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

aSchool of Biological Sciences, Seoul National University, Seoul, 151-742, Republic of Korea
bHelixmith Co. Ltd., 21, Magokjungang 8-ro 7-gil, Gangseo-gu, Seoul, 07794, Republic of Korea

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 co-culture system 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 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 vivo studies, probably by suppressing AR signaling through the upregulation of Ca2+/CAMKKβ and
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 of *fi*cinale 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 (KSF) 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 the upper chamber of the 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
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-1 cells 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, Minneapolis, 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
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 dependent 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 < 0.001 (one-way ANOVA) compared with control, ***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. 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.
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 therapeic 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 hyper trophy [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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.

G. Kramer, G.E. Steiner, A. Handisurya, U. Stix, A. Haitel, B. Knerer, A. Geisl, C. Lee, M. Marberger, Increased expression of lymphocyte-derived cytokines in benign prostatic hyperplasia tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation, Prostate 52 (2003) 1715–1719.

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, a stromal and epithelial growth factor in benign prostatic hypertrophy, lower urinary tract symptoms and risk of acute urinary retention, J. Urol. 196 (2016) 1493–1498.
[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. 81 (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. Izami, 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.