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Review Article

Role of prostate stem cells and treatment strategies in benign prostate hyperplasia

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Abstract: Benign prostate hyperplasia (BPH) is a progressive disease with a direct correlation between incidence and age. Since the treatment and management of BPH involve harmful side effects and decreased quality of life for the patient, the primary focus of research should be to find better and longer-lasting therapeutic options. The mechanisms regulating prostate stem cells in development can be exploited to decrease prostate growth. BPH is defined as the overgrowth of the prostate, and BPH is often diagnosed when lower urinary tract symptoms (LUTS) of urine storage or voiding symptoms cause patients to seek treatment. While multiple factors are involved in the hyperplastic growth of the stromal and epithelial compartments of the prostate, the clonal proliferation of stem cells is considered one of the main reasons for BPH initiation and regrowth of the prostate after therapies for BPH fail. Several theories explain possible reasons for the involvement of stem cells in the development, progression, and pathogenesis of BPH. The aim of the current review is to discuss current literature on the fundamentals of prostate development and the role of stem cells in BPH. This review examines the rationale for the hypothesis that unregulated stem cell properties can lead to BPH and therapeutic targeting of stem cells may reduce treatment-related side effects and prevent the regrowth of the prostate.

Keywords: Prostate, BPH, stem cells, androgen

Introduction

Prevention strategies for BPH need further investigation. The current treatment strategies to reduce BPH symptoms are falling short, given the increasing incidence of BPH as men age and the current rising aging population. Strategies to pharmacologically reduce BPH symptoms are treatment with α-adrenergic antagonists, that relax smooth muscles and improve urine flow, either alone or in combination with 5-α reductase types 1 and 2 inhibitors, that block the conversion of T to DHT and reduce prostate size [1-5]. If BPH symptoms do not improve with pharmacologic treatment, then surgical options are explored, including transurethral resection of the prostate (TURP), transurethral incision of the prostate (TUIP), prostatic urethral lift, aquablation, prostatic stenting, and prostatic artery embolization [6, 7]. Discontinuation of pharmaceutical treatment in BPH can result in regrowth of the prostate and return of symptoms in both humans and rodents [8, 9], suggesting there is a component of the disease that is never targeted or is not sufficiently treated. We propose that the role of prostate stem cells in BPH is similar their role in prostate development and therapeutic strategies that target prostate stem cells would provide a needed prevention strategy.

Targeting prostate stem cells alone is unlikely to be curative, in our opinion, but when used in combination with current strategies could enhance therapeutic response and reduce the incidence of regrowth when treatment is stopped. Specificity of targeting prostate stem cells, without targeting all stem cells remains a barrior, in our opinion. There are several ways to target stem cells, but two key mechanisms are: inhibition of key stem cell signaling pathways

and direct targeting of stem cells via surface markers. Signaling pathways involved in stem cell biology include hedgehog, phosphoinositide 3-kinase (PI3K)-AKT, and Wnt [10]. Surface markers can be used for targeted delivery of therapeutic agents to stem cells. In recent studies antibodies against stem cell surface markers are currently being used to deliver nanoparticles with cytotoxic agents specifically to stem cells expressing CD44 and CD133 [11]. In addition to targeting stem cell signaling pathways and directing therapy with stem cell surface markers, several naturopathic medicinal therapies are thought to target prostate stems cells. Qianliening capsule, saw palmetto, *Pygeum Africanum*, and *Hypoxis rooperi* which have been used to target prostate stem cells in BPH with few side effects, may provide an opportunity for early prevention strategies [12, 13]. Even though BPH is a very prevalent disease in older men, not much is known about the biological factors involved in its pathology. In our opinion, given the high incidence of BPH in the aging population, treatment strategies should be developed based on the role of stem cells in prostate development to prevent BPH with aging. In this article the role of prostate stem cells in the etiology and treatment options of BPH in relationship to prostate development are reviewed.

Etiology of BPH

BPH is a common disease in elderly men and is the most frequent disease treated by urologists. While BPH has not been associated with progression to prostatic carcinoma [14-17], several key regulatory functions are shared between BPH and prostate cancer.

The frequency and incidence of benign nodular hyperplasia increase with age and have a higher prevalence in men over 50 years of age [18-21]. BPH is often accompanied by LUTS, which, if left untreated, can eventually lead to acute urinary retention that necessitates surgical intervention [21-24]. BPH is caused primarily by hyperplasia of the prostatic stromal and epithelial cells paired with a decrease in apoptosis of prostatic epithelial cells. BPH arises in the periurethral region of the transitional zone of the human prostate, whereas prostate cancer mainly arises in the peripheral zone of the prostate [25-30].

Although the symptoms of BPH cannot be simplified as just urethral resistance, a primary symptom caused by BPH is urinary outlet obstruction. Urinary outlet obstruction, also known as bladder outlet obstruction (BOO), can cause changes in the anatomical features of the prostate gland. Specifically, this occurs as changes in the transitional zone and not in the overall volume of the prostate [29, 30]. The volume of the periurethral region may correlate with the degree of outlet obstruction. Assessment of BPH is based upon clinical history, digital rectal examination (DRE), and urodynamic studies that evaluate urinary flow patterns [31]. Several problematic symptoms of BOO include: poor flow of urine, terminal dribbling, straining, hesitancy, nocturia, and increased frequency and urgency of urination [31, 32]. In addition to DRE, the size of the prostate can also be estimated by a perirectal ultrasound, which is critical in both determining treatment modalities for BPH and evaluating potential underlying prostate cancer. If left untreated, BPH can eventually lead to acute urinary retention and chronic kidney problems [33-35].

Even though BPH is a very prevalent disease in men, not much is known about the biological factors involved in its pathology. Several theories in the scientific community attempt to explain the possible etiology and pathology of BPH. A primary concept suggests that more dihydrotestosterone (DHT) is produced in the aging prostate, which then leads to enlargement [33, 36-38]. However, Walsh et al. demonstrated that the amount of DHT in the hyperplastic prostate is not different from that seen in the normal prostate [39]; suggesting that the presence of a critical amount of DHT is not the only requirement for developing BPH. Several studies have suggested that the amount of DHT present in the prostate is critical in developing BPH by promoting stromal-epithelial interactions and by increasing the number of prostate cells due to stem cell proliferation [16, 33]. Prostate stem cells are thought to undergo clonal expansion and produce transit-amplifying cells which give rise to terminally differentiated luminal cells that are then programmed to undergo apoptosis. In BPH, this process is unbalanced resulting in an enlargement of the prostate. Thus, targeting of prostate stem cells to manage BPH at the source of the increased number of cells could be an effective therapeutic strategy.

In BPH, some cells in the basal compartment have stem cell features, including genomic protection and suppression of cell death. Even though genomic mutations are rare in BPH, epithelial and stromal cell proliferation is increased several-fold in BPH [40]. The stromal to epithelial cell ratio can increase 5-fold in BPH (10:1) compared to normal prostate (2:1), resulting in common BPH symptoms [41]. The interaction between the epithelial compartment and the stroma increases signaling factors that promote epithelial expansion. Signaling between the stromal and epithelial cells is critical in prostatic development, as demonstrated by Cuhna et al. [42]. The theory of stromal and epithelial cell interaction is postulated as "embryonic reawakening" whereby stromal cells secrete growth factors that stimulate adjacent epithelial cell proliferation and vice versa [16] and will be further discussed in section 3b) Prostate Stem Cells.

Aging and androgens

Aging and hormone variations are two main etiological factors of BPH. Evidence suggests that BPH is a hormonally regulated disease. In studies by Bianch-Frias et al., aging was identified as another risk factor for BPH that may affect the microenvironment and promote pathology in the human prostate. The authors observed that with aging, the prostate stroma shows increased smooth muscle disorientation and decreased expression of collagen-related genes, further suggesting extracellular matrix disorientation [43]. Furthermore, Bianch-Frias et al. observed an increase in the infiltration of inflammatory cells into the inter-glandular space of the aging stroma; with an increase in B-cells, T-cells, macrophages, the chemokine (C-C motif) ligand 8 (CCL8), and the stress response protein, apolipoprotein D (ApoD) [43]. Several studies have suggested a delicate balance of androgens and growth factors within the microenvironment that regulates autocrine/paracrine signaling is critical for maintenance of normal cellular proliferation and tissue homeostasis [43, 44]. Alterations in the cytoskeletal framework can lead to prostate pathologies, including BPH and prostate cancer [43, 45]. Cytokines, growth factors, and matrix components can be considered critical in maintaining the prostate microenvironment and are potential targets for treating BPH.

Prostatic growth is dependent on androgens. Men castrated before puberty do not develop BPH in their later age [33, 46, 47]. Geller further described how high levels of DHT, an active form of testosterone (T), plays a central role in the development and pathogenesis of BPH, including: "embryonic re-awakening" as explained by McNeal [16], increasing NADPH, and increasing the estrogen-to-testosterone ratio in plasma [47]. Despite a low concentration of testicular androgens in aging men, there is increased activity of 5-α reductase, an enzyme that metabolizes T to DHT, and elevated DHT levels promote the stromal and epithelial growth that leads to BPH [46]. 5-α reductase deficiency, or inhibition of the enzyme, reduces conversion of testosterone to DHT and disrupts many androgen-controlled developmental processes [46, 48]. In adult men inhibition of 5-α reductase results in a decrease in the size of the prostate [46, 48]. The DHT levels and conversion from testosterone were increased in prostatic hypertrophy further supporting the role of DHT in BPH [49]. Later several studies measured elevated levels of DHT in BPH prostates when compared to normal prostates [46, 49-53]. Studies conducted by Walsh et al. refute the idea that low levels of DHT are simply an artifact in a normal post-mortem prostate [39]. Furthermore, separate studies conducted by Morfin [50] and Isaacs' groups [36] support the idea that elevated DHT in BPH prostatic tissue is related to an increase in 5-α reductase enzyme activity, thus demonstrating the effects of androgen-induced metabolic activities on normal and hyperplastic prostates. In addition to androgens, Devlin et al. review the roles of estrogens, insulin, and growth factors in prostate development and growth regulation associated with BPH [54] and are discussed below in section 3) Role of prostate compartments in development and BPH. Although the circulating levels of testicular androgens decrease with age, levels of DHT and AR signaling are high in the aging prostate. Studies show a role for androgens and AR in promoting BPH development [55, 56].

AR regulation of the prostate microenvironment includes the immune system and vascularization within the stroma. Lai and colleagues demonstrated that AR expression in both epithelial and stromal cells attracts infiltrating macrophages [57-60]. The eventual interac-

tions between macrophages and epithelial/stromal cells lead to increased expression of transforming growth factor β-2 (TGFβ-2) and chemokine CCL3 respectively [55, 56]. Androgens also regulate the vascular system, where epithelial cellular death during prostatic involution following castration was first described by Kerr et al. [61] and was confirmed as apoptosis [62], and this has been modeled in the human prostate [63]. The epithelial cell apoptosis induced by castration is actually preceded by a dramatic change in the stroma vasculature that may facilitate directly, or indirectly, the signaling of apoptosis of epithelial cells [64]. While dysregulation of androgen signaling is clearly a major contributing factor to BPH, the specific effects on and between multiple cellular compartments within the prostate still need elucidation, and the role of androgen signaling in the prostate stem cell compartment is discussed below.

Inflammation

Inflammation is a risk factor in BPH by many mechanisms in addition to androgen signaling. McLauren et al. proposed that inflammation and the presence of stem cells in the prostate are the mechanisms triggering the re-awakening of developmental growth in the prostate [65]. Inflammation of the prostate can lead to activation of interleukins-triggered growth pathways in the stroma and epithelium of the prostate [65]. Jerde et al. showed that interleukin-1 (IL-1) induces growth signaling in BPH via activation of insulin-like growth factor (IGF)-dependent signaling during prostate development [66]. Along with IL-1, IL-6, IL-8, IL-12, other interleukins are involved in growth signaling during prostate development, and reactivation of growth signaling during inflammation results in regenerate of prostate tissue. Separate studies have emphasized the role of IGF-1 signaling in prostate development and growth [67-69]. Shah et al. reviewed molecular pathways to target in BPH, although targeting inflammation in BPH is not currently thought to be an appropriate therapeutic target [70]. Growth factors and interleukins secreted into the prostate stroma promote several proliferation pathways such as mitogen-activated protein kinase (MAPK), PI3K signaling pathways, and the activation of transcription factors that induce proliferation promoting genes leading to stroma proliferation [71]. Effector molecules, along with autocrine and paracrine growth factors, work together to cause increased stromal and epithelial cell proliferation, regulated by stem cell maintenance in our opinion, thus leading to the development and progression of BPH.

Abdominal obesity

Increased waist circumference correlates with BPH and is associated with increased inflammation. Abdominal obesity is defined as a waist circumference that is >102 cm (40 inches) and has been linked to the progression of BPH [72]. As the prevalence of a large waist circumference has increased, so has awareness of the associated adverse health factors [73]. A meta-analysis of cohort and case-control studies has identified a positive association with BMI and increased risk of BPH and LUTS [74]. While the mechanisms are not fully understood, Gotera et al. have been studying possible etiologies. They have concluded that waist circumference >102 cm indirectly increases the risk of developing BPH through both IL-6 and hyperinsulinemia secondary to insulin resistance [75]. IL-6, an acute phase reactant, is increased with inflammation, an established risk factor for BPH, and elevated IL-6 is common in abdominal obesity [65]. Thus, an increased waist circumference, which is associated with inflammation, promotes growth signaling pathways that further reinforce the epithelial/stromal interactions and potentially stem cell proliferation that are hypothesized to be a driving factor in developing BPH.

Role of prostate compartments in development and BPH

Stromal and epithelial paracrine signaling

Prostate development is initiated through paracrine interactions between the stromal and epithelial compartments. Epithelial and stromal cells show heterogeneous expression of AR [76, 77]. AR expression in the stroma is instrumental in regulating paracrine signaling to induce epithelial cell proliferation. In classic studies utilizing tissue recombination with androgen-insensitive AR^{tfm} and androgen-sensitive AR^{wt} prostate tissue, Cunha et al. demonstrated that a functional AR is not required in the epithelium but is required in the mesenchyme for androgen-induced growth and bran-

ching morphogenesis [42]. However, a functional AR in the epithelium is required for the differentiation of the epithelium into mature prostatic secretory cells [42]. Thus, AR is required for functional differentiation in the adult prostate, and during prostatic development AR regulates many stages including proliferation, branching morphogenesis, and maturation of secretory epithelial cells. Estrogens have long been associated with hyperplasia in rodent models [78] and BPH risks in men [79]. Stromal estrogen receptor (ER)-α and ER-β signaling are both required to regulate androgen signaling [80, 81]. Dr. Cunha thoroughly reviewed the androgen and estrogen-regulated epithelial/mesenchymal interactions [82], and Delvin et al. provide a robust review of the current understanding of the roles of androgen, estrogen, and growth factors in BPH initiation and progression [54].

AR signaling in stromal cells also promotes the secretion of growth factors that act on the epithelial compartment, the immune system, extracellular matrix remodeling, and neovascularization. Paracrine signaling from the prostate stromal compartment is a key regulator of epithelial stem cells during epithelial branching in prostate development [83, 84]. Recently, single-cell RNA sequencing of the developing prostate has further elucidated specific cell populations that express developmental regulators [85]. Regulation of prostate epithelial branching during development initially occurs through paracrine signaling of growth factors from the stroma. Conversely, paracrine signaling from the epithelium also triggers stromal stem cells to differentiate into stromal smooth muscle cells and fibroblasts [22, 86]. The stromal stem cell-derived smooth muscle cell progeny express AR protein and 5-α reductase enzyme [22]. In smooth muscle cells, DHT binding to the AR receptor triggers the expression of several growth and differentiation factors, including IGF-1 [57], fibroblast growth factor (FGF-7) [87], nerve growth factor-β (NGF-β) [42, 88], and vascular endothelial growth factor (VEGF) [89]. TGFβ inhibits androgen signaling in prostate stromal cells [89], and FGF-7 can induce epithelial branching independent of androgen signaling [90]. Additionally, prostate epithelial stem cells initiate prostate proliferation and epithelial branching by Nkx3.1 [91], Notch [81, 92, 93], Foxa1 [83, 94], Shh [95-97], and Wnt [83, 94] signaling. Thus, stromal and epithelial cell proliferation control is a multifactorial process regulated by several growth factors that interact at multiple levels including through the stem cell compartment further discussed below.

Prostate stem cells

In the prostate both the stromal and epithelial compartments contain stem cells, and adult prostate stem cells play a critical role in differentiation and maintenance. Expansion of prostate stem cells in both the epithelial and stromal compartments can lead to hyperplasia in the prostate gland; thus, the size of the prostate is dictated by both the stromal and epithelial compartments. As postulated by Coffey and Walsh, the increase in the size of an organ depends on the balance between cell proliferation and cell death [17, 33]. Studies conducted by Coffey et al. suggested that androgens regulate cellular proliferation and cell death in the prostate and that BPH results from an unregulated stem cell compartment [17, 22, 33]. For example, in the epithelial compartment, stem cells divide and give rise to a compartment of transit-amplifying cells that eventually terminally differentiate into mature luminal cells with an average lifespan of 500 days [98]. The terminally differentiated luminal cells have a finite lifespan and eventually undergo apoptosis. However, due to either a delay or a block in the maturation process as postulated by Isaacs and colleagues [22, 33], this may lead to an increased number of transit-amplifying cells, thus leading to an enlargement in the size of the gland. As a result, the size of the prostate may be defined by the presence of epithelial stem cells and transit-amplifying cells [33]. Historically, prostate epithelial stem cells were thought to reside in the basal layer and give rise to distinct types of progenies which have varying proliferation potentials. Transit-amplifying cells divide and give rise to intermediate cells [22, 33]. Intermediate cells respond to androgens and terminally differentiate into luminal cells [22, 23]. There is evidence of multiple types of epithelial stem cells, including a common basal and luminal stem cell and only luminal stem cells that have been proposed to be a source of recurrent prostate cancer [99-101]. Thus, the plasticity of the prostate cell compartments is a likely contributor to different cell types identified in BPH by single-cell RNA sequencing [102].

The stromal stem cells in the prostate have demonstrated mesenchymal stem cell properties. The stromal stem cells give rise to smooth muscle cells, fibroblasts, adipose, and myoadipose cells with mesenchymal stem cell markers [22, 86]. Additionally, prostate stroma derived from BPH specimens has multipotent properties upon treatment with differentiation agents, suggesting the presence of adult stromal stem cells in the bulk of the prostate stroma [85]. Alternatively, Brennen et al. demonstrated that BPH tissue was infiltrated with mesenchymal stem cells [103]. While the presence of stem cells is hypothesized to be one of the factors leading to BPH, the factors that stimulate differentiation into mature stromal and epithelial cells are not fully understood, particularly as associated with BPH.

Androgen regulation of the prostate stem cell compartment

The response of epithelial prostate stem cells to androgen levels was shown in seminal work conducted by English et al. and others in the late 1980s. English et al. showed that the prostate gland can undergo regression upon castration and re-growth with subsequent re-administration of testosterone [9]. This regression and re-growth suggest that stem cells not only survive androgen deprivation but also have the capability to respond to androgen stimulation and regenerate an entire prostate gland [9, 104, 105]. Thus, prostate stem cells that survive androgen deprivation therapy (ADT) can regulate prostate size through the uncontrolled generation of stromal and epithelial cells.

ADT interrupts stromal-epithelial interactions and interrupts cell expansion in BPH. Interruption of stromal-epithelial interactions decreases the level of several growth factors, hierarchical differentiation and the total number of stromal stem cells [22, 106]. There are prostate stem cells that survive ADT, and prolonged ADT exposure can conversely cause the stroma to induce proliferation of epithelial cells. ADT alone is not sufficient as a treatment for BPH since resistance mechanisms do not eliminate the stem cells, the cells responsible for prostate growth and stroma signaling. Therefore, understanding the basic mechanisms that trigger the proliferation and/or apoptosis of cells are critically needed to increase our understanding of factors that control the existence and number of stem cells in the prostate. While basal epithelial stem cells of the prostate respond to proliferative signaling and give rise to transit-amplifying/intermediate cells which terminally differentiate into luminal cells, distinct prostate luminal stem cells that bypass the traditional differentiation path were first described in 2014 [107, 108], and were later analyzed with single-cell RNA sequencing [109]. Defects in the maturation process of epithelial stem cells to luminal cells can cause an increase in the number of epithelial cells.

BPH initiation and progression depend on the fine balance between stromal and epithelial cell proliferation and apoptosis. An imbalance in the paracrine signaling that promote proliferation and/or induce apoptosis can lead to abnormal proliferation of stromal cells leading to BPH [110-113]. In BPH, “reawakening” of the embryonic growth, development, and differentiation occurs wherein the stromal compartment reacquires the properties of its embryonic anlagen. In development, the urogenital mesenchyme actively induces the development of the urogenital epithelium into the prostate in the presence of androgens [16, 103]. The proliferation and cell death mechanisms are tightly regulated in the developing prostate, but in BPH, these mechanisms are thought to be deregulated. Uncontrolled proliferation led to the continued proliferation and clonal expansion of stromal and epithelial cells. Additionally, androgen ablation may affect stromal cell function but not the actual number of stromal cells. In BPH nodules, stromal cells have high levels of proliferation and low levels of apoptosis. For example, in BPH the expression of the apoptotic inducer TGF-β1 is decreased, and the anti-apoptotic protein Bcl-2 is increased [110]. Zhang et al. observed a positive correlation between the density of stromal cells and proliferation with an inverse relationship between the density of epithelial cells and apoptosis [114]. Many additional mechanisms regulating epithelial and stromal proliferation and apoptosis have been associated with BPH, including estrogen and hedgehog signaling. Differential regulation of ER-α and ER-β on AR signaling has been shown to regulate prostate growth [81, 92, 93]. Stromal hedgehog signaling is also required for prostate growth and regeneration [95-97] and is currently under investigation in

BPH and in development as potential therapeutic target to treat BPH [115, 116].

We hypothesize that the total number of stem cells present in the prostate modulates prostatic growth and BPH development. A pool of stem cells is accumulated in the postnatal prostate, which is critical for the development of the prostate to its normal size. Critical physiological levels of androgens are also important for generating the pool of stem cells. The importance of these stem cells in the prostate is evidenced by experiments by Coffey and his colleagues. Boys castrated before puberty do not get BPH even if exposed to high levels of androgens later in life [33, 46, 47]. This suggests that the presence of a critical number of stem cells is important for abnormal development and that this number of prostate stem cells is reached only after puberty. There are also molecular factors that help maintain the pool of stem cells in the prostate. Targeting these molecular factors could help deplete the stem cell pool and reduce prostate size when combined with ADT. BPH patients may be given interventions that target prostate stem cells in combination with ADT to reduce prostate size. The pool of stem cells in the adult prostate is maintained through stromal-epithelial interactions, mediated by growth and survival factors regulated by androgens. Thus, in our opinion targeting prostate stem cells in combination with ADT or other therapeutic interventions like 5-α reductase inhibitor to deplete the stem cell pool to a critical level may result in improved treatment of BPH.

Rationale for targeting stem cells to treat BPH

Given the decrease in androgen levels with age, a subsequent decrease in proliferation of prostatic epithelium and stroma would be expected. However, Bushman et al. observed that castration of adult mice led to the induction of cells expressing various stem cell markers, including CD44, CD133, Sca-1, and CD117 indicating that a decrease in androgens with age may be compensated by an increase in proliferation of stem/progenitor cells [65]. Crowell et al. demonstrated that Trop2+ luminal progenitor cells expand in the prostate of aged mice and men [100]. PSCA-expressing prostate luminal progenitor cells were increased in BPH tissue compared to normal prostate [100]. These studies suggest that prostate stem cells increase with age and in BPH and that castration, or ADT, alone may not be sufficient for effective BPH treatment. We propose that inhibiting mechanisms that promote proliferation of prostate stem cells, such as hedgehog signaling and Wnt signaling, may increase the efficacy of BPH treatment.

Current BPH treatment considerations and options

Once BPH is diagnosed, there are two different options for the patients with bothersome symptoms: (i) medicinal treatments are suggested for patients with symptoms that are bothersome and impede quality of life; and (ii) surgical treatment is usually suggested for patients with recurrent disease who can tolerate a surgical procedure [117, 118].

Current BPH pharmaceutical treatments include finasteride (a form of ADT), and surgical interventions, including TURP and TUIP [119, 120]. ADT and TURP/TUIP have quality of life impacting side effects such as impotence, decreased libido, and abnormal ejaculation. Finasteride or other pharmacologic forms of ADT are used continuously to keep the prostate size under control [119, 120]. Discontinuation of pharmaceutical treatment often results in regrowth of the prostate and return of symptoms, suggesting there is a component of the disease that is never targeted or is not sufficiently treated. Stromal-epithelial interactions and prostate stem cells are two components of the prostate we propose are resisting/surviving the effects of hormone ablation. Though ADT reduces the prostate size, the number of stem cells in the prostate is not decreased since most stem cells are not affected by ADT. Thus, the remaining stem cells regenerate the prostatic tissue when androgen levels return to normal [22]. In addition, prostate stem cells are resistant to apoptosis by α-adrenergic antagonists. Resistance to apoptosis prevents a reduction in prostate size and/or increases the chances of recurrence [121-123]. Therefore, we propose targeting the prostate epithelial and stromal stem cells is important to circumvent prostate regeneration. As suggested by Isaacs [9], ADT treatment followed by radiation therapy is one approach that could inhibit the stromal-epithelial interactions and thereby

inhibit signaling that triggers proliferation of the stem cell compartment. The enlarged prostate should be targeted in a way as to reduce stem cell numbers to a critically low level, without complete depletion of the stem cell pool. This critically low level of the stem cell pool will leave the remaining stem cells presumably unable to induce BPH development in the future, even if triggered by different autocrine and paracrine signaling factors. Thus, we propose that BPH treatment should focus on reducing prostate size, reducing the number of prostatic stem cells and relieving the associated clinical symptoms of lower urinary tract. Though different options are available for BPH treatment, some significantly impact the patient's quality of life. Several avenues that can be targeted pharmacologically to reduce BPH symptoms by reducing the size of the enlarged prostate include: i) targeting DHT synthesis; ii) targeting smooth muscle function; iii) surgical interventions; iv) targeting stromal-epithelial interactions; v) targeting stem cells.

i) Targeting DHT synthesis: Several clinical trials compromised of patients with moderate-to-severe clinical BPH symptoms demonstrated a reduction in BPH symptoms and prostate size by treatment with α-adrenergic antagonists in combination with either 5-α reductase types 1 and 2 inhibitors [1-5]. α-Adrenergic antagonists are drugs that block α-adrenoceptors that function to constrict smooth muscles surrounding the urethra and bladder. Relaxation of smooth muscles with α-adrenergic antagonists improves urine flow. Examples of α-adrenergic blockers include tamsulosin (FlomaxÒ), alfuzosin, terazosin, and doxazosin. 5-α reductase types 1 and 2 inhibitors inhibit the 5-α reductase enzymes, thereby blocking the conversion of T to DHT. 5-α reductase inhibitors, finasteride and dutasteride, further reduce prostate size, thus, improving the BPH symptoms. Clinical trials demonstrated that treating patients with BPH with α-adrenergic antagonists and/or 5-α reductase inhibitors may have long-term control at reducing the prostate size and improving BPH symptoms. However, such treatments are not effective at completely eliminating the need for surgery or the risk of acute urinary retention. BPH is a clinically progressive disease. While pharmaceutical treatment alleviates symptoms of BPH, the chances of return of symptoms are high, suggesting targeting additional mechanisms of BPH disease would be more successful.

ii) Targeting smooth muscle function: a. α-adrenergic antagonists are drugs that block α-adrenoceptors that function to constrict smooth muscles surrounding the urethra and bladder. Relaxation of smooth muscles with α-adrenergic antagonists improves urine flow and relieves BPH symptoms. Examples of α-adrenergic blockers include tamsulosin (FlomaxÒ), alfuzosin, terazosin, and doxazosin. b. Other medications that target smooth muscle activity in the urinary track include anticholinergic drugs that inhibit muscarinic receptors in the bladder urothelium which limit detrusor overactivity associated with BPH. While these medications can show some benefit, they need to be used with caution, especially in elderly patients, due to the numerous side effects, most notably confusion [6].

iii) Surgical interventions: In addition to TURP procedures, multiple surgical interventions have been developed to treat BPH, including endoscopic procedures such as a prostatic urethral lift. This procedure can alleviate adverse effects such as erectile and ejaculatory dysfunction but is less efficacious than a TURP [120]. Other surgical procedures with limited evidence of benefit include aquablation, prostatic stenting, and prostatic artery embolization. Each of these procedures is less invasive than TURP; however, long-term data and success rates have not been sufficiently measured [6].

iv) Targeting stromal-epithelial interactions: Stromal-epithelial interactions regulate prostate development and contribute to prostatic diseases. Animal and *in vitro* models provide evidence that targeting growth factor signaling pathways between stromal-epithelial compartments, such as hedgehog, notch, and estrogen receptor signaling, would decrease prostate growth and BPH symptoms. Growth factor signaling regulates stromal-epithelial interactions leading to stromal and epithelial cell proliferation [124]. For instance, stromal and epithelial cell proliferation and differentiation were observed to be dependent on Notch signaling [125, 126]. ER-α promotes proliferation, and ER-β induces apoptosis in normal prostate epithelium and prostate cancer [92]. ER-α is overexpressed in BPH stroma, whereas in BPH

ER-β is minimally expressed in stromal cells and highly expressed in basal and luminal cells [92]. Furthermore, ER-α, not ER-β, increases urine retention in mice which can be relieved with treatment with the ER-α antagonist, raloxifene [127]. Additionally, in the prostate of aging men, the levels of prostatic testosterone decrease while the levels of estrogens increase [92, 128, 129]. A loss of ER-β expression and increased expression of ER-α is observed in BPH and prostate cancer [129]. Therefore, targeting the estrogen receptor pathway is another strategy to treat BPH. Paracrine signaling, such as PI3K/AKT/mTOR, hedgehog signaling, and IGF signaling also plays a role in regulating stromal-epithelial interactions. Deregulation of these pathways that mediate paracrine signaling can lead to BPH [10]. Studying the signaling pathways involved in stromal-epithelial proliferation and differentiation may identify new targets for more effective therapies for BPH.

v) Targeting stem cells: While most therapies focus on reducing prostate size and temporarily alleviating the patient's symptoms, an emphasis on targeting stem cells may help prevent regrowth of the prostate and return of BPH symptoms, in our opinion. BPH stem cells can be targeted by several mechanisms. In studies from our laboratory, prostate stem cells that have a functionally active ABCG2 transporter and aldehyde dehydrogenase 1A1 (ALDH1A1) can serially regenerate the prostate when combined with embryonic rat urogenital mesenchyme [130, 131]. Additionally, we showed ABCG2 regulates the maintenance of mouse and human prostate stem cells and can be pharmaceutically inhibited to decrease regeneration of the mouse prostate [132, 133]. Additionally, targeting stem cells can be accomplished by inhibiting signaling pathways such as hedgehog, PI3K/AKT and Wnt, which promote self-renewal and proliferation of prostate stem cells [10], and can be investigated as potential targets for therapeutic interventions. Recent studies take advantage of linking antibodies against cancer stem surface markers to nanoparticles to target cytotoxic agents specifically to cancer stem cells as described in a recent review [134]. Many of the surface markers used to target nanoparticles are found on prostate stem cells, including CD44 and CD133 [11]. Specifically, nanoparticles linked with CD44 antibodies have successfully targeted therapy to prostate cancer stem cells [135]. We propose that nanoparticles linked to antibodies to prostate stem cell markers would target prostate stem cells in BPH and have therapeutic benefits.

Future directions

While there are several treatment options for BPH, most are not curative for the majority of patients and significantly decrease in quality of life of patients with BPH. Given the significant number of men affected by BPH, prevention and curative treatments should be the focus of future research. Targeting prostate stem cells provides an opportunity to develop curative treatments. While some studies have focused on preventing the growth of the prostate and reducing the symptoms of BPH, or finding therapies with fewer side effects; these studies have not focused on targeting prostate stem cells. More studies are needed to evaluate novel therapies in preclinical models with the goal of rapid movement toward clinical trials. Silymarin is a phytoestrogen compound that can prevent testosterone-induced BPH in rats [136]. In this study, silymarin is both pro-apoptotic and anti-oxidative to prevent BPH [136]. Better nutrition and exercise are also indicated in the prevention of BPH. Dietary supplements including zinc, β-sitosterol, saw palmetto, *Pygeum Africannum,* and Cernilton can help relieve symptoms of BPH and have fewer side effects. Additionally, studies have shown that intake of dietary supplements, such as isoflavones (genistein) or medicinal herbs (saw palmetto), significantly improve symptoms of BPH, including a decrease in LUTS [137]. Diets containing a high intake of vegetables, polyunsaturated fats, and moderate alcohol intake also decrease the risk of BPH. In contrast, diets associated with a high intake of starches and red meats increase the risk of BPH [138, 139]. More BPH prevention studies need to be performed to assess the role of diet in disrupting prostate stem cell regulation of prostate growth. Specifically, more studies are needed examining the impact of dietary supplements specifically on prostatic stem cells are need.

Naturopathic medicinal therapies, including qianliening capsule (a natural product and a Chinese formulation) and saw palmetto are

options that target prostate stem cells in BPH and have fewer side effects. Qianliening, in capsule form, acts through suppression of the EGFR/STAT3 signaling pathway leading to decreased expression of Bcl-2 resulting in decreased prostate epithelial proliferation [12]. Other natural products, including saw palmetto, *Pygeum Africanum*, and *Hypoxis rooperi,* have few side effects and are being used to successfully treat BPH [13]. Disulfiram is a naturopathic therapy that inhibits ALDH detoxification of alcohol and has been used as an anti-alcoholic drug for decades. Disulfiram directly targets stem cells with high ALDH activity and has been shown to specifically target cancer stem cells, recently reviewed [140]. The ALDH3A1 isoform is elevated in BPH, making disulfiram an exciting new treatment to test in BPH [141]. The role of ALDH in stem in BPH warrants further studies. Targeting prostate stem cells in BPH can help manage the disease and delay the growth of the prostate. Additionally, in our opinion, therapy strategies that target prostate stem cells are likely effective therapies in the prevention of BPH.

Conclusions

In the absence of symptoms that disrupt lifestyle, management of BPH by watchful waiting is an option for patients [117]. Current BPH therapeutic and surgical therapies reduce the prostate burden and alleviate the associated symptoms of BPH but do not reduce the risk of disease progression and return of BPH symptoms with the regrowth of the prostate. Furthermore, current pharmaceutical and surgical therapy options have harmful side effects that can greatly impact the patient's quality of life. In our opinion, there is a need for better therapeutic options to effectively prevent and treat BPH. To achieve better therapeutic outcomes, BPH treatment strategies should focus more on the root problem in the development, progression, and pathogenesis of BPH [9, 16, 21, 103] and focusing on stem cell biology in BPH provides an attractive therapeutic target. The vast knowledge of the prostatic stem cells in development and growth needs to be exploited in order to better manage the inevitable emergence of BPH in aging men.

Disclosure of conflict of interest

None.

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