

## CHAPTER 1

# Etiology and Pathogenesis

Robert H. Getzenberg<sup>1</sup> & Prakash Kulkarni<sup>2</sup>

<sup>1</sup>GTx Inc., Memphis, TN, USA

<sup>2</sup>James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

### Key points

- Lower urinary tract symptoms (LUTS) corresponding to benign prostatic hyperplasia (BPH) are a complex disease that may represent distinct etiologies.
- By deciphering the molecular underpinnings, we can begin to delineate the distinct causes and identify different readouts, and therefore formulate and individualize therapies.
- BPH/LUTS involves the cellular components of the prostate including the epithelial and stromal cells.
- A number of steroid hormones including androgens, estrogens, and progesterone, along with various growth factors and chemokines have been demonstrated to contribute to the abnormal regulation of prostatic growth.
- Although inflammation has been demonstrated to be associated with BPH/LUTS, anti-inflammatory treatment approaches have, in general, not been shown to be effective.
- Cancer/testis antigens have been shown to be associated with BPH with more severe symptoms and therefore may serve as novel biomarkers thereof.

### Introduction

Diseases of the prostate are some of the most common and devastating diseases in men, especially as they age. Indeed, the prevalence of BPH is estimated to begin its increase in the third decade of life from 5–10% to greater than 90% for men above 85 years of age [1]. One in four males will undergo surgery at some time in their life to relieve symptoms of BPH, which compresses the urethra and produces urinary-outflow obstruction. Although the use of pharmacologic agents has increased in the treatment of this disease, transurethral

resection of the prostate (TURP) is still a leading surgical procedure in the United States, second only to cataract extraction, with an annual cost to the health-care system in excess of \$5 billion [2].

Although we currently have a great deal of knowledge regarding the prostate, there are still many questions that need to be answered. Several of these questions, relating to clinically relevant prostatic diseases, such as prostatitis, BPH, and so on, involve normal prostate growth, differentiation, and aging, or aberrations in these processes, or both. Indeed, the earliest manifestation of BPH is the appearance of

---

*Male Lower Urinary Tract Symptoms and Benign Prostatic Hyperplasia*, First Edition.

Edited by Steven A. Kaplan and Kevin T. McVary.

© 2014 John Wiley & Sons, Ltd. Published 2014 by John Wiley & Sons, Ltd.

the mesenchyme in periurethral nodules, which has a similar morphology to the prostatic mesenchyme during embryogenesis [3]. In later stages of BPH development, glandular budding and branching toward a central focus lead to further nodule growth [3]. Such morphological evidence suggests that BPH is intrinsically a mesenchymal disease that results from a reawakening of embryonic inductive interactions between the prostatic stroma and epithelium [3]. Therefore, it is critical to understand the elements of prostatic regulation that play a role in the normal growth and differentiation of the prostate that can then be applied to the diseased gland.

### What is BPH/LUTS? The biology

In this chapter, we will focus on the disease known historically as BPH but perhaps more appropriately termed LUTS. BPH or LUTS is one of the most common diseases occurring in aging men in the United States. Pathologically diagnosed BPH is characterized by the non-malignant proliferation of the epithelial and stromal components of the prostate. Such histological BPH may or may not be associated with clinical BPH, which is characterized by the progressive development of LUTS. LUTS primarily results from constriction of the urethra and resulting resistance to urinary flow, and may take the form of urgency, frequency, nocturia, and a weak urine stream with incomplete emptying. If left untreated, LUTS can result in acute urinary retention, urinary incontinence, recurrent urinary-tract infections, and or obstructed uropathy [4]. Interestingly, some men with significantly enlarged prostates do not present with LUTS, while some men with normally sized prostates experience severe LUTS.

BPH is a chronic condition that increases in its prevalence and severity with age. The presence of histological BPH in men is estimated to be 8%, 50%, 70%, and 90% in their fourth, sixth, seventh, and eighth (and older) decades of life, respectively. The presence of moderate to severe LUTS (i.e. clinical BPH) is estimated to be 26%, 33%, 41%, and nearly 50% for the same respective age groups [5]. The extremely high prevalence of BPH and its associated symptoms can lead to a severe impact on quality of life, making it one of the nation's major health expenditures. In 2006, the management of BPH/LUTS was estimated to cost \$4 billion/year in the United States alone [6]. Inclusion of prescription and non-prescription medication costs, and indirect costs associated with morbidity (e.g. work limitations), increases this estimate significantly.

Medical treatment for clinical BPH has evolved over the last decade with a growing focus on pharmacological management of LUTS over more invasive therapies. A steady decline in surgical treatments for clinical BPH has been reported since the 1990s and is concomitant with an increase in nonsurgical interventions designed to manage symptoms [7,8]. This is likely due, at least in part, to the increased use of two largely effective drug categories in the treatment of LUTS, 5 $\alpha$ -reductase inhibitors, which in effect shrink the prostate by inducing prostatic epithelial apoptosis and atrophy, and  $\alpha_1$ -adrenergic receptor antagonists, which reduce prostatic urethral smooth muscle tone [9]. A number of short-duration clinical trials have compared the relative efficacy of these drug modalities individually and in combination. In these trials, 5 $\alpha$ -reductase inhibitors and  $\alpha_1$ -adrenergic receptor antagonists proved effective in treating clinical BPH symptoms but in combination showed no increased effect in alleviating symptoms or improving flow rate [7].

A relatively recent trial was performed to fully determine the efficacy of these approaches. To further investigate the effectiveness of individual and combination drug therapy for the medical management of clinical BPH, the National Institute of Diabetes and Digestive and Kidney Diseases conducted a long-term, randomized trial known as the Medical Therapy of Prostatic Symptoms (MTOPS) study. The MTOPS trial investigated whether finasteride, a 5 $\alpha$ -reductase inhibitor, and doxazosin, an  $\alpha_1$ -adrenergic receptor blocker, alone or in combination would specifically delay or prevent clinical progression of BPH. The results demonstrate that dual-drug therapy significantly reduced the risk of overall BPH clinical progression more than either drug monotherapy alone or placebo with a mean follow-up of 4.5 years [8]. Importantly as a component of the study protocol, serum samples were collected from MTOPS patients prior to randomization and at yearly intervals during the trial as well as at the end of the study. Prostate biopsy samples were also collected at baseline at year 1 and at the end of the study from a patient subgroup. These bio-samples were collected and banked in anticipation of analyses of potential molecular changes associated with patient responses to the MTOPS clinical protocol.

A number of theories have been proposed to explain the biology of the prostatic changes associated with BPH/LUTS. These include embryonic awakening, as described above [3], hormonal changes, and inflammation. Although there are significant data to support each of these that are summarized in this chapter, today, we still do not understand the full etiology of the prostatic changes and their associated symptoms. In all likelihood, it appears to be a combination of these changes that contribute to BPH/LUTS.

## Regulation of the normal prostate

The human prostate is a walnut-sized gland, located at the base of the bladder and surrounding the urethra. The prostatic epithelial cells contribute secretions that empty through ducts into the urethra to form a major component of seminal plasma. There are 15–30 excretory ducts from the prostate that enter the urethra as it passes through the prostate, and each of these is surrounded by four to six prostatic lobules that contain acini lined by tall columnar epithelial cells. The endocrine system has been extensively documented to affect the prostate via testosterone, which is the major serum androgen that stimulates prostatic growth. During development, androgens and the androgen receptor regulate several key events that include development and differentiation of major target tissues such as the prostate, seminal vesicles, and epididymis [10]. Furthermore, it is generally held that androgens are not only required for normal function of the prostate gland but also implicated in prostate disease. Thus, identifying novel target genes, particularly those that are androgen regulated, may help to better understand the molecular basis of prostate physiology during health and disease.

## Androgen regulation of the prostate

The prostate is composed principally of stromal and epithelial cells that are in close proximity to one another. BPH is a disease that is thought to involve stromally induced hyperplastic changes in the epithelium [1] and clearly demonstrates the interrelationships between stromal and epithelial cells.

Interactions between the stroma and the epithelium have been shown by a number of investigators to be critical in the regulation of prostatic growth and differentiation, and many of these stromal–epithelial interactions have been shown to work through soluble and structural signaling systems [11]. BPH has been compared with the fetal prostate, at which time the gland is also highly proliferative, and upon histological examination of BPH sections of the prostate gland, the morphology is similar to the fetal prostate. In the developing prostate, the effects of androgens have been demonstrated to be primarily on the underlying stromal cells, which, in the developing prostate, are the only cell type found to contain androgen receptors [12,13]. As the prostate matures, androgen receptors are found in both epithelial and stromal cells, suggesting that androgen action at this time may occur directly in both cell types [14]. However, 5 $\alpha$ -reductase, the enzyme that is responsible for the conversion of testosterone to dihydrotestosterone (DHT), is localized only in the stromal cells, again demonstrating the importance of stromal cells in the hormonal regulation of prostatic growth. Androgen-receptor complexes affect prostatic function by interacting with androgen-response elements, specific DNA sequences located in the regulatory regions of a number of androgen-responsive genes. In addition, DHT has been demonstrated also to influence the expression of other prostatic growth factors.

### Estrogens, progesterone, prostatic regulation, and BPH

While estrogens have been shown to diminish prostatic growth therapeutically, the classical thinking is that this is believed to be an

indirect effect, mediated by blocking pituitary function and decreasing LH, which subsequently inhibits testicular testosterone production [15]. More recent studies have demonstrated more direct effects of estrogens on prostatic regulation and diseases. The impact of estrogens on the prostate and on BPH has recently been reviewed [16]. In classic studies, administration of androgens and estrogens to male beagles resulted in more highly symptomatic BPH in these animals as they aged [17]. In humans, while testosterone levels have been shown to decrease with age, estrogens do not follow this pattern. Therefore, correlations between serum estrogen levels and prostatic volume have been observed [18]. While estrogens have been shown to be direct contributors to the regulation of both stromal and epithelial cells within the prostate, the specific impact on the development of the prostatic changes associated with BPH is less clear. The principal estrogen receptors, ER $\alpha$  and ER $\beta$ , have both been shown to have roles in the differential regulation of the prostate, which is even more complex based upon the contextual and temporal nature of these interactions [16].

### Growth factors and chemokines in BPH/LUTS

Over the past three decades, several lines of evidence have emerged that strongly suggest that prostatic growth is under the immediate control of specific autocrine and paracrine growth factors and their receptors, and is indirectly modulated by steroids. Thus, the complex milieu includes members of the fibroblast (FGF), insulin and insulin-like growth factor (IGF), transforming growth factor (TGF) families, and several other growth-regulatory proteins. Several studies have observed that

these proteins and their downstream effector molecules are overexpressed in BPH and create a landscape of increased stromal and epithelial growth, and mesenchymal transdifferentiation that leads to disease progression [19]. Interestingly, in contrast to the prevailing notion that BPH is due to a pathological proliferation of prostatic fibroblasts/myofibroblasts and epithelial cells, Gustafsson and coworkers [20] have suggested that BPH is due to an epithelial-to-mesenchymal transition (EMT) that results in the accumulation of mesenchymal-like cells derived from the prostatic epithelium and endothelium. Since TGF- $\beta$  is thought to play a key role in EMT, the authors suggest that TGF- $\beta$ /Smad should be considered as targets for treatment of BPH [20].

In addition to growth factors, a variety of chemokines are actively secreted by the prostatic microenvironment. The primary driving forces behind the chemokine secretion appears to be the accumulation of senescent stromal fibroblasts, and possibly epithelial cells in the aging and enlarged prostate. Furthermore, chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and histological inflammation could also serve as rich sources of chemokine secretion in the prostate. By binding to their cognate receptors, chemokines can stimulate powerful proproliferation signal transduction pathways and thus function as potent growth factors in the development and progression of BPH/LUTS [21]. A few reports in the literature also suggest that chemokine-mediated angiogenesis may be a contributing factor to BPH/LUTS development and progression. Thus, low-level secretion of multiple chemokines within the aging prostatic microenvironment may promote a concomitant and cumulative overproliferation of both stromal fibroblastic and epithelial cell types associated with increased prostatic volume. Though the accumulated

evidence is rudimentary and fragmented, it argues favorably for the conclusion that chemokines can, and most likely do, promote BPH/LUTS, and justifies further investigations examining chemokines as potential therapeutic targets to delay or ablate disease initiation and progression [21].

Diabetes is another significant risk factor for BPH/LUTS due to the resulting hyperinsulinemia. Hyperinsulinemia stimulates the liver to produce more IGF, another mitogen and an anti-apoptotic agent that binds insulin receptor/IGF receptor and stimulates prostate growth. The levels of IGFs and IGF-binding proteins in prostate tissue and in blood are associated with BPH risk, with the regulation of circulating androgen and growth hormone [22].

Two other growth-regulatory molecules that have been implicated in prostatic growth and enlargement are sonic hedgehog and Cyr61. Hedgehog (Hh) signaling has long been recognized for its role in axial patterning, mesenchymal-epithelial inductive signaling, and growth regulation during fetal development. In many embryonic tissues, Hh functions as a proliferative stimulus. Robust Hh signaling is commonly found in the adult human prostate, and sonic hedgehog and Indian hedgehog are both expressed by the urothelium of the fetal prostate anlage where they regulate cell proliferation and differentiation, and play a role in prostate ductal budding [23]. Cyr61, a member of the CNN family of secreted regulatory proteins that is upregulated in BPH, has also been shown to be induced by lysophosphatidic acid and act as a secreted autocrine and/or paracrine mediator in stromal and epithelial hyperplasia [24]. As we begin to unravel the precise mechanisms involved, new treatments for BPH aimed at these interacting pathways involving various growth factors may emerge. Therefore, targeting growth factors potentially represents an

attractive therapeutic approach to the regulation of abnormal enlargement of the prostate and the amelioration of other symptoms associated with BPH.

### Inflammatory changes associated with BPH

Among the biomarkers that have been evaluated, those associated with inflammation appear to have taken center stage [1]. It seems that a form of inflammation may be activated in more highly symptomatic BPH [25]. As discussed above, these chemokines that are released by the prostatic environment in response to inflammation have been shown to be associated with increased prostatic cellular growth and have been proposed perhaps to play a role in the enlargement of the prostate as well as LUTS [21].

Inflammatory infiltrates were identified in more than 80% of men with complicated and/or symptomatic BPH, and both International Prostate Symptom Score and prostatic volume were higher in men with these inflammatory cells [26]. The prognostic significance of utilizing inflammation and tissue necrosis scores has been investigated, and while they seem promising, they require further study [27].

In the prostate cancer prevention trial, while there were modest associations between the use of nonsteroidal anti-inflammatory agents (NSAIDs) and the risk of BPH, NSAID use was not directly correlated with it [28]. Similar findings resulted from the Prostate, Lung, Colorectal, and Ovarian Screening Trial [29]. Several studies have now evaluated the utilization of anti-inflammatories as modulators of the symptoms associated with BPH.

Macrophages have been proposed to be a target for some of the inflammation that has been associated with BPH development. CD68(+)

macrophages have been found in both the stromal and epithelial compartments in men with BPH/LUTS [30]. Monocyte chemoattractant protein-1 (MCP-1/CCL2) has been associated with BPH as well, suggesting at least one mechanism through which macrophages are attracted to the prostate [31].

The stress response involves not only the epithelial components of the prostate but also the stromal elements. In fact, it may be the stromal components that are the key regulators of the prostatic changes associated with BPH, regardless of whether the disease presents as more epithelial or stromal predominant. The stress response may be either a driver or a passenger in the process that results in the prostatic changes corresponding to the observed symptoms, but regardless, it seems to be an important contributor.

### Prostate-associated Gene 4 as a stress modulator within the prostate

Since most BPH is diagnosed not pathologically but as part of a spectrum of symptoms, there is an obvious need for a relatively noninvasive tool that can aid in the personalization of BPH treatment. In an effort to characterize molecular changes associated with symptomatic BPH, we performed an analysis of patterns of gene expression associated with highly symptomatic disease as defined by their American Urological Association symptom score [32]. From this analysis, we identified a series of proteins encoded by the differentially expressed genes that were associated with severe symptoms. Among these proteins was a Cancer/Testis Antigen, Prostate-associated Gene 4 (PAGE4), alternatively termed JM-27. PAGE4 was demonstrated to be relatively specific to the prostate and was approximately

18-fold higher in expression in BPH associated with significant symptoms [32]. When PAGE4 protein levels were examined, they were found to be associated not with the prostatic epithelium, like most prostatic biomarkers, but with the prostatic stroma. The relatively high level of expression within the stromal cells of the prostate associated with symptomatic BPH makes PAGE4 a unique protein. In preliminary studies, PAGE4 expression appears to be expressed within the fetal prostate but then turned off in the normal adult prostate and is reactivated in the stroma of the men with symptomatic but not asymptomatic BPH. When we artificially overexpressed the protein, we found that the PAGE4-overexpressing cells were able to protect themselves from stresses including glucose deprivation, tumor necrosis factor- $\alpha$ , and adriamycin challenge [33]. Thus, it appears that PAGE4 may represent a marker of the stress-associated changes that appear to accompany symptomatic BPH. This stress reaction may include inflammation, which, as described above, has been associated with more highly symptomatic disease.

In an effort to identify noninvasive biomarkers of BPH, we have been measuring PAGE4 in the blood as a potential indicator of disease type. These studies should reveal whether PAGE4 has the potential to serve as a serum-based biomarker of symptomatic BPH.

### The need for biomarkers of BPH

BPH is a term used to describe an enlargement of the prostate that is associated with symptoms that have been described as LUTS. Although the term BPH refers to the prostate, it is known that other organs, including the bladder, are centrally involved in many of the symptoms that are associated with the disease. Despite the fact that BPH is among the

most common urologic conditions affecting aging men, we still know very little about its etiology, and the frequently utilized medical therapies are focused on symptom improvement rather than on the biology of the disease(s). It is clear that not all BPHs are created the same. Some men present with large prostates, and others present with prostates within the normal size range. Furthermore, BPH is currently treated differently than most diseases as a result of its symptomatic description. As opposed to diseases like cancer, BPH is not diagnosed pathologically and is described by the reported symptoms and their severity. Typically a disease presents, and earlier treatment is better than later. In opposition to the approach of catching and treating a disease early, in BPH, treatments are usually reserved for those with some of the most severe symptoms, as opposed to those with histologic disease. Therefore, there is an urgent need to improve our molecular understanding of BPH so that we can discern novel biomarkers that could identify early on in their disease course men with severe disease that could have or may go into urinary retention. No such biomarkers exist or are currently being used. Furthermore, biomarkers are needed that can stratify patients into categories of potential response to therapies, that is, which patients may respond better to a particular therapy. This type of approach would allow us to focus potentially efficacious therapies on those with the disease type known to be most responsive rather than our current strategy of treating and seeing if symptoms improve.

### Conclusions

We need to move beyond the currently used model where we treat BPH as merely a collection of symptoms rather than focusing on the

### Dos and Don'ts

- There are currently no available serum or tissue biomarkers with clinical utility in stratifying patients with BPH/LUTS.

biology that underlies them. Personalization is necessary to use the currently available treatments more wisely as well as to understand the potential of novel therapies to treat subgroups of patients. While we do not yet have the biomarkers to discern personalization, our increased understanding of the genetics, epidemiology, and microenvironmental stress associated with BPH should provide us with valuable tools to begin down this road.

### Bibliography

- 1 Oesterling JE. Benign prostatic hyperplasia: a review of its histogenesis and natural history. *Prostate*. 1996;6:67–73.
- 2 Graverson PH, Gasser TC, Wasson JH, Hinman F Jr., Bruskewitz RC. Controversies about indications for transurethral resection of the prostate. *J Urol*. 1989;141:475–81.
- 3 McNeal J. Pathology of benign prostatic hyperplasia. Insight into etiology. *Urol Clin North Am*. 1990; 17(3):477–86.
- 4 Roehrborn CG, McConnell JD, Saltzman B, Bergner D, Gray T, Narayan P, et al. Storage (irritative) and voiding (obstructive) symptoms as predictors of benign prostatic hyperplasia progression and related outcomes. *Eur Urol*. 2002;42:1–6.
- 5 McVary KT. BPH: Epidemiology and comorbidities. *Am J Manag Care*. 2006;2:S122–8.
- 6 Taub DA, Wei JT. The economics of benign prostatic hyperplasia and lower urinary tract symptoms in the United States. *Curr Urol Rep*. 2006; 7(4):272–81.
- 7 Chapple CR. Pharmacological therapy of benign prostatic hyperplasia/lower urinary tract symptoms: an overview for the practising clinician. *BJU Int*. 2004;94:738–44.
- 8 McConnell JD, Roehrborn CG, Baustista OM, Andriole GL Jr., Dixon CM, Kusek JW, et al. The long term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *N Engl J Med*. 2003; 349:2387–98.
- 9 Sarma AV, Jacobson DJ, McGree ME, Roberts RO, Lieber M, Jacobsen SJ. A population based study of incidence and treatment of benign prostatic hyperplasia among residents of Olmsted County Minnesota: 1987–1997. *J Urol*. 2003;173:2048–53.
- 10 Marker PC, Donjacour AA, Dahiya R, Cunha GR. Hormonal, cellular, and molecular control of prostatic development. *Dev Biol*. 2003;253(2):165–74.
- 11 Lee C. Role of androgen in prostate growth and regression: stromal–epithelial interaction. *Prostate*. 1996;6:52–6.
- 12 Cunha GR, Fujii H, Neubauer BL, Shannon JM, Sawyer L, Reese BA. Epithelial–mesenchymal interactions in prostatic development. Morphological observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. *J Cell Biol*. 1983;96:1662–70.
- 13 Cunha GR, Lung B. The importance of stroma in morphogenesis and functional activity of urogenital epithelium. *In Vitro*. 1979;15:50–71.
- 14 Sar M, Lubahn DB, French FS, Wilson EM. Immunohistochemical localization of the androgen receptor in rat and human tissues. *Endocrinology*. 1990;127:3180–6.
- 15 Coffey DS. Androgen action and the sex accessory tissues. In: Knobil E, Neill J, et al., editors. *The physiology of reproduction*. New York: Raven Press; 1988. pp. 1081–19.
- 16 Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: past, present and future. *Differentiation*. 2011;82(4–5):184–99.
- 17 Coffey DS, Walsh PC. Clinical and experimental studies of benign prostatic hyperplasia. *Urol Clin North Am*. 1990;17:461–75.
- 18 Belanger A, Candas B, Dupont A, Cusan L, Diamond P, Gomez JL, et al. Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year old men. *J Clin Endocrinol Metab* 1994;76:1086–90.

- 19 Lucia MS, Lambert JR. Growth factors in benign prostatic hyperplasia: basic science implications. *Curr Urol Rep.* 2008;9(4):272–8.
- 20 Alonso-Magdalena P, Brössner C, Reiner A, Cheng G, Sugiyama N, *et al.* A role for epithelial-mesenchymal transition in the etiology of benign prostatic hyperplasia. *Proc Natl Acad Sci USA.* 2009;106(8):2859–63.
- 21 Macoska JA. Chemokines and BPH/LUTS. *Differentiation.* 2011;82(4–5):253–60.
- 22 Wang Z, Olumi AF. Diabetes, growth hormone-insulin-like growth factor pathways and association to benign prostatic hyperplasia. *Differentiation.* 2011;82(4–5):261–71.
- 23 Vezina CM, Bushman AW. Hedgehog signaling in prostate growth and benign prostate hyperplasia. *Curr Urol Rep.* 2007;8(4):275–80.
- 24 Sakamoto S, Yokoyama M, Zhang X, Prakash K, Nagao K, Hatanaka T, *et al.* Increased expression of CYR61, an extracellular matrix signaling protein, in human benign prostatic hyperplasia and its regulation by lysophosphatidic acid. *Endocrinology.* 2004;145(6):2929–40.
- 25 Chughtai B, Lee R, Te A, Kaplan S. Inflammation and benign prostatic hyperplasia: clinical implications. *Curr Urol Rep.* 2011;12(4):274–7.
- 26 Robert G, Descazeaud A, Nicolaiew N, Terry S, Sirab N, Vacherot F, *et al.* Inflammation in benign prostatic hyperplasia: a 282 patients' immunohistochemical analysis. *Prostate.* 2009;69(16):1774–80.
- 27 Willder JM, Walker VC, Halbert GL, Dick CP, Orange C, Qayyum T, *et al.* The prognostic use of inflammation and tissue necrosis in benign prostatic hyperplasia. *Urol Int.* 2013 Jan 10. [Epub ahead of print]
- 28 Schenk JM, Calip GS, Tangen CM, Goodman P, Parsons JK, Thompson IM, *et al.* Indications for and use of nonsteroidal antiinflammatory drugs and the risk of incident, symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *Am J Epidemiol.* 2012;176(2):156–63.
- 29 Sutcliffe S, Grubb Iii RL, Platz EA, Ragard LR, Riley TL, Kazin SS, *et al.* Non-steroidal anti-inflammatory drug use and the risk of benign prostatic hyperplasia-related outcomes and nocturia in the Prostate Lung Colorectal, and Ovarian Cancer Screening Trial. *BJU Int.* 2012;110(7):1050–9.
- 30 Lu T, Lin WJ, Izumi K, Wang X, Xu D, Fang LY, *et al.* Targeting androgen receptor to suppress macrophage-induced EMT and benign prostatic hyperplasia (BPH) development. *Mol Endocrinol.* 2012;26(10):1707–15.
- 31 Fujita K, Ewing CM, Getzenberg RH, Parsons JK, Isaacs WB, Pavlovich CP. Monocyte chemotactic protein-1 (MCP-1/CCL2) is associated with prostatic growth dysregulation and benign prostatic hyperplasia. *Prostate.* 2010;70(5):473–81.
- 32 Prakash K, Pirozzi G, Elashoff M, Munger W, Waga I, Dhir R, *et al.* Symptomatic and asymptomatic benign prostatic hyperplasia: molecular differentiation by using microarrays. *Proc Natl Acad Sci USA.* 2002;99(11):7598–603.
- 33 Zeng Y, Gao D, Kim JJ, Shiraishi T, Terada N, Kakehi Y, *et al.* Prostate-associated gene 4 (PAGE4) protects cells against stress by elevating p21 and suppressing reactive oxygen species production. *Am J Clin Exp Urol.* 2013;1(1):39–52.