis through enhanced macrophage recruitment via CCL2/CCR2-induced STAT3 activation, EMBO Mol. Med. 5 (2013) 1383–1401.
[29] J.C. Chow, D.W. Young, D.T. Golenbock, W.J. Christ, F. Gusovsky, Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction, J. Biol. Chem. 274 (1999) 10689–10692.
[30] A. Sciarra, F. Di Silverio, S. Salciccia, A.M. Autran Gomez, A. Gentilucci, V. Gentile, Inflammation and chronic prostatic diseases: evidence for a link? Eur. Urol. 52 (2007) 964–972.

[31] G. Penna, B. Fibbi, S. Amuchastegui, C. Cossetti, F. Aquilano, G. Laverny, M. Gacci, C. Crescioli, M. Maggi, L. Adorini, Human benign prostatic hyperplasia stromal cells as inducers and targets of chronic immuno-mediated inflammation, J. Immunol. 182 (2009) 4056–4064.

[32] J.E. Elkahwaji, R.J. Hauke, C.M. Brawner, Chronic bacterial inflammation induces prostatic intraepithelial neoplasia in mouse prostate, Br. J. Canc. 101 (2009) 1740–1748.

[33] G.E. Steiner, U. Stix, A. Handisurya, M. Willheim, A. Haitel, F. Reithmayr, D. Paikl, R.C. Ecker, K. Hrachowitz, G. Kramer, C. Lee, M. Marberger, Cytokine expression pattern in benign prostatic hyperplasia infiltrating T cells and impact of lymphocytic infiltration on cytokine mRNA profile in prostatic tissue, Lab. Invest. 83 (2003) 1131–1146.

[34] J. Li, Y. Tian, S. Guo, H. Gu, Q. Yuan, X. Xie, Testosterone-induced benign prostatic hyperplasia rat and dog as facile models to assess drugs targeting lower urinary tract symptoms, PloS One 13 (2018), e0191469.

[35] K.P. Lai, C.K. Huang, L.Y. Fang, K. Izumi, C.W. Lo, R. Wood, J. Kindblom, S. Yeh, C. Chang, Targeting stromal androgen receptor suppresses prolactin-driven benign prostatic hyperplasia (BPH), Mol. Endocrinol. 27 (2013) 1617–1631.