



Published in final edited form as:

*Differentiation*. 2011 ; 82(4-5): 184–199. doi:10.1016/j.diff.2011.04.006.

## Androgens and estrogens in benign prostatic hyperplasia: past, present and future

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### Abstract

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are common clinical problems in urology. While the precise molecular etiology remains unclear, sex steroids have been implicated in the development and maintenance of BPH. Sufficient data exists linking androgens and androgen receptor pathways to BPH and use of androgen reducing compounds, such as 5 $\alpha$ -reductase inhibitors which block the conversion of testosterone into dihydrotestosterone, are a component of the standard of care for men with LUTS attributed to an enlarged prostate. However, BPH is a multifactorial disease and not all men respond well to currently available treatments, suggesting factors other than androgens are involved. Testosterone, the primary circulating androgen in men, can also be metabolized via CYP19/aromatase into the potent estrogen, estradiol-17 $\beta$ . The prostate is an estrogen target tissue and estrogens directly and indirectly affect growth and differentiation of prostate. The precise role of endogenous and exogenous estrogens in directly affecting prostate growth and differentiation in the context of BPH is an understudied area. Estrogens and selective estrogen receptor modulators (SERMs) have been shown to promote or inhibit prostate proliferation signifying potential roles in BPH. Recent research has demonstrated that estrogen receptor signaling pathways may be important in the development and maintenance of BPH and LUTS; however, new models are needed to genetically dissect estrogen regulated molecular mechanisms involved in BPH. More work is needed to identify estrogens and associated signaling pathways in BPH in order to target BPH with dietary and therapeutic SERMs.

### Keywords

androgen; estrogen; BPH; prostate; hormone action; hormone therapy

### Introduction

#### Background

Benign prostatic hyperplasia (BPH) also commonly called benign prostatic hypertrophy can be described clinically or pathologically. Clinical BPH is commonly viewed as benign enlargement of the prostate, which contributes to an array of urinary voiding difficulties that can range from bothersome to significantly impacting quality of life among older men [1].

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Pathologic BPH is the histological determination of non-neoplastic new prostatic growth in adult men. Autopsy studies have revealed that the prevalence of pathologic BPH increases markedly after the 4<sup>th</sup> decade and is found in up to 90% of men over age 80 [2]. The high prevalence of BPH in older men has led some to consider prostatic hyperplasia to be a ubiquitous result of aging [3]. The precise molecular mechanisms underlying the induction, maintenance, and development of clinical sequelae resulting from BPH are incompletely understood. This article will review the BPH literature and consider the widely accepted permissive role of androgens and the emerging importance of estrogens in BPH. A greater understanding of sex steroid signaling in BPH will be necessary for novel and effective prevention and treatment strategies for this very common clinical problem.

### Clinical BPH

The human prostate consists of three distinct histologic zones: central, peripheral, and transition [4]. While prostate cancer is found primarily in the peripheral zone, nearly all clinically significant BPH develops in the transition zone of the prostate [5]. Macroscopic growth of the transition zone can cause narrowing of the urethra as it passes through the prostate, leading to a bladder outlet obstruction (BOO), which may affect the flow of urine. In men the prostate is the most common cause of obstruction. BPH and subsequent BOO contributes to a spectrum of urinary voiding problems that can significantly impact quality of life and are commonly known as lower urinary tract symptoms (LUTS) [6]. In large studies of men with BPH and LUTS, there is not a strong correlation between prostate size, symptoms and urinary flow rates [1]. However, serum prostatic specific antigen (PSA) does correlate with prostate volume, and men with larger prostates and high serum PSA are at higher risk of experiencing more significant symptoms, including ultimate progression to acute urinary retention [1]. LUTS include bladder storage symptoms such as nocturia, urgency, increased urinary frequency, and difficulty starting the stream of urine; decreased urinary flow and incomplete emptying are generally attributed to problems with bladder emptying [7]. Other important causes and contributing factors to LUTS are age-related declines in detrusor function and systemic medical conditions [7, 8]. Unlike earlier times in man's history, with contemporary treatment strategies, BPH is now a rare direct cause of mortality. Experiencing an acute episode of urinary retention and nocturia are clinical markers of increased mortality risk that likely represent the high comorbidity of BPH with other systemic medical conditions [9, 10]. Taken together with high morbidity and financial burden, BPH and LUTS are serious medical matters.

A number of treatment options exist for the care of men with bothersome LUTS due to an enlarged prostate. The standard of care for BPH is treatment with  $\alpha$ -blockers, 5- $\alpha$ -reductase inhibitors, surgery, or a combination thereof [1]. Drugs that block  $\alpha$ -1 adrenergic receptors are effective in BPH mainly by blocking sympathetically mediated contraction of prostatic smooth muscle, relieving BOO, and leading to improved urine flow. Alpha-1 adrenergic receptors are present throughout the smooth muscle of the male urinary tract, and at the level of the spinal cord, ganglia and nerve terminals, and therefore, are likely to have a wide spectrum of favorable effects on these extra-prostatic sites [11]. While  $\alpha$ -blockers are effective at improving LUTS for many patients, they do not affect prostate growth and therefore do not decrease the risk of BPH complications such as acute urinary retention and the need for surgical intervention [12]. Hormonal treatment of BPH with 5- $\alpha$ -reductase inhibitors blocks the conversion of testosterone (T) to dihydrotestosterone (DHT), shrinks the prostate and results in improved urinary flow rates, underscoring the well-known dependence of BPH on DHT. The gold-standard treatment for BPH is transurethral resection of the prostate (TURP) which relieves obstruction by removing BPH nodules in the transition zone; however, a variety of minimally invasive surgical approaches exist [6]. Some patients do not respond well to these standards of care and it is difficult to predict

which patients will respond to a particular treatment. Thus, there remains a need for advances and improvements in treatment and prevention of BPH and LUTS based on a better understanding of their pathophysiology.

### **Pathological BPH**

Pathological BPH is characterized by hyperplastic epithelial and stromal growth that coalesces into microscopic and macroscopic nodules in the prostate gland [13]. There are five generalized types of BPH nodules: 1. Fibromyoadenomatous (common), 2. Fibroadenomatous, 3. Fibrous/fibrovascular, 4. Fibromuscular, and 5. Muscular (uncommon) [14]. More commonly BPH is described as epithelial (containing mostly prostate epithelial cells), mixed (containing stromal and epithelial cells), and stromal (containing only stromal cells) [15]. The earliest nodules that develop in BPH are found in the periurethral region and are typically stromal, composed of fibrous tissue mixed with some smooth muscle [16]. Somewhat less commonly, BPH nodules may be found in the peripheral zone, which may be palpable with digital rectal examination, and are typically composed of epithelial glandular elements [17]. The lack of glandular elements in stromal BPH nodules, and the observation of zonal differences in the inception of BPH nodules suggest a distinct etiology of stromal nodules compared to BPH with glandular components. When the transition zone enlarges macroscopically, due to BPH nodular growth, it can impede the flow of urine through the prostatic urethra and hence contribute to LUTS. Understanding the key molecular mechanisms for how distinct BPH nodules develop and are associated with LUTS is essential to developing clinical management tools for these diseases.

### **Theories of the etiology of BPH**

The precise molecular etiology of BPH is complicated and unknown, but several theories have been proposed. These include embryonic reawakening, aging, androgens, estrogens, oxidoreductase, and inflammation theories [18, 19]. One prominent theory of BPH pathogenesis was proposed by McNeal and is termed the embryonic reawakening theory [5]. In 1978, McNeal posited that prostatic hyperplasia represents an awakening of hormonally-mediated developmental processes [5]. Through precise study of 63 autopsy prostates, he noted that BPH nodules arose from the transition zone, which he attributed to the “unique mingling of prostatic glands with sphincteric stroma” [5]. Consistent with this observation, Cunha demonstrated that androgen regulation and paracrine interactions were necessary for prostate glandular development and maintenance, establishing the key role of stromal-epithelial cell interactions in the prostate [20–23]. A hormonal etiology involving dysregulation of stromal-epithelial cell interactions is recognized as important for BPH development, but the precise pathogenesis remains to be elucidated. All of the proposed theories above require the presence of androgens; however, androgens appear to be permissive but not inductive reviewed by [3]. Age is an associated factor for the development of both histologic and clinically significant BPH. Although nearly all men will develop histologic BPH with age, it is thought that BPH initiation occurs in younger men (~30 years of age) [2]. Furthermore, younger men develop BPH but at much lower rates compared to aged men [2]. As noted above, there are different types of BPH nodules, which are likely to be initiated by different molecular mechanisms and as such multiple theories may explain the distinct types of BPH. Improved understanding of mechanisms of sex steroid hormone action in the induction and maintenance of BPH could lead to rational design of hormonally-based targeted therapies. Further research is needed to elucidate the mechanisms behind each type of BPH, as well as, BPH as a whole.

## Models of BPH

### Humans and human cells

There are many *in vitro* and *in vivo* models of BPH and as with all model systems each has its own strengths and weaknesses (Table 1) [24]. Perhaps the best organism to evaluate BPH is man; after all it is man whom all other models emulate. However, there are ethical issues that make human BPH studies difficult. Additionally, human genetics are highly variable between populations with distinct rates of BPH (e.g. African American, Caucasian, and Asian) making interpretation of key molecular events associated with the disease difficult. Another confounding issue in man as an experimental unit is the lack of ability to control the experimental environment. Unlike in animal studies of lower phylogeny where temperature, lighting, housing, air, water, and food are tightly regulated, controlling the environment is challenging in human studies. This is due in part to different socioeconomic backgrounds, personal choices, beliefs, and lifestyles. Finally, the cost associated with human research is high. For these reasons and others, use of humans are not ideal for early stages of BPH research.

Although there are inherent problems with human experimental studies of BPH, biological and genetic processes may be inconsistent among species and as such use of human cells and tissues are advantageous. For example, prostatic PSA and adrenal androgens such as DHEA are not present in rodents, yet they are important in androgen action and prostate research. This has led a number of researchers to utilize human cells or tissues in BPH research. Specifically, human xenografts [25–27] or human tissue recombination xenograft models [28] have been developed and studied extensively. The use of xenografts is particularly well suited for studies evaluating maintenance or treatment of BPH, however with all xenograft studies several drawbacks apply. They are less suitable for researching the development and prevention of BPH. Additionally, use of immunocompromised mouse or rat hosts make xenograft studies less appealing for evaluating BPH in the context of an intact immune system. Lastly, although no animal model can evaluate LUTS directly, analysis of secondary complications due to BPH (e.g. BOO) is not possible with xenograft models.

Tissue recombination, a technique that utilizes epithelia and stroma from various species or organs, has successfully been used for the study of a wide range of normal and pathogenic states [21, 22, 29–33]. In this regard, Barclay and colleagues utilized tissue recombination methods using benign human prostatic epithelial cells (BPH-1 cell line [34]) and human stroma from BPH or normal prostates [28]. In those experiments it was found that BPH stroma significantly increased epithelial proliferation relative to control normal stroma, but importantly, malignant transformation did not occur in the BPH tissue recombinants [35]. These data are consistent with the important growth promoting role of stroma in BPH. There are distinct advantages of utilizing tissue recombination technology in BPH research. First, human cells can be employed; second, cells are commonly grown in culture first and then recombined and grown in mouse hosts. While the cells are in culture it is possible to manipulate gene expression (e.g. use of shRNA or forced expression of gene of interest) and hence evaluate its consequences, such as growth and differentiation. Furthermore, *in vitro* experiments can be inexpensively performed as proof of principle prior to *in vivo* experiments. Lastly, tissue recombination is especially useful in evaluation of stromal-epithelial interactions, which are likely to play a central role in the manifestation and maintenance of BPH.

## Spontaneous Models

Models where spontaneous BPH occur are highly desirable because they likely recapitulate the underlying pathophysiology of human disease. The only animals other than man that develop spontaneous BPH are dogs [36] and nonhuman primates [37, 38]. The logistics and costs of carrying out such experiments with these species are typically high, and as such they are used less frequently. Another limitation of spontaneous models is a lack of genetic manipulation, which restricts the use of these models for key mechanistic questions.

## Hormone induction models

Men as they age develop an increased estrogen to androgen ratio [39] coincident with the development of BPH. This concept has led to hormone induction models of BPH. Like man, dogs and rodents have hormone responsive prostates making them particularly important in BPH research. The administration of androgens and estrogens to recreate a hormonal environment similar to men as they age, reliably produces prostatic growth in dogs [24, 36, 40–46] and rats [47, 48]. Key research utilizing these models have significantly moved the field of BPH research forward although prostate anatomy in dogs and rats differs significantly from the human prostate. In particular, these prostates may grow outwardly and away from the prostatic urethra, making prostatic growth less likely to cause obstruction and affect urine flow, a key feature of human BPH. As such, BOO due to BPH has not been sufficiently described in these models. Nonetheless, obstructive voiding has been described in the dog [49]. Interestingly, encapsulating the canine prostate with a physical mesh wrapping to prevent outward expansion of the prostate leads to BOO [50]. Possibly the biggest obstacle to the utilization of many BPH models is the lack of genetic manipulation. The ability to alter the genetics of cells, tissues, and whole organisms have greatly advanced the scientific understanding of molecular mechanisms in developmental biology, cancer, and many other disciplines. Although transgenic rats and dogs are possible [51, 52] they are unlikely to surpass the mouse in availability of genetically altered pathways. Further complications with the usage of dog and rat hormone induction models are the associated cost and special housing needed for these studies. Taken together anatomic differences, limitations of transgenic technology, and high cost have made the use of dogs and rats in BPH research less ideal. Certainly many aspects of dog and rat models, as with all models, have and will continue to move the field of BPH research forward; however, new genetically tractable models of BPH with putative “LUTS” are needed.

## Mouse models

Perhaps the best genetically workable organism for BPH and BOO research is the mouse, in which gain and loss of function are easily regulated. In general, mouse models for BPH have been poorly received due to their prostatic anatomy which is similar to the rat; therefore, the challenges of modeling human BPH are similar to those encountered with rats and dogs. The prostatic lobes of the mouse, although hormonally responsive, grow outwardly from the urethra into the abdominal cavity. Thus, it is intuitive that regardless of prostate size, it may be impossible for BPH related BOO and subsequent “LUTS” to occur. In an era where genomic information is freely available and genetic alterations are easily attained, it would be beneficial to have genetically tractable models and hence utilize the power of mouse genetics in BPH research. This may be especially important for determining molecular mechanism(s) associated with specific types of BPH (i.e. epithelial, mixed, and stromal). Advantages of transgenic mouse models are the genetics can be altered via specific breeding to other genetically modified animals to evaluate specific gene interactions in the development or maintenance of prostatic hyperplasia. Additionally, costs are relatively low compared to those of larger animals and the design of experiments using many animals is comparably easy. Transgenic technology has opened the mouse model field for BPH research. Mouse models dependent upon prolactin and prostatic epithelial secretion of

prolactin have been generated and they replicate certain aspects of BPH [53–55]. The conditional prolactin mouse model develops benign prostatic growth by 10–15 months of age. In this model both prostate epithelia and stroma proliferate leading to prostates approximately 20 times the size of controls. Although dysplasia is observed in mice with prolactin overexpression, frank carcinomas have not been found. The fact that prostatic hyperplasia and dysplasia coexist in the prostate is consistent with what is found the aged prostate in man. Another BPH mouse model utilizes the ARR2PB promoter driven keratinocyte-derived chemokine; this mouse developed both epithelial hyperplasia and reactive stroma phenotypes [56]. Other models of prostatic hyperplasia include gene overexpression of FGF3 [57], FGF7 [58] and p27Kip1-gene knockout mice [59]. Interestingly, LXR null mice develop prostatic stromal hyperplasias and provide a unique model for this type of prostatic hyperplasia [60]. However, it should be recognized that effects of one gene on the induction of multifactorial disease(s) such as BPH are likely only to enhance the knowledge of the particular pathway or process evaluated. Thus, utilization of transgenic mouse models of BPH should be used with this caveat in mind.

In addition to transgenic mice, wild-type mice treated with hormones have been used to study the lower urinary tract [61]. Although, BPH was not evaluated, those experiments indicated that treatment of castrated male mice with estradiol-17 $\beta$  (E<sub>2</sub>) and DHT induced urodynamic changes consistent with BOO. Advantages of a wild-type hormone-induced mouse model over transgenic models are their ease of use, management, and cost. However, currently no such mouse models of BPH have been described, in part, because the anatomy of the prostate remains an issue in mouse models. To this end, whereas, mouse distal prostatic lobes have been extensively studied and characterized, a careful examination of the prostatic urethra has not been sufficiently examined in mice. In man, BPH-associated new prostatic growth around the urethra is a major contributor to BOO and the impediment of urine flow. In mice the prostate lobes loosely surround and grow in apposition to the rhabdosphincter, which is a striated muscular tube surrounding the murine urethra (Figure 1). As such, the prostate lobes can physically impinge upon the rhabdosphincter as shown in Figure 1. Although the prostate lobes are not rigidly encapsulated within a fibromuscular capsule (as in the human), if the prostates become hyperplastic, they may grow away or towards the rhabdosphincter. As with dogs and rats, it is unlikely that prostatic growth external to the rhabdosphincter affects urine flow, which supports the idea that the mouse is a less useful model for BPH leading to BOO. In the mouse, perhaps a more anatomically analogous situation to transition zone growth found in man would be the new prostatic growth within the periurethral space that is encompassed by the rhabdosphincter (Figure 1). It is relatively easy to imagine that expansion of prostate tissue within the periurethral space and immediately surrounding the urethral lumen could affect urethral kinetics and hence urine flow. However, to date analysis of glandular growth immediately surrounding the prostatic urethral lumen and subsequent urine flow has not been scrutinized in mice. Interestingly it has previously been demonstrated that hormones induce prostate proliferation in mice [62] and altered urodynamics are associated with hormone treatment [61]. However, in those studies periurethral proliferation and uroflow were not evaluated. Future experiments will determine if sex hormones induce new prostatic proliferation and subsequent events similar to BOO within the prostatic urethra and putative mouse “LUTS.” Such an addition to the BPH field may open new areas of basic and preclinical research in which to evaluate hormone action, therapies, and other molecular mechanisms.

## Androgens

### Androgens and BPH - past

It is now well accepted that androgens are essential for the maintenance of BPH, but this was first demonstrated in the late 19<sup>th</sup> century. In “The results of double castration in

hypertrophy of the prostate,” Dr. J. William White in 1895 elaborated his theories of the prostate’s dependence on a then-unknown energy source from the testes [63]. Two main points about the testes were suggested: that they were needed for reproduction of the species and that they were needed for preservation of male characteristics. More importantly, it was posited that some dysregulation of gonadal function coincided with hypertrophies of the prostate as a “result of misdirected energy” [63]. White presented a series of 111 cases demonstrating that bilateral castration led to decreased prostate size and improvement in urinary function in some patients [63]. These early attempts to treat enlarged prostates with castration established the critical role of androgens in the maintenance of BPH. This series claimed success rates of approximately 80%, but did not distinguish among patients with BPH and advanced prostate cancer [49]. The essential role of androgens in prostate pathology was exemplified by Huggins and Hodges, who showed that castration was an effective treatment for metastatic prostate cancer, a discovery for which Huggins shared a Nobel Prize with Peyton Rous in 1966 [64]. The basic concept that androgens are important in the maintenance of prostate disease remains critical to the standard of care for both BPH and prostate cancer today [65].

### Androgens and androgen signaling

Androgens and other sex steroid hormones are synthesized from the sterol precursor cholesterol. Testosterone (T), produced by Leydig cells of the testes, is the main circulating androgen in men, and is critical for virilization of Wolffian-duct structures, spermatogenesis and hypothalamic-pituitary-testes feedback inhibition of sex steroid production. The majority of circulating T in men is produced by the testes, with metabolism of adrenal androgens contributing to less than 1% of circulating T in eugonadal men [66]. Dihydrotestosterone (DHT) is an important metabolite of T and is considered the most potent androgen in men. During fetal development it is DHT that drives differentiation of the urogenital sinus into prostate and is responsible for virilization of the external genitalia and secondary sexual characteristics. As such, DHT may be the most important prostatic androgen in development and aging. In the prostate, T is converted to DHT by type II 5 $\alpha$ -reductase. Other androgens important in men include adrenally-derived dehydroepiandrosterone (DHEA), androstenedione, and 5 $\alpha$ -androstenedione, which can be converted to higher potency sex steroids to affect the prostate directly [39, 67–69].

Many lines of evidence support that androgens are permissive but insufficient for the induction and maintenance of BPH. For example, anti-androgen therapy with Flutamide or 5 $\alpha$ -reductase inhibitors and surgical castration causes rapid reduction in prostate size emphasizing androgen necessity [70–72]. Additionally, in castrated animals treatment with androgens induces prostatic regrowth, proliferation and increased prostate size. Contrasting this, androgens may not influence prostate growth because supplementation of men with androgens does not appear to increase the incident risk of BPH or LUTS [73, 74]. Furthermore, BPH prevalence increases with age, while levels of serum androgens decline. Thus while androgens are clearly important in BPH, other factors are likely involved.

### Androgen signaling via AR

Androgens affect gene expression in a wide variety of tissues and cell types by binding with the androgen receptor (AR), which functions as a hormone-inducible transcription factor that is a member of the nuclear hormone receptor superfamily. There are two reported isoforms of the AR protein, AR-A and AR-B and multiple splice variants are possible [75–77]. Androgen receptor-B is the predominant isoform expressed in the prostate [78], and AR variants have been linked to prostate cancer. Binding of androgens to AR triggers the receptor to form complexes with chaperone proteins and undergo post-translational modifications including phosphorylation. Simultaneously, AR dissociates from heat shock

proteins, homodimerizes and subsequently is translocated from the cytoplasm to the nucleus. Binding of co-activators and co-repressors coordinate AR regulation of gene expression in the nucleus. In addition to classical AR signaling, non-genomic signaling of the AR has also been reported. Extra-nuclear signaling induces extremely rapid changes in cellular function [79]. The effects of genomic and extra-nuclear AR signaling in prostate and BPH are unknown, however, it is likely that they are important mediators of androgen action.

Androgens during development, puberty and aging are necessary but not sufficient for development of BPH in men [29, 49, 73, 74]. Men castrated before puberty do not develop nodular hyperplasia [49] and men with hypopituitarism leading to low serum androgens do not develop BPH [80]. Nearly 100 years after the first therapeutic castrations for BPH, chemical castration with luteinizing hormone-releasing hormone agonist therapy was demonstrated to cause reversible regression of prostate growth and improvement of LUTS and peak urinary flow rates in some patients [49]. Nonsteroidal anti-androgens, such as Flutamide, suppress prostatic epithelial hyperplasia in rats implanted with mouse urogenital sinus tissue and treated with testosterone [71]. Flutamide and Bicalutamide can be used for the treatment of BPH in men, causing decreased prostate volume and PSA despite increased serum testosterone, but tend to be poorly tolerated due to side effects [72]. Alternatively anti-androgens have not fared well in the treatment of BPH and LUTS suggesting that other factors are involved in these processes [81–83].

The most important androgen in prostate and BPH may be DHT, which is converted from T by the enzyme 5 $\alpha$ -reductase. There are three types of 5 $\alpha$ -reductases: types I, II, and III; and type II is found primarily in prostatic luminal epithelial and stromal cells [84]. Compared to T, DHT has a higher affinity for AR [85]. In the prostate, interaction of DHT with AR, and the subsequent binding of DHT/AR dimerized complex to androgen response elements in the DNA causes transcription of various genes, resulting in the production of proteins such as prostate specific antigen (PSA) and regulatory proteins important for cellular growth and function. DHT stimulates several growth factors that drive cellular proliferation in the human prostate, including growth-stimulatory epidermal growth factor (EGF), keratinocyte growth factor (KGF) and insulinlike growth factors (IGFs). The activity of transforming growth factor- $\beta$  (TGF- $\beta$ ), which modulates apoptosis, is also affected by DHT [80]. More recently, the importance of DHT is recognized to be two-fold: one, to act as an androgen and two, to be metabolized into 5 $\alpha$ -androstane-3 $\beta$ -17 $\beta$ -diol (3 $\beta$ Adiol), an androgen which is also a ligand for ER $\beta$  [68, 69].

Type II 5 $\alpha$ -reductase is the predominant isozyme in the human prostate and is targeted by Finasteride, which decreased prostate size by 25% on average in men with BPH, improved LUTS, and increased urinary flow rates [86]. Another 5 $\alpha$ -reductase inhibitor, Dutasteride, inhibits 5 $\alpha$ -reductase types I and II; because type I 5 $\alpha$ -reductase is the predominant isozyme in the hair follicle it has the added benefit of increasing hair growth in male pattern hair loss [87]. Less is known about 5 $\alpha$ -reductase type III but it may also participate in prostatic proliferation because it is expressed in prostate cancers [88]. Therapy with 5 $\alpha$ -reductase inhibitors also has the favorable effect of decreased risk prostate cancer in men who are regularly screened [89]. The efficacy of 5 $\alpha$ -reductase inhibitors may be due to the dependence of prostate luminal epithelial cells on DHT for maintenance of terminal differentiation and secretory function [90] or alternatively the effects of DHT on stromal derived growth factors [91]. Men and mice with reduced expression of 5 $\alpha$ -reductase do not develop normal sized prostates [92, 93]. Furthermore, in men with congenital 5 $\alpha$ -reductase deficiency, palpable prostates are not found and normal prostatic epithelium is rare, reaffirming DHT's role in differentiation of the urogenital sinus into prostate [92]. Despite several earlier studies that reported higher levels of DHT in BPH specimens [94, 95], when compared to fresh cadaveric control prostates, BPH specimens do not contain increased



levels of DHT [96]. Despite the important role of 5 $\alpha$ -reductase inhibitors in treatment of BPH, anti-androgen based therapies are not effective in all men with BPH, and androgen supplementation does not appear to increase the risk of BPH [49, 73, 74], further suggesting that factors other than androgens are involved in BPH pathologies.

The role of AR in the prostate remains incompletely understood. In both normal human prostates and BPH specimens, AR is located in the nuclei of virtually all luminal epithelial cells, most stromal cells, and occasional basal epithelial cells [84, 97]. In normal dog prostates, all epithelial cells stain for nuclear AR [98]. Studies of canine BPH induced by treatment with androgens and estrogens showed that AR staining is present in the nucleus and cytoplasm of prostatic epithelial cells, and withdrawal of hormone treatment causes decreased epithelial and stromal AR staining [99]. Androgens and hence AR are important prostatic growth factors, as they are necessary for prostate maintenance and epithelial proliferation in castrated hosts. To further support the proliferative role of AR, prostatic over-expression of epithelial AR in mice causes increased prostate proliferation [100]. However, there is evidence contrary to the growth-promoting role of AR and androgens. For example, androgens do not stimulate prostate proliferation in intact animals with normal prostates and loss of prostatic epithelial AR in conditional knockout mice and tissue recombinants results in increased epithelial proliferation and decreased glandular differentiation [23, 101, 102]. These latter experiments suggest that epithelial AR is important in maintenance of prostatic differentiation, but acts as a suppressor of epithelial proliferation. The latter idea is consistent with AR functions in mature normal prostates, because the normal adult prostate is growth quiescent in a high androgenic environment. Further research is needed to delineate the role of various androgens, AR-signaling, and therapies targeting the androgen pathway in BPH.

### AR polymorphisms and BPH

Unlike single gene diseases such as sickle cell anemia and cystic fibrosis, BPH is a clinically heterogeneous disease that most likely results from multiple genetic, epigenetic, and environmental influences. The importance of genetic polymorphisms in BPH risk, prostate size, and clinical outcomes is incompletely understood but identification of men with high risk genotypes would be helpful for preventative measures and better understanding of biological mechanisms in BPH [103]. Expansion of polymorphic CAG tandem-repeats in exon 1 of the AR gene leads to a rare X-linked polyglutamine disorder called Kennedy's disease, or X-linked spinal and bulbar muscular atrophy [104]. Exon 1 of the AR encodes the amino-terminal transactivation domain, which is essential for transcription of AR target genes [105]. Much attention has also been paid to the role of normal variants of CAG tandem-repeats of exon 1 of AR in androgen responsive diseases, such as prostate pathologies, but the importance of this polymorphism in BPH is conflicted. The Physician's Health Study demonstrated that men with fewer CAG repeats in exon 1 of the AR gene, which is inversely correlated with AR's transcriptional activity, are more likely to have an enlarged prostate, exhibiting moderate to severe obstructive voiding symptoms and needing BPH surgery [106, 107]. However, similar studies in Japanese, Dutch, Australian and Chinese populations failed to consistently demonstrate this relationship [108–110]. A cohort study of men with BPH in Finland showed an association between the short CAG repeats in the AR gene and small prostate volume [111] supporting the findings of an earlier study of a Finnish cohort that found shorter CAG repeats were significantly less common in men with BPH [112]. These inconsistent results may be secondary to genetic differences between populations studied [111]. However, given the importance of CAG repeats on AR activity in other diseases [113] this would suggest a relevant role for AR polymorphisms in BPH.

## Estrogens

### Estrogens in men

Estradiol-17 $\beta$  (E<sub>2</sub>) is considered the most potent estrogen in men and is important for a variety of physiologic processes including bone maturation and mineralization, peak bone mass, and skin and lipid metabolism [114]. In men, the majority of circulating E<sub>2</sub> is formed from aromatization of T, mainly in fat and muscle, while up to 20% is secreted by Leydig cells of the testes [114]. However, serum levels of E<sub>2</sub> do not necessarily reflect tissue levels of E<sub>2</sub> [114]. In this regard, prostate *in situ* E<sub>2</sub> production may influence local estrogen regulated processes. Such local production of E<sub>2</sub> has been implicated in prostatic hyperplasia and loss of aromatase expression causes decreased estrogen-induced prostate proliferation [62, 115]. However, an important question remains: which estrogens or estrogen interactions affect prostate pathologies?

The prostate is commonly thought of as an androgen target tissue, but it is also an important target of estrogens. Although E<sub>2</sub> is the primary estrogen evaluated in prostate research, a number of other potential estrogenic sources may play significant positive or negative roles in the prostate, as outlined in Figure 2. These estrogens can be divided into multiple categories including those that are found systemically (in serum) or those produced *in situ* in the prostate. Local steroids with estrogen receptor agonist activity include E<sub>2</sub>, 5 $\alpha$ -androstane-3 $\beta$ , 17 $\beta$ -diol (3 $\beta$ Adiol), and 7 $\alpha$ -hydroxy-DHEA (7HD). The effects of these sex steroids are not fully appreciated but are likely to influence prostate hyperplasia. Their mechanism of action, including promotion or suppression of proliferation and differentiation is dependent upon their specificity and activation of estrogen receptors (ERs).

Estrogens within the circulation can be endogenous or exogenous (Figure 2). Commonly found endogenously derived estrogens include estrone (E<sub>1</sub>), E<sub>2</sub> and estriol (E<sub>3</sub>). E<sub>1</sub> is a weak estrogen formed mainly from peripheral aromatization of adrenal androstenedione, and as such is considered to have minimal influence on estrogenic pathways within the prostate. However, E<sub>2</sub> (as discussed above) has been shown to be a potent estrogen and a powerful inducer of prostatic proliferation. In men, serum concentrations of E<sub>3</sub>, the predominant estrogen of pregnancy, are minimal, and the potential role of E<sub>3</sub> in male physiology and in the prostate is not well understood. Recently another endogenous estrogen, 27-hydroxy-cholesterol (27HC), an oxysterol, was found to bind ERs and regulate ER mediated transcription [116]. Although the affinity of 27HC for estrogen receptors is lower compared to E<sub>2</sub>, the concentrations of 27HC in serum and tissues are significantly higher [116–120]. This suggests that 27HC may be an important regulator of ER activity in estrogen target organs such as the prostate. Circulating exogenous estrogens may also affect estrogen action in men. Serum levels of xenoestrogens are dependent upon dietary and other environmental exposures and as such, levels may vary among different populations. Such estrogens include phytoestrogens, therapeutic selective estrogen receptor modulators (SERMs), and endocrine disruptors (e.g. BPA, insecticides, etc.) (Figure 2).

Phytoestrogens are commonly associated with various diets (e.g. Western vs. Eastern diets) and are generally assumed to have a positive effect on the prostate. Phytoestrogens include polyphenols, flavonoids, and isoflavanoids, reviewed by [121]. An example of a polyphenol is resveratrol, which is commonly found in grape skins and red wine. Flavonoids are subgrouped into flavanones, flavones, flavonols, and catechins and are found in many foods including fruits, parsley, celery, kale, broccoli, chocolate, and green tea. Isoflavanoids are categorized into isoflavones, isoflavans, and coumestans and are found in foods like legumes, clover, and spinach. Phytoestrogens have been suggested have a role in the prevention of estrogen associated diseases such as prostate cancer [122]. The role of phytoestrogens in BPH remains unclear, but they may act as inhibitors of proliferation.

Therapeutic SERMs, including Raloxifene, Toremifene and diethylstilbesterol (DES), have been used for prevention of prostate cancer progression and amelioration of side effects of androgen ablation therapy, as well as, other medical conditions [123–125]. In general, SERMs are compounds which modulate the activity of ERs and may have agonist or antagonist effects in different cells types, depending on the activation or inactivation of different ERs or the differential stabilization of the conformation of ERs by individual SERMs. The anti-estrogen Toremifene has been shown to prevent prostate cancer progression and has been reported to have few side effects [124]. The utility of anti-estrogenic SERMs in BPH remains to be evaluated; however, given the importance of estrogens in the manifestation and maintenance of BPH and toleration of antiestrogens in men, therapeutic SERMs may be ideal preventatives and therapies for BPH.

The effects of various estrogens on the prostate are complex, as estrogens have both indirect and direct effects. In 1941, Huggins and Hodges demonstrated that injection of estrogens caused marked reduction of elevated acid phosphatase and improvement of prostate cancer bony metastases, similar to treatment with bilateral orchiectomy [126]. High doses of exogenous estrogens cause chemical castration due to suppression of pituitary gonadotropin secretion, leading to decreased testicular androgen secretion, lower plasma androgens and prostatic epithelial atrophy [127]. Exogenous estrogen administration also causes release of prolactin from the anterior pituitary, which is a mitogen that induces prostatic dysplasia in rats that is preventable by simultaneous administration of the dopamine agonist bromocriptine [128]. Exogenous estrogens, unopposed by androgens also act directly on the prostate to induce squamous metaplasia of the epithelium, by inducing proliferation of basal epithelial cells, which then differentiate into cells with a squamous cell phenotype [30, 129, 130]. DES, a potent synthetic exogenous estrogen, used to be administered to men with advanced prostate cancer and to pregnant women for potential prevention of miscarriage and premature birth [131]. Among men with prostate cancer the effect of DES is primarily to decrease circulating T by feedback inhibition of the hypothalamus-pituitary-gonadal axis, ultimately inhibiting neoplastic growth of the prostate. The direct effects of DES on the prostate and signaling pathways are largely unknown. The administration of DES to pregnant women exposed their fetuses, *in utero*, to this potent estrogen. Female fetuses exposed to DES have a higher risk of vaginal clear cell adenocarcinoma, and multiple teratogenic effects on the reproductive tract [131]. Male fetuses exposed to DES have a variety of urogenital malformations, including testicular hypoplasia, cryptorchidism and epididymal cysts [131]. DES exposure *in utero* has been modeled in rodents and shown to have profound effects on the prostate including increased prostate size and dysplasia later in adulthood [132–134]. Accumulating data is now becoming available for “DES sons” [135], but further studies are needed. Estrogenic exposure may have positive and/or negative effects on an organism but the exposure is dependent upon the type of estrogens/SERMs, time of life of the exposure, and stage of disease.

Another group of xenoestrogens found in the circulation are endocrine disruptors. Insecticides, in general, and in particular metabolites of dichlorodiphenyltrichloroethane (DDT), act as weak estrogens or as anti-androgens and have potentially adverse effects on male reproduction [136]. In addition, endocrine disruptors such as Bisphenol-A (BPA) have demonstrated adverse effects on the lower urogenital tract [133, 137–139]. BPA, which acts as a weak estrogen, is nearly ubiquitous in the environment and is commonly found in plastics and food containers. As such, BPA is found in high concentrations (2–3 ng/ml) in the serum of nearly all Americans [140]. Developmental BPA, DES, or E<sub>2</sub> exposure in rodents causes increased prostate susceptibility to adult-onset of dysplasia and hormonal carcinogenesis [137]. The role of BPA and other environmental estrogens in BPH has not been fully determined but the effects of endocrine disruptors are likely to be observed in estrogen target tissues such as the prostate.

## Estrogen signaling through ERs

Estrogens exert their effects on target cells and tissues through interaction with estrogen receptors (ERs), notably ER- $\alpha$  and ER- $\beta$  (Figure 3). ERs, like ARs, are ligand-modulated nuclear transcription factors (Figure 3)[141]. ER- $\beta$  is highly homologous to ER- $\alpha$  in the DNA-binding and ligand-binding domains, but differs with respect to the N-terminal A/B transactivation domain [142]. Binding of estrogen ligand to ER in the cytoplasm induces conformational changes in the ligand-binding domain, leading to interactions with co-activators, co-repressors and dimerization [143]. The estrogen-ER complex can bind directly (classical signaling) to estrogen response elements (EREs) in the promoters of target genes or indirectly (non-classical signaling) by interacting with AP1 and SP1 sites in promoters of estrogen-regulated genes [144]. Extra-nuclear signaling (i.e. non-genomic signaling) of ERs results in rapid biochemical effects, such as increased intracellular calcium or nitric oxide, or induction of enzymes, especially by phosphorylation [79].

## ER dimerization

Perhaps the least understood area of ER regulation in biological processes is ER dimerization [145]. As mentioned above, ligand bound ERs ( $\alpha$  and  $\beta$ ) form dimers. These dimers are either homodimers (ER- $\alpha/\alpha$ , ER- $\beta/\beta$ ) or heterodimers (ER- $\alpha/\beta$ ). Depending upon the ER dimerization state, whether ER- $\alpha/\alpha$ , ER- $\beta/\beta$ , or ER- $\alpha/\beta$ , it is expected that profound differences in gene expression and cell biological outcomes will be observed [145]. Although incompletely understood in the prostate, ER- $\alpha/\alpha$  homodimers and estrogens that promote their dimerization and activity are considered growth promoting, whereas ER- $\beta/\beta$  dimerization is generally considered growth inhibitory. The role of ER- $\alpha/\beta$  heterodimerization is less understood. One possibility is that an ER within a heterodimer may act in a dominant fashion: for example, in ER- $\alpha/\beta$  heterodimers, if ER- $\alpha$  acts dominantly, then ER- $\alpha$  target genes will be transcribed and *visa-versa* if ER- $\beta$  is dominant acting. Alternatively, ER- $\alpha/\beta$  heterodimers may serve to activate transcription of a unique set of genes altogether [145–148]. Thus, dimer states may dictate proliferation and differentiation in estrogen target tissues in the prostate. Which ER acts as the dominant receptor in a heterodimer is likely dependent upon the cell type, interactions with cofactors, and the stage of progression (e.g. normal or diseased cell). For many years the difficulty of evaluating dimerization states lay within the inability of methodologies to accurately determine dimerization states particularly *in vivo*. Fluorescence resonance energy transfer (FRET) has typically been used to determine receptor dimerization *in vitro*, however due to technical inaccuracies, including photobleaching and autofluorescence, other techniques have evolved including bioluminescence resonance energy transfer (BRET) [145]. Use of BRET reduces these problems associated with FRET, resulting in lowered background, increased sensitivity and quantification. Thus, *in vitro* and *in vivo* analyses of homodimerization and heterodimerization may be measured. The balance of homodimers and heterodimers within the same cell may hold the key to estrogen regulation in cell biological processes including the pathogenesis of BPH. Moreover, identifying the dominant acting ER partner in ER- $\alpha/\beta$  heterodimers may lead to the identification of new and unique target genes involved in estrogen hormone action. To date dimerization analyses in BPH disease progression is unknown, however the elucidation of dimerization states will provide a better understanding of the molecular mechanism involved in estrogen hormone action and may pave the way for future therapies for BPH and other estrogen mediated diseases.

## Estrogens in BPH

The most compelling implication of estrogens in the pathogenesis of BPH is that treatment of male dogs with androgens and estrogens leads to earlier and more extensive BPH and obstructive voiding [49]. In humans, as serum androgens decline with advancing age, serum levels of E<sub>2</sub> remain relatively constant but the net effect is an increased serum E<sub>2</sub> to T ratio,

which is associated with the development of BPH and LUTS [39, 149–151]. Some studies have demonstrated correlation of serum estrogens with prostate volume and other features of BPH [152–154] while others have failed to demonstrate this relationship [155]. There is evidence for a correlation between transition zone volume and total prostate volume with serum estrone [154]. Furthermore, a study of 49 men who underwent radical prostatectomy for low volume prostate cancer demonstrated that the volume of BPH histology in these specimens correlates with serum free T, E<sub>2</sub>, and estrone levels [156]. A strong trend for increased risk of surgical intervention for BPH was found across quintiles for serum E<sub>2</sub> concentrations in the Physician's Health Study [157].

Estrogen hormone action in the prostate is dependent upon types of estrogens and also the type of estrogen receptor. In this regard, stromal cells from normal prostates may respond differently to estrogenic ligands than BPH stromal cells. In an *in vitro* study of cells isolated from normal organ donors or from BPH specimens, normal stromal cells predominantly used rapid E<sub>2</sub> signaling, mediated by G-protein coupled receptor-30 (GPR30), but BPH stromal cells used classical ER-signaling, which was inhibited by treatment with ER antagonist [158]. This suggests that the mechanism of estrogen regulated cell growth and the role of stromal cells may be different in normal versus BPH prostates. Elucidation of the estrogen-regulated pathways in BPH may lead to better therapies targeted towards stromal components of the prostate.

### Role of ER- $\alpha$ in BPH

The precise roles of ER- $\alpha$  and ER- $\beta$  in the pathogenesis of BPH are not fully understood. It is likely that the two ER subtypes mediate diverse functions through interactions with different ligands, changes in the balance of classical versus non-classical signaling, and interactions with different co-repressors and co-activators [130]. In general, ER- $\alpha$  stimulation in the prostate results in hyperplasia, inflammation and dysplasia [115]. Induction of squamous metaplasia of the prostate depends on ER- $\alpha$ , not ER- $\beta$  [30]. Estrogens have also been postulated to mediate prostatic cancer progression via ER- $\alpha$ , as prostatic intraepithelial neoplasia does not occur in ER $\alpha$ KO mice and SERMs that bind and inhibit ER- $\alpha$  prevent prostate cancer progression in mice and men [62, 124, 159]. Collectively, these data support the role of ER- $\alpha$  as a key mediator of prostatic proliferation and differentiation.

### Role of ER- $\beta$ in BPH

A main distinction of ER- $\beta$  in the normal prostate is its localization in epithelial cells, unlike ER- $\alpha$ , which is primarily located in stromal cells. In general, ER- $\beta$  inhibits proliferation in the prostate. ER- $\beta$  knockout (ER $\beta$ KO) mice can develop prostatic hyperplasia with age, which is not observed in wild-type or ER- $\alpha$  knockout mice [160]. The prostatic hyperplasia observed in some strains of ER $\beta$ KO mice is generally attributed to the unopposed action of ER- $\alpha$  [161]. This would suggest that the ratio of ER- $\alpha$  to ER- $\beta$  is an important factor in estrogen-induced proliferation. Neonatal exposure of rats to DES causes prostatic hyperplasia and dysplasia, which likely results from concomitant upregulation of ER- $\alpha$  and downregulation of ER- $\beta$ . ER- $\beta$  has also been shown to activate apoptosis in BPH in an androgen-independent manner [162]. Given that loss of ER- $\beta$  is associated with increased proliferation and selective activation of ER- $\beta$  is associated with inhibition of proliferation in the prostate, a better understanding of the precise roles of ER- $\beta$  in BPH will likely provide insight into strategies for prevention and based on modulation of the activity of ER- $\beta$ .

### Stromal-epithelial interactions in BPH

Given the important role of the stroma in prostate organogenesis, epithelial identity, and cancer progression, its role in BPH seems likely [21–23, 163, 164]. Furthermore, steroid

hormones have important roles in prostate growth and maturation and prostate development is mediated by androgen-regulated stromal factors [21, 165]. The role of estrogens and stromal-epithelial interactions in the prostate is less clear. Prins and colleagues suggested that estrogen imprinting and prostate pathologies are mediated through stromal ER- $\alpha$  [132]. This conclusion is consistent with ER- $\alpha$  localization in the normal prostate, where ER- $\alpha$  is found primarily in the nuclei of stromal cells; however, ER- $\alpha$  is also found in epithelial cells [62, 166]. The estrogen regulated stromal-epithelial interactions involved in prostatic growth are complicated in part due to the variable temporal and spatial localization of ERs within the prostate (Figure 4). If estrogen mediated glandular growth events are controlled via the stroma it is likely that estrogen regulated stromal factors, also known as “estromedins”, are the primary mediators. Alternatively, ER- $\alpha$  may affect stromal cell proliferation directly and hence affect stromal BPH nodular growth. If estrogen mediated events are controlled by epithelia, then estrogens may mediate epithelial proliferation directly or alternatively affect epithelial estromedins which may promote stromal hyperplasias. ER- $\beta$  is found almost exclusively in the nuclei of prostatic epithelial cells [167]. However, in estrogen-treated mice, the localization of ER- $\alpha$  in the prostatic epithelium increases, while epithelial ER- $\beta$  decreases suggesting that estrogens may mediate epithelial proliferative effects directly through ER- $\alpha$  (stromal, epithelial, or both) and less through ER- $\beta$  [62]. In support of this idea, ER- $\alpha$  but not ER- $\beta$ , is necessary for prostate proliferation in estrogen treated mice [62]. Determination of estrogen hormone action in BPH remains to be elucidated.

## Therapies, hormones, and BPH

### Aromatase inhibitors

Atamestane (1-methyl-1,4-androstadiene-3,17-dione) is a competitive and specific inhibitor of aromatase, and blocks the conversion of androstenedione and T to estrone and E<sub>2</sub>, respectively [43]. Atamestane blocks the induction of prostatic hyperplasia in dogs and monkeys treated with estrogen and androstenedione [43]. In men with BPH, Atamestane causes decreased serum E<sub>2</sub> and estrone and a 15% reduction in prostate size, but does not improve LUTS or improve urine flow rates [168, 169]. Another aromatase inhibitor, TZA-2237, administered to beagles treated with testosterone and androstenedione, inhibited hormonally-induced prostate growth, and caused a lower E<sub>2</sub> concentration in the prostate, but increased levels of DHT and 5 $\alpha$ -reductase in the prostate [170]. However, combination therapy with TZA-2237 and the anti-androgen chlormadinone acetate induced decreased intraprostatic E<sub>2</sub> and decreased the smooth muscle component of the beagle prostate, but did not decrease the prostate volume or amount of the glandular epithelium [170]. In a randomized clinical trial of 88 hypogonadal men over the age of 60, treatment with the aromatase inhibitor Anastrozole for 12 months caused increased serum testosterone and DHT, modestly decreased serum E<sub>2</sub>, but did not result in changes in urinary obstructive symptoms or PSA [171]. These trials in dogs and men demonstrate that while aromatase inhibitors decreased serum concentrations of estrogens, they also produced a dose-dependent increase in peripheral androgen concentration, which may counteract the beneficial anti-estrogen effect [168]. This suggests that best role of aromatase inhibitors in the treatment of BPH may be in combination with anti-androgen therapies, such as 5 $\alpha$ -reductase inhibitors.

Trials of anti-estrogen therapies for BPH in humans are limited. In one study, 10 men were treated with Tamoxifen for 10 days prior to undergoing TURP [172]. Compared to the prostates from untreated patients, TURP specimens from the men treated with Tamoxifen have significantly fewer nuclear ARs and progesterone receptors (PRs) [172]. Anti-estrogen treatment prevents the development of BPH in castrated dogs with experimental prostatic hyperplasia induced by treatment with androstanediol and E<sub>2</sub>. When injected with Nafoxidine, a potent anti-estrogen, dogs treated with androstanediol and estradiol have smaller prostates with atrophy rather than glandular hyperplasia, which was observed in

dogs treated only with hormones [173]. Meparticin, which interferes with entero-hepatic circulation of estrogens, leading to lower serum estrogen concentration and decreased prostatic estrogen and estrogen receptor levels, decreased voiding symptoms and increased urinary flow rates [174]. Although more studies are needed, the use of antiestrogenic SERMs in the treatment or prevention of BPH holds high potential as a therapeutic in men.

### Isoflavones and phytoestrogens

Genistein is an isoflavonoid found in soybeans, and is present in high levels in the traditional Japanese and Chinese diets [175]. Genistein is a phytoestrogen, which affects both estrogen and androgen signaling pathways. Substantial efforts are now underway to characterize the potential chemoprevention roles of isoflavones in hormone responsive malignancies such as breast and prostate cancer [176]. Japanese and Chinese men also have lower rates of BPH than observed in Western populations, and given the importance of estrogen and androgen signaling in BPH, some studies have examined the potential for soy isoflavones in prevention and treatment strategies for BPH. Treatment of BPH cells derived from TURP specimens with genistein versus vehicle control revealed that genistein inhibits the growth of BPH cells in culture in a dose-dependent manner [175]. Using benign prostate epithelial cells (BPH-1), Hsu et al. showed that the soy isoflavones genistein and Daizein induced apoptosis [177]. Isoflavones are known to bind with higher affinity to ER- $\beta$  than to ER- $\alpha$  which may account in part for their distinct action compared to endogenous estrogens [178]. Given that stimulation of ER- $\beta$  inhibits proliferation in the prostate, this is consistent with isoflavone-mediated inhibition of prostatic hyperplasia in both *in vitro* and *in vivo* settings. Isoflavones may also affect availability of endogenous sex steroid hormones by modifying the activities of 3 $\beta$ -hydroxysteroid dehydrogenase, aromatase, 17 $\beta$ -hydroxysteroid dehydrogenase and 5 $\alpha$ -reductase, thus potentially altering growth and differentiation [178]. Recent evidence suggests that in addition to their anti-proliferative properties in prostate, isoflavones isolated from red clover (genistein, formononetin and biochanin A) can inhibit smooth muscle contraction in *ex vivo* rat prostate lobes [179]. Armed with a greater understanding of the role of ER- $\beta$  in BPH, and their direct effects on prostatic stroma, isoflavones could represent promising strategies for prevention of BPH.

### Resveratrol

Resveratrol (3,4,5-trihydroxystilbene) is a phytoalexin found in many plant species and products, including red wine, and is thought to be responsible for the French Paradox: a low rate of cardiovascular mortality in Southern French populations despite consumption of a diet high in saturated fat [180]. As demonstrated in numerous studies of ER- $\alpha$ -positive breast cancer cell lines, resveratrol acts as an ER- $\alpha$  agonist at low concentrations and antagonist at high concentrations [180]. This has led to the characterization of resveratrol as having a “hormetic” dose-response: stimulation at low doses and inhibition at high doses [180]. In human prostate cancer cell lines, resveratrol has consistent anti-proliferative effects [181]. Given that resveratrol represses both AR transcriptional activity and down regulates AR in a post-translational manner, without affecting levels of AR protein, this may serve as a potential mechanism for its anti-proliferative action in prostate [182, 183]. While studies of the effect of resveratrol on BPH *in vivo* and *in vitro* are limited, it remains a potential strategy for SERM treatment of prostate hyperplasia.

### Saw palmetto

Extract from the *Serenoa repens* plant, colloquially known as saw palmetto, is a common ingredient in herbal medicines for BPH, which are extremely popular. There are at least 70 varieties of saw palmetto extract on the market in the United States today [184]. Native Americans reportedly used saw palmetto berries for genitourinary disturbances, and in the 1870s first investigations of the medicinal properties of saw palmetto berries reported that

they could remedy lower urinary tract inflammation, obstruction and cystitis [184]. Permixon is the most widely studied saw palmetto extract, and contains long chain fatty acids and several phytosterols, including beta-sitosterol, campesterol and stigmasterol [184]. Permixon inhibits both isozymes of 5 $\alpha$ -reductase, but interestingly does not affect PSA expression and this effect is due to the fatty acids (lauric, linoleic, linolenic and myristic) in the extract [184]. Permixon also inhibits the binding of DHT to AR [185] and reduces the concentration of ERs in prostate tissues from patients with BPH [186]. Treatment of male rats with testosterone and E<sub>2</sub> causes growth of all prostate lobes and inflammation, particularly in the dorsal lobe, but co-treatment with Permixon inhibits the hormone-induced prostate growth and inflammation [187]. A recent Cochrane review found that while there are few trials evaluating preparations of clinically relevant doses of saw palmetto, this herbal supplement appears to be well tolerated in men, but is no better than placebo in improving overall urinary symptom scores, reducing prostate size, or affecting peak urine flow [188]. It is possible that focusing on active compounds found in saw palmetto with well-characterized hormonal effects may be a high-yield strategy for treatment in BPH. In fact, a Cochrane meta-analysis of four trials comparing a beta-sitosterol preparation to placebo in the treatment of men with BPH showed that they improved urinary symptom scores and urinary flow rates, but did not affect prostate size [189].

### Pygeum africanum

The role of herbal supplements in the treatment of BPH is debated, and this topic has been reviewed elsewhere [190]. However, we will consider the herbal supplement components with well-characterized hormonal mechanisms in the treatment of BPH. Plant extracts are extremely popular in the treatment of BPH, with American urologists estimating that up to 90% of newly referred patients with LUTS have tried or are using a form of alternative and complementary medicine, typically marketed as herbal prostate supplements [190]. Extracts of the African evergreen tree *Pygeum africanum* are a popular ingredient in herbal prostate supplements, with a mechanism related to androgen signaling in BPH. One of the active compounds of *Pygeum africanum* is N-butylbenzene-sulfonamide (NBBS), which has recently been shown to inhibit transactivation of AR by binding and inhibiting AR translocation into the nucleus [191]. Studies of the *Pygeum africanum* preparation, Tadenan, have shown that it inhibits fibroblast proliferation and has anti-estrogenic and anti-inflammatory effects mediated by inhibition of leukotrienes [192]. Further complicating the mechanism is the finding that in rabbits with experimentally induced partial BOO, high doses of *Pygeum africanum* extract protect the ischemic detrusor muscle dysfunction by protecting bladder cell membranes from lipid peroxidation [193]. A recent Cochrane review of 18 trials of *Pygeum africanum* versus placebo revealed that it causes a moderate improvement in urinary symptom scores, increases urinary flow rates and men taking it are more than twice as likely to report overall symptoms to be improved [194]. It is likely that the clinical benefit experienced by BPH patients are due to the multiple mechanisms targeting hormonal, inflammatory and bladder components of male LUTS, and is highly dependent on the brand of the supplement and the active components.

### Future challenges and directions

There is great potential in targeting androgen and estrogen pathways in the treatment and prevention of BPH. However, multiple challenges exist including: determination of ER and AR ligands, ratios of total ligands, determination of hormone action, and underlying molecular mechanisms involved in distinct types of BPH (epithelial, stromal, and mixed). To overcome these obstacles, better models of BPH are needed that reliably represent BPH in men both biologically and clinically. In this regard, the mouse, although macroscopically and anatomically distinct from men, may serve as an important model for BPH. Future studies will likely determine the relevance of mouse models of BPH.



An important area of study that is lacking in BPH research is prevention. Since the manifestation of BPH takes decades, preventatives, especially those that target ARs and/or ERs can be utilized over time to determine their efficacy for a particular individual or type of BPH. In this regard, the androgen pathway has been the most studied and clinically used. However use of SERMs in the prevention and/or treatment of BPH [129] has merit. For example, Ellem and Risbridger argued in 2007 that potential therapies based on anti-estrogen action in the prostate will depend critically on understanding the “differential effects of ER- $\alpha$  versus ER- $\beta$  activation” [129]. A potential strategy to inhibit proliferation in the prostate would be to block ER- $\alpha$  but promote ER- $\beta$  activation. Perhaps the greatest promise in future anti-estrogen strategies for BPH lies in therapy with SERMs. Tamoxifen, 4-hydroxy-Tamoxifen, Toremifene, and Raloxifene inhibit proliferation of both prostatic epithelial and stromal cells *in vitro* [195, 196]. In another study, Raloxifene and Tamoxifen did not affect proliferation induced by E<sub>2</sub> of a stromal cell line (WPMY-1) or BPH epithelial cell line (BPH-1), but both SERMs inhibited proliferation of prostate stromal cells in a rat model of prostatic hyperplasia induced by estrogen and androgen treatment [197]. In a study of seven beagles with spontaneous BPH, treatment with the SERM Tamoxifen resulted in decreased prostate volume [165]. A randomized trial of Tamoxifen in dogs with BPH showed an average 28.5% decrease in prostate volume compared to placebo [198]. SERMs are well-tolerated in men, and have been shown to ameliorate side effects of androgen ablation therapy in men with metastatic prostate cancer, reduce fracture risk and improve lipid profiles [199, 200]. The added prostate cancer chemopreventive benefit of SERMs furthers the attractiveness of these compounds as potential adjuvant or alternative therapies for men with BPH and LUTS [124].

## Summary

The roles of sex steroid hormones in the multifactorial pathogenesis of BPH are well established yet their molecular mechanisms have yet to be elucidated. Androgens signaling through androgen receptors act permissively and are necessary for the development of BPH. Androgens may also serve as a potential “pool” for metabolism to estrogens that can promote or inhibit prostatic proliferation. Estrogens, whether systemic or local, are numerous and may have beneficial growth-inhibiting effects or negative growth promoting effects in the prostate depending upon the estrogen type, timing of exposure, stage of prostate disease, ER-signaling methods, and cell type. Determination of the molecular mechanisms involved in estrogen hormone action, especially on dimerization states, affected downstream pathways, and stromal epithelial-interactions will lead to a better understanding of these processes in prostate biology, BPH pathogenesis, and other diseases. Novel and genetically tractable models for BPH are sorely needed and will assist in the genetic dissection of pathways involved with specific pathologic subtypes of BPH, as well as, BPH overall. Ultimately, a better understanding of molecular mechanisms involved in the induction and maintenance of BPH will lead to the development of better therapies in prevention and treatment of this disease.

## Acknowledgments

This work was supported by NIH grants CA123199, 1RC2 ESO18764 (WAR). Tristan Nicholson is a trainee in the Medical Scientist Training Program funded by NIH T32 GM07356. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or NIH.

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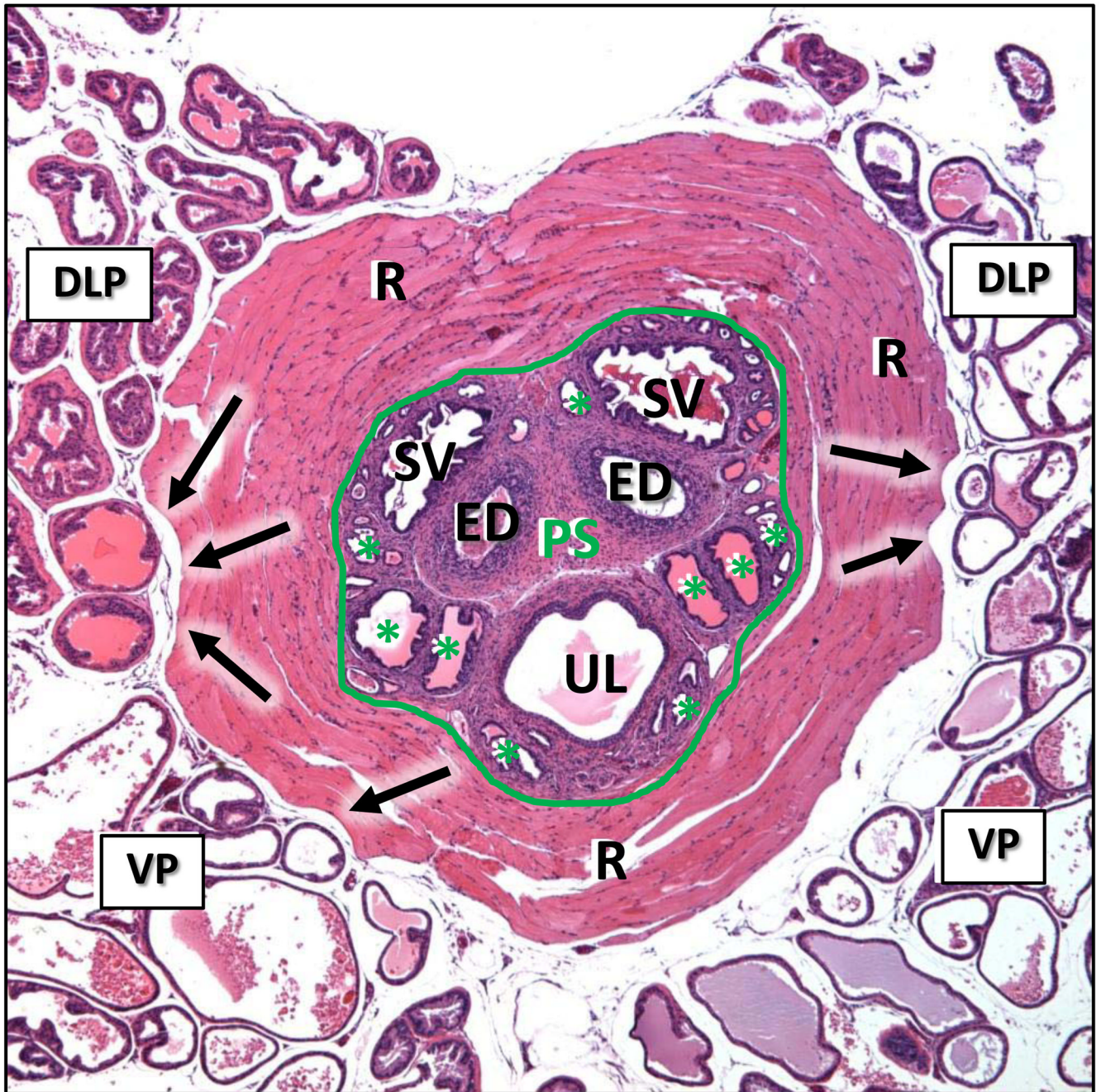


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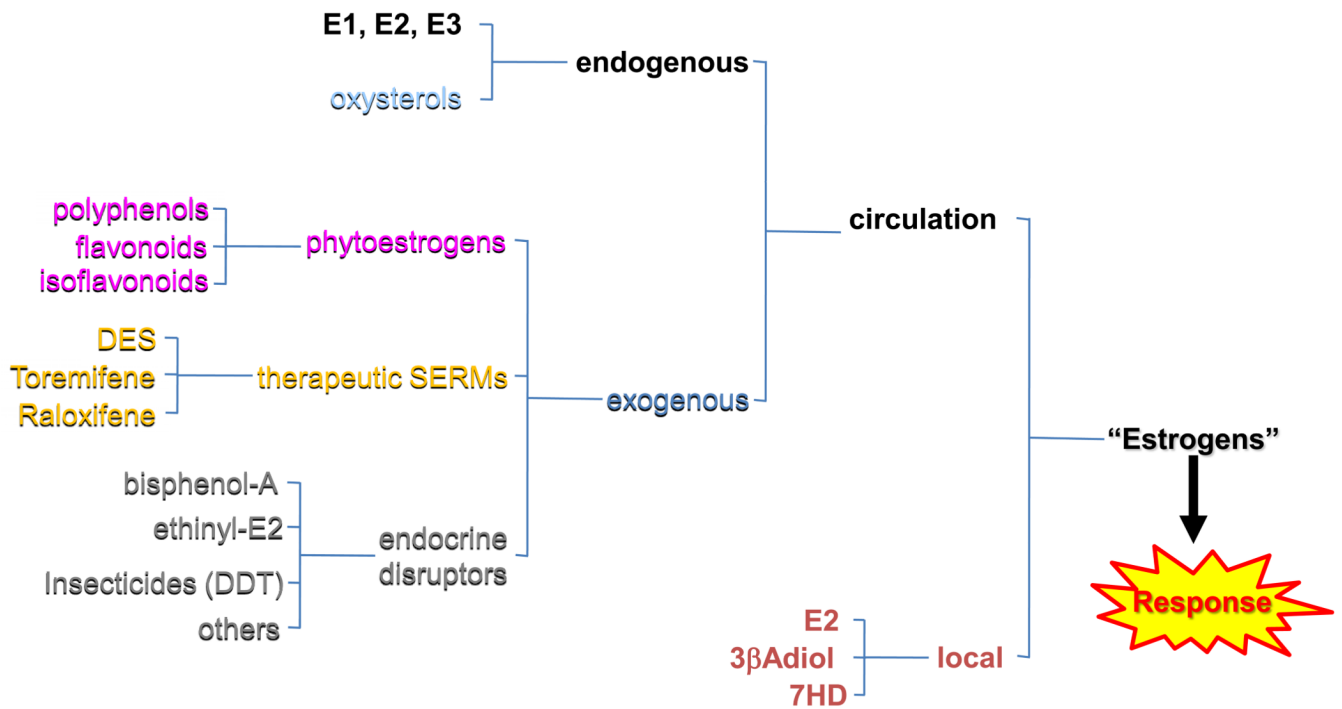
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**Figure 1.**

Anatomy of the mouse prostatic urethra. Hematoxylin and eosin stained prostatic urethra from intact adult male mouse. The mouse prostatic urethra contains 4 paired prostatic lobes: dorsolateral prostates (DLP), ventral prostates (VP) and anterior prostates (located cranially to this region), which surround the muscular rhabdosphincter (R). The prostatic lobes can grow significantly larger under experimental conditions and as such it may grow outwardly away from rhabdosphincter and urethra. The outward prostatic growth has been the rationale for not utilizing rodents as models of BPH. Note that prostatic lobes do impinge upon rhabdosphincter (black arrows). The rhabdosphincter encompasses the periurethral space (PS), which contains seminal vesicle ducts (SV), ejaculatory ducts (ED), urethral lumen

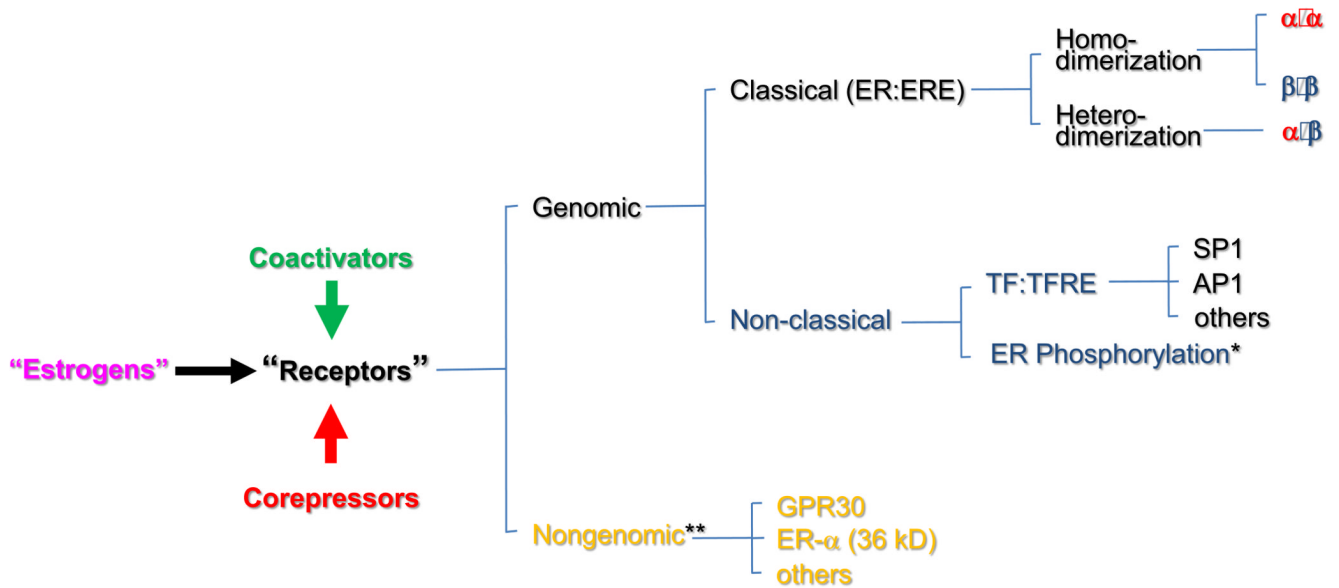
(UL), and the remaining periurethral space containing stroma and numerous prostatic glands/ducts (\*). Note not all prostatic glands within the PS are denoted).



**Figure 2.**

Estrogenic sources

Estrogenic molecules induce receptor-mediated responses throughout the body including the prostate. Estrogenic molecules can be found systemically within the circulation and locally within the prostate. Prostatic estrogen receptor ligands include E<sub>2</sub>, 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ Adiol), and 7 $\alpha$ -hydroxy-DHEA (7HD). Circulating estrogens can be further divided into endogenous estrogens: estrone (E<sub>1</sub>), E<sub>2</sub>,estriol (E<sub>3</sub>), and oxysterols, and exogenous estrogens: phytoestrogens, therapeutic selective estrogen receptor modulators (SERMs) and endocrine disrupters.

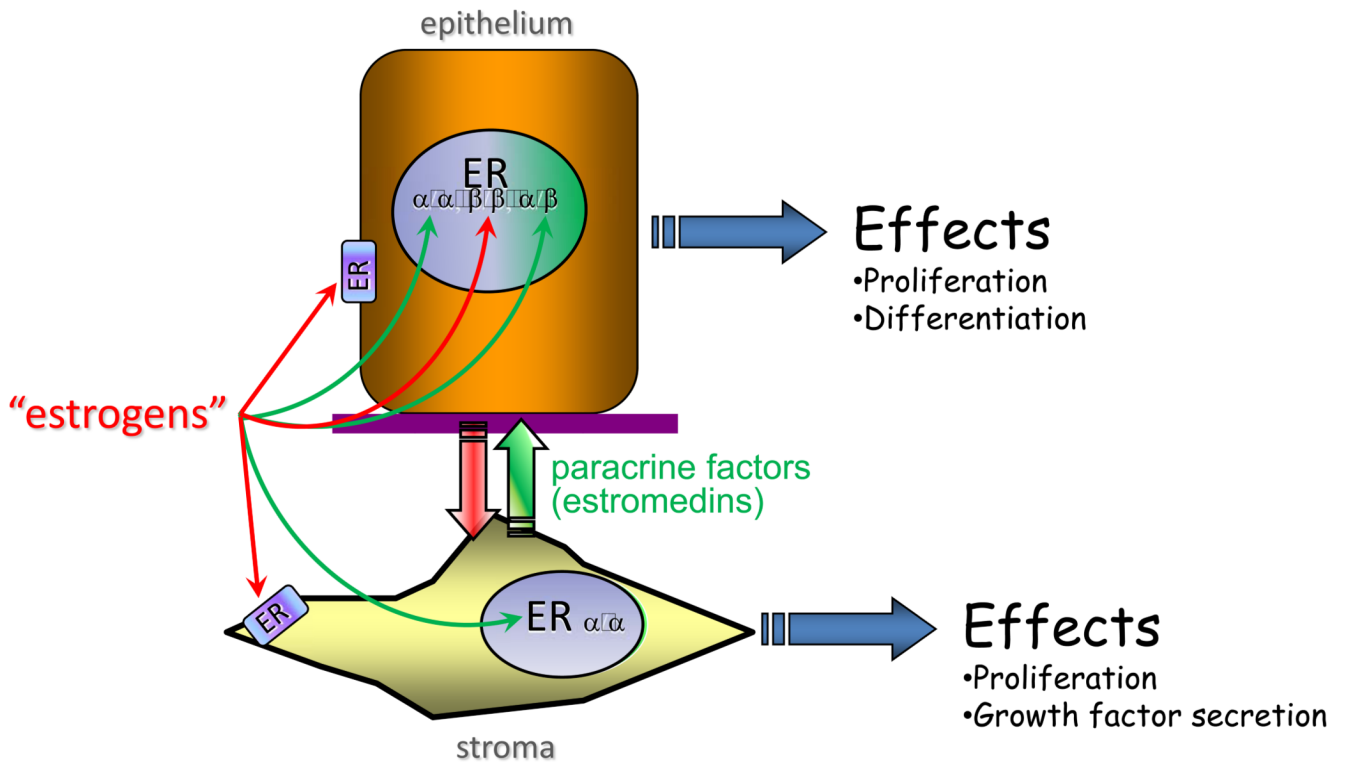


**Figure 3.**

**Mechanisms of estrogen hormone action**

Estrogens mediate their responses through estrogen receptors (ERs) via multiple methods. Ligand activated ERs are influenced by coregulators (activators and/or repressors), which affect ER activity. Activated ERs can respond genomically or non-genomically. Non-genomic responses may be mediated by G-protein linked 30 (GPR30), ER- $\alpha$ -36, or by other putative ERs. Genomic responses occur by classical and non-classical pathways. The classical ER pathway involves ERs directly binding to estrogen response elements (ERE), whereas non-classical methods involve ERs binding to various transcription factors (TF), which in turn bind to various transcription factor response elements (TFRE) such as AP-1 and SP-1. Alternatively, ERs may be phosphorylated and directly bind EREs. The classical pathway involves ligand activation of ER- $\alpha$  and/or ER- $\beta$  and a chief regulatory aspect involves dimerization. Three potential dimers can be generated ( $\alpha/\alpha$ ,  $\beta/\beta$ ,  $\alpha/\beta$ ), each with profound differences in specificity of gene transcription. \* phosphorylated ER may be ligand independent and bound to ERE. \*\*non-genomic responses are typically rapid but ultimately lead to genomic responses.





**Figure 4.**

Tissue specific estrogen hormone action

Estrogens mediate their effects through estrogen receptors (ER)- $\alpha$  and - $\beta$ , found in the epithelial and stromal compartments of the prostate. ER- $\alpha$  and ER- $\beta$  may be found in the prostate epithelium and thus ER homodimers ( $\alpha/\alpha$ ,  $\beta/\beta$ ) and heterodimers ( $\alpha/\beta$ ) may form. ER- $\alpha$  is the principal ER found in prostate stroma and thus only ER- $\alpha/\alpha$  homodimers are likely to form. Estrogens acting through epithelial or stromal receptors produce paracrine acting factors that may influence estrogen mediated events like promotion (green line) or inhibition (red line) of proliferation. Non-genomic ERs are found in stroma and epithelial cells and act to decrease proliferative events.

**Table 1**

## Benefits and drawbacks of various BPH models

Model	Benefit	Drawback
Xenograft	Human cells, BPH types	Immune function, genetics, SE
Tissue recombination	Human cells, SEI, <i>in vitro</i> and <i>in vivo</i>	Immune function, SE
Chimpanzee	Anatomy, spontaneous, <i>in situ</i>	Genetics, SH, cost, anatomy
Dog	Literature, spontaneous, <i>in situ</i>	Genetics, SH, cost, anatomy
Rat	<i>In situ</i>	Genetics, anatomy
Mouse (transgenics)	Pathway analysis, <i>in situ</i>	Lacks multifactorial initiation, anatomy

Key: stromal-epithelial interactions (SEI), special housing (SH), secondary events (SE)