CHAPTER -1

INTRODUCTION

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1. Introduction and Review Literature

The prostate is a fibromuscular exocrine gland. It is a male accessory reproductive gland which secretes a complex proteolytic solution into the urethra during ejaculation. Human prostate is a walnut shaped organ and located beneath the urinary bladder. Adult human prostate weigh around 20 grams at the age of 20.

1.1 Anatomy of Prostate Gland:

The gland surrounds the first 3 cm of the urethra (prostatic urethra) as it leaves the urinary bladder. The ejaculatory ducts enter dorsally and join the urethra within the gland either side of the prostatic utricle. The most caudal aspect of the gland, which opposes the urinary bladder, is termed the base of the gland. The walls of the prostatic urethra are highly convoluted and lined with transitional epithelium. In its resting (not distended) state, the ureter has a longitudinal ridge (the urethral crest) running the length of the gland. The majority of the ductal glands secrete into longitudinal grooves (the urethral sinuses) formed on either side of the ridge. Near the junction of the ejaculatory tubes and the urethra is a short diverticulum in the urethral crest. This is the prostatic utricle, the male vestigial remnants of the female uterus and vagina. The human prostate is a complex ductal-acinar gland, divided into three anatomical zones: peripheral, transitional and central zones, surrounded by a thick-continuous fibromuscular stroma (McNeal 1984; McNeal and Bostwick 1984; Timms et al. 1994) and covered by a thin vascularised fibrous sheath. This fibromuscular layer extends within the organ as septae, dividing the gland into ill defined lobules and functional areas. *(Fig. 1.1)*

There are three defined concentric layers of the secretory components of the gland. (I) The innermost comprises mucosal glands, concentrated around and secrete into the upper region of the prostatic urethra, (II) the middle or internal area contains submucosal gland, secrete via short ducts into the urethral sinuses and (III) the outer or peripheral area constitutes the majority of the gland, secrets via long ducts into the urethral sinuses. The anterior isthmus is an area of the gland ventral to the urethra, relatively free of glands and rich in fibromuscular tissues.

1.2 Histology of Prostate gland

At histological level, human prostate contains mainly two major cell types that are called epithelial and stromal cells. The stromal to epithelial ratio in normal prostate of human is 2:1

(Bartsch et al. 1987; Timms et al. 1994). Within the acini and tubules, the epithelium forms complex folds and papillae supported by a thin highly vascularised loose connective tissue. The most part of secretory epithelium is pseudostratified which comprises tall columnar cells and basal cells supported by a fibro-elastic stroma containing randomly orientated smooth muscle bundles. The epithelium is highly variable and containing cuboidal or squamous epithelium, which is also present with transitional epithelium in the distal regions of the longer ducts. Densely packed basal nuclei are characteristic of the prostatic epithelium. The tall columnar secretory cells have an extensive basal golgi complex, apical lysosomes and secretory granules. The epithelium contains scattered neuroendocrine cells, which partly control release and expulsion of prostatic secretions during ejaculation. *(Fig 1.2)*

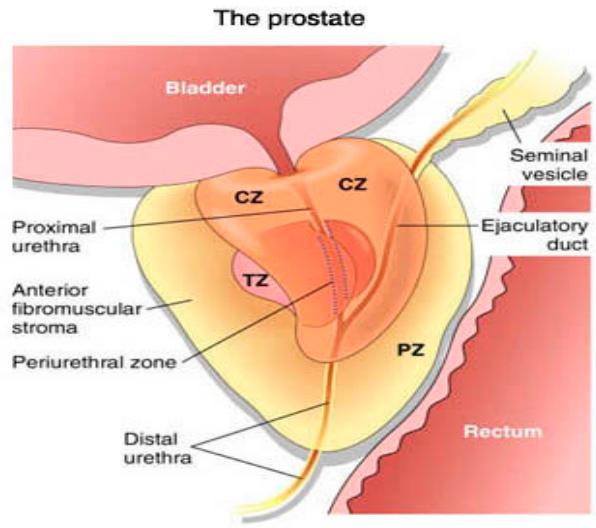

Figure 1. 2: *Prostatic Zones: PZ= Peripheral Zone, CZ= Central Zone, TZ= Transitional Zone*

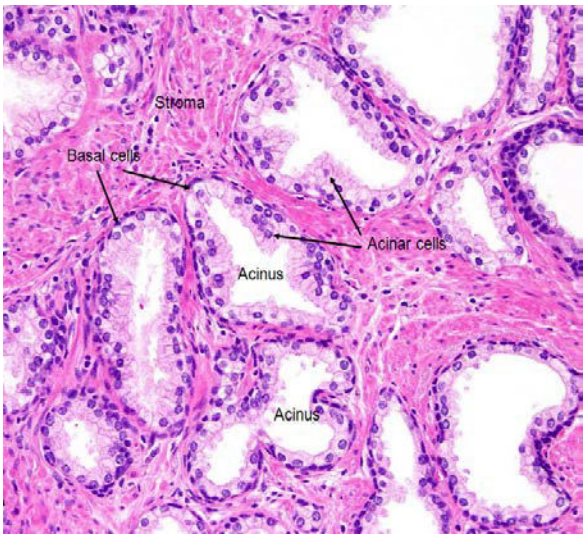

Figure 1. 1: *histology of normal prostate gland*

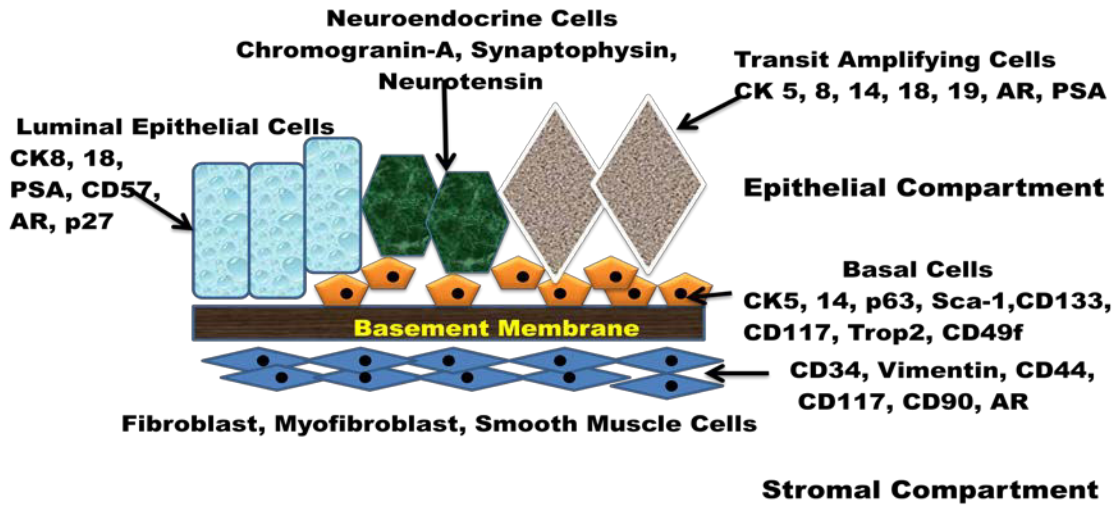

Figure 1. 3: *Prostate gland cells organization and their identity markers.*

1.3 Cellular organization and their molecular markers in adult prostate

Prostatic Epithelial cell layer is composed of four differentiated cell types known as basal, secretory luminal, neuroendocrine (NE) and transit-amplifying (TA) cells that are identified by their morphology, location and distinct marker expression *(Fig 1.3)*. The basal cells form a layer of flattened to cuboidal shaped cells above the basement membrane and express p63 (a tumor suppressor gene - p53 homolog), Bc1-2 (a pro-apoptotic factor), cluster designation (CD) 44, hepatocyte growth factor (HGF) and the high molecular weight cytokeratins (CK) 5 and 14. The expression of androgen receptor (AR) is low or undetectable in the basal cells, which makes the basal cells independent of androgens for their survival (Bonkhoff and Remberger 1993; Prins et al. 2002; Long et al. 2005). The luminal cells are the major cell type of the prostate that form a layer of columnar shaped cells above the basal layer constituting exocrine compartment that secretes prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) into lumen. They are terminally differentiated, androgen dependent, non-proliferating cells, expressing low molecular weight CK8 and 18, CD57 and p27Kip1 (a cell cycle inhibitor) (Prins et al. 2002; Long et al. 2005; De Marzo et al. 2010) along with high levels of AR . NE cells are rare cells scattered in the basal and luminal layers of the prostate. They are terminally differentiated and androgen-insensitive cells, expressing chromogranin-A, synaptophysin and neuron-specific enolase (NSF) (Bonkhoff et al. 1995; Mahapokai et al. 2000; Long et al. 2005). The NE cells also produce and secrete neuropeptides such as bombesin, calcitonin and neurotensin that are believed to support epithelial cell growth and differentiation (Abrahamsson 1999; Abate-Shen and Shen 2000; Amorino and Parsons 2004). Additionally, there is a small group of intermediate cells referred as TA cells that express both basal as well as luminal cell markers (CK5, CK8, CK 14, CK 18, AR and PSA) (Bonkhoff et al. 1994; Bonkhoff and Remberger 1996; Xue et al. 1998; Hudson et al. 2000). The epithelial layer is surrounded by a stromal layer, which forms a peripheral boundary of the prostate gland. The stromal cell layer consists of several types of cells that include smooth muscle cells (the most abundant cell type in stroma), fibroblasts and myofibroblasts. Stromal cells express mesenchymal markers like CD34, vimentin, CD44, CD117 and CD90 (Takao and Tsujimura 2008).

1.4 Prostate Gland Development in human and rodent:

In contrast to the most male accessory sex glands, which develop at embryonic stage from the Wolffian ducts (mesodermal), the gland is an endodermal structure and originates from the urogenital sinus (UGS). Although the developmental process is continuous, it can be categorized in five distinct stages involving determination, initiation or budding, branching

morphogenesis, differentiation, and pubertal maturation *(Fig.1.4)*. In human, the prostate gland development begins during the second and third trimester and completed by the time of birth (Lowsley 1912; Prins 1993). Final growth and maturation occur at puberty when circulating androgen levels rise sharply. However, as testosterone levels fall during the third trimester, the gland enters a quiescent state. The quiescent state persists until puberty, when testosterone levels again increase and the epithelium proliferates, giving rise to the complex infoldings seen in the mature gland. The prostate doubles in size during this phase of development, ARs are expressed by the epithelial cells and the full secretory phenotype is established. By 45 to 50 years, testosterone levels are in decline again and the prostate undergoes a period of involution *(Fig 1.5)*. With increasing age, atrophication of the gland may continue, though commonly, benign prostatic hypertrophy occurs.

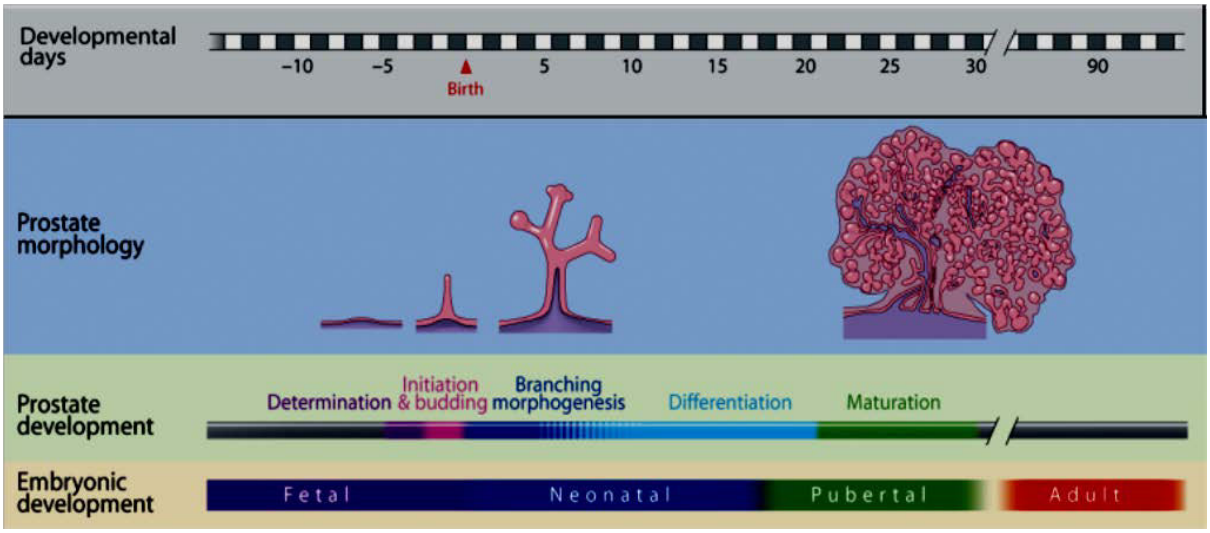

Figure 1. 4: *Prenatal to postnatal morphology and development of prostate*

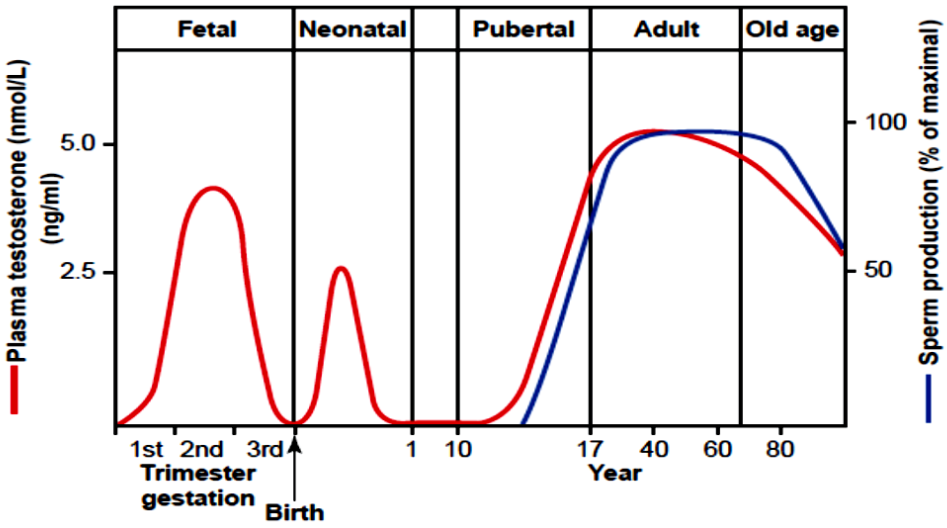

Figure 1. 5: Age dependant Testosterone level in human

In the mouse, the initial outgrowth of epithelial buds occurs between fetal days 16.5–17.5 (f16.5–17.5) in a 19 day gestational period (Sugimura et al. 1986), while in the rat it occurs at f18.5 in a 21 day gestation (Hayashi et al. 1991).

Prostate branching morphogenesis patterns of rat and mouse are lobes specific and coordinated by cell differentiation of epithelial and mesenchymal cell in the proximal-to-distal direction (Hayashi et al. 1991; Prins and Birch 1995; Hayward et al. 1996a; Hayward et al. 1996b). Epithelial differentiation from progenitor cells into differentiated basal and luminal cells has been documented in the rat prostate with changing patterns of cytokeratins as well as alterations in AR expression, an early marker of epithelial cell differentiation (Prins and Birch 1995; Hayward et al. 1996a). Concomitant with epithelial differentiation, the prostatic mesenchyme undergoes differentiation postnatally. As the prostate undergoes branching morphogenesis, a branched vascular bed forms in parallel with neovascularization forming within the prostatic stromal elements and capillary beds extending to the ductal basement membrane (Shabsigh et al. 1999)

1.5 Physiological function of the prostate

The main role of the prostate as a male reproductive organ is to produce prostatic fluid, which accounts for up to 30 percent of the semen volume *(Fig 1.6)*. Sperm motility and nourishment are aided by the prostatic fluid constituents. Prostatic fluid is a thin, milky alkaline (pH- 7.3-7.5) fluid containing citric acid, calcium, zinc, acid phosphtase and fibrinolysin (Donkervoort et al. 1977). Prostate specific antigen (PSA) is also a constituent found in prostatic secretion. PSA is a proteolytic enzyme which liquefies semen for the motility of the sperm to fertilize the ovum (egg) *(Table-1.1)*. Interestingly, only human and dogs experience abnormal growth of the prostate while other mammals are spared (Partin and Rodriguez 2002).

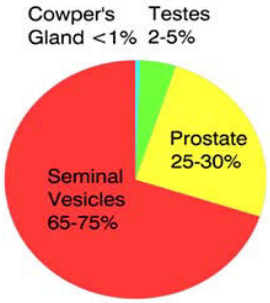

Figure 1. 6: *semen volume portion distribution*

COMPONENT	***SIGNIFICANCE***
Citric Acid	*ATP production via the Krebs cycle for sperm motility*
Proteolytic enzymes (PSA, pepsinogen, amylase, etc.)	*Break down the clotting proteins from the seminal Vesicles*
Prostatic Acid phosphatase	*Hydrolyze a broad variety of small organic phosphomonoesters under acidic conditions*
Seminalplasmin	*An antibiotic that destroys bacteria*
Prostaglandins	*Smooth Muscle contraction for sperm transport*

Table 1. 1*: Composition and significance of Prostatic fluid*

1.6 Prostate Endocrinology

The prostate is a hormonally regulated glandular organ whose growth accelerates at sexual maturity due to androgen action on both stromal and epithelial cells (Sugimura et al. 1986; Prins and Korach 2008). At embryonic stage the determination and initiation of prostatic development in the human and rodent fetus is entirely dependent upon androgens produced by the fetal testes. Surgical or chemical castration (i.e. anti-androgen administration) of rodents during critical periods of fetal life results in inhibition of prostate development (Price 1936; Jost 1953; Price and and Williams-Ashman 1961; Cunha 1973; Lasnitzki and Mizuno 1977). However, the extent of inhibition depends on the timing of androgen ablation relative to bud initiation. In the 1970s, it was determined that the primary androgen responsible for prostatic development is dihydrotestosterone (DHT), the reduced metabolite of testosterone (Wilson and Gloyna 1970). DHT is formed in the prostate epithelium by 5α-reductase and has been shown to have higher affinity for the AR as compared with the parent compound, testosterone (Fang et al. 1969). AR are highly expressed in the UGS mesenchyme before and during prostate morphogenesis whereas epithelial AR expression is induced after budding and branching morphogenesis (Shannon and Cunha 1983; Takeda et al. 1985; Husmann et al. 1991; Prins and Birch 1995).

Study has shown that AR induction in prostate epithelium begins as early as postnatal days 1–2 (before cytodifferentiation of the epithelium and mesenchyme) (Prins and Birch 1995), it is possible that androgen-driven epithelial signals contribute to morphogenesis of the prostate by affecting the differentiation of adjacent mesenchymal cells. It is noteworthy that AR expression does not vary along the proximal distal axis of the developing and adult prostate (Prins et al. 1992; Prins and Birch 1995), thus differential gene expression along this axis is likely driven by factors other than androgens. Other steroid hormones also play a crucial role during prostate development including estrogens and retinoids (Prins et al. 2001; Huang et al. 2004; Prins and Korach 2008). Specific steroid hormone receptors have been identified to understand the prostate morphogenesis in rat, which vary in a time and cell-specific manner (Prins et al. 2002). *(Fig. 1.7)*

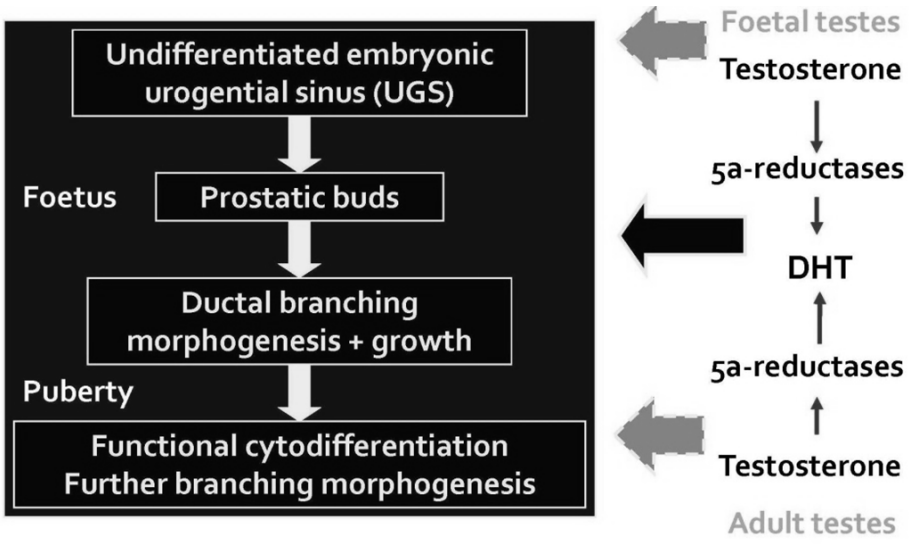

Figure 1. 7: *Steroid hormone action through specific receptors during prostatic development*

1.6.1 Androgens

The growth and maintenance of the prostate gland for its functional and structural integrity, require the constant support of an adequate level of circulating androgens (Bruchovsky et al. 1975). The testes secrete several male sex hormones, which are collectively called androgens, including testosterone, DHT, and androstenedione.

Testosterone is formed by the interstitial cells of Leydig, which lie in between the seminiferous tubules and constitute about 20 percent of the mass of the adult testes. Testes secrete large quantities of testosterone twice during life span, first when the male infant is born and the second after the puberty stage. After secretion from the testes, about 97 percent of the testosterone becomes either loosely bound with plasma albumin or more tightly bound with a beta globulin called sex hormone binding globulin and circulates in the blood in these states for 30 minutes to several hours. By that time, the testosterone either is transferred to the tissues or is degraded into inactive products that are subsequently excreted. Much of the testosterone that becomes fixed to the tissues is converted within the tissue cells to DHT by 5α-reductase enzyme, a membrane-bound enzyme that catalyzes the irreversible conversion of testosterone to DHT, with NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) as a cofactor (Chen et al. 1998), especially in certain target organs such as the prostate gland in the adult and the external genitalia of the male fetus. *(Fig 1.8)* The testosterone that does not bind to the receptors in tissues is rapidly converted, mainly by the liver, into androsterone and dehydroepiandrosterone (DHEA) and simultaneously conjugated as either glucuronides or sulfates. These are excreted either into the gut by way of the liver bile or into the urine through the kidneys.

DHT has more affinity for the AR other then testosterone. AR, (NR3C4) (subfamily 3, group C, member 4), is a nuclear receptor. AR is nuclear transcriptional regulator and activated upon binding of DHT or testosterone (Locke et al. 2008). The location of *AR* gene is Xq11-12 (X-Chromosome-qArm-11-12) (Heemers and Tindall 2007; Zhu and Kyprianou 2008) and made of 8 axons containing five AR regulatory domains *(Fig 1.9).*

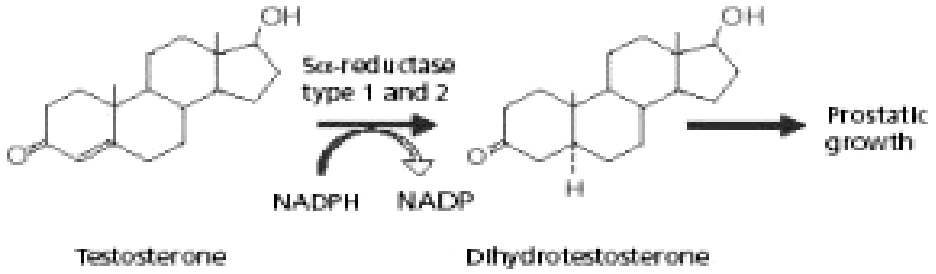

Figure 1. 8: *Conversion of testosterone into dihydrotestosterone (DHT)*

1. **N-terminal regulatory domain (NTD) A/B (Axon-1)**- N-Terminal domain is responsible for ligand activated transcriptional activity and the constitutive activity without bound ligand (Bergerat and Ceraline 2009).

2. **DNA binding domain (DBD) (Axon-2 and 3)**- This Domain contain two zinc finger domains, which functions as a DBD/LBD binding site for receptor homodimer formation (Umesono and Evans 1989).

3. **Hinge region (Axon-4)-** It is a connecting bridge between DBD with the LBD; Also contains a ligand dependent nuclear localization signal (NLS) for AR nuclear translocation by interacting with Filamin-A (Fln A) (Claessens et al. 2008).

4. **Ligand binding domain (LBD) (Axon-5, 6)-** It interacts with AF-2 and facilitates the binding of androgens, hence represents the primary key of AR signaling (Lonergan and Tindall 2011).

5. **C-terminal domain (CTD) (Axon-7, 8)-** Its interaction with NTD causes the dimerization of AR via FxxLF motifs (Lonergan and Tindall 2011).

The interaction of each domain for nuclear import of AR is regulated by interplay between each domain of the gene. These interactions aid the nuclear targeting of AR and its homodimer formation. Once inside the nucleus, AR binds to specific recognition sequences known as Androgen Response Elements (AREs) in the promoter and enhancer regions of

target genes. The AR transcriptional complex is completed by recruitment of co-regulators, which ultimately results in modulation of gene expression (Dehm and Tindall 2006).

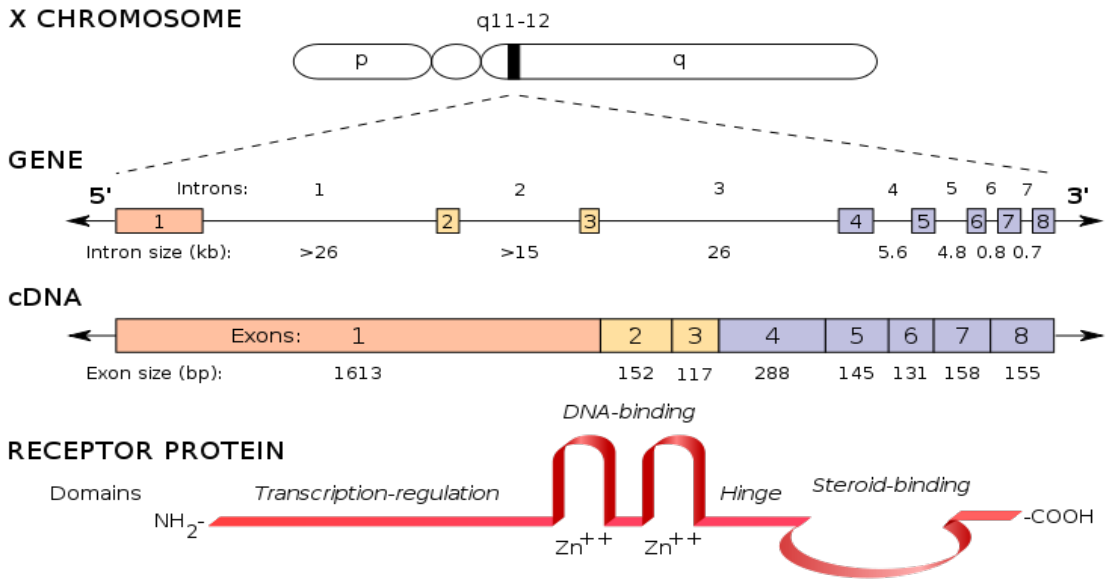

Figure 1. 9: *AR Gene Structure*

There are two isoforms of the AR protein, AR-A and B (Roberts et al. 2004; Guo et al. 2009; Watson et al. 2010) out of which, AR-B is the predominantly expressed in the prostate (Wilson and McPhaul 1996). In prostate, AR is located in the nuclei of luminal epithelial and stromal cells. It is absent in basal cells (Chodak et al. 1992; Pelletier 2008). Binding of androgen leads to AR-Heat shock proteins (HSP) complex formation in cytoplasm and stabilized by phospho-activation of AR (Zoubeidi et al. 2007). Later, AR dissociates from HSP, homodimerizes, translocates inside the nucleus from the cytoplasm and bind to the ARE, resulting in up or down regulation of specific gene transcription (Heemers and Tindall 2007). The process includes various chaperone proteins, undergo post-translational modifications including phosphorylation, co-activation and co-repression (Nicholson and Ricke 2011). Gene expression results in the production of PSA and other various regulatory proteins important for cellular growth and function. DHT stimulates several growth factors like epidermal growth factor (EGF), keratinocyte growth factor (KGF) insulin-like growth factors (IGFs) and transforming growth factor-β (TGF-β) (Carson and Rittmaster 2003) that drive cellular proliferation in the human prostate. More recently, the importance of DHT is recognized to be two-fold: one, to act as an androgen and two, to be metabolized into 5α-androstane-3β-17 β-diol (3βAdiol), an androgen which is also a ligand for ERβ (Weihua et al. 2002; Oliveira et al. 2007) *(Fig 1.10)*.

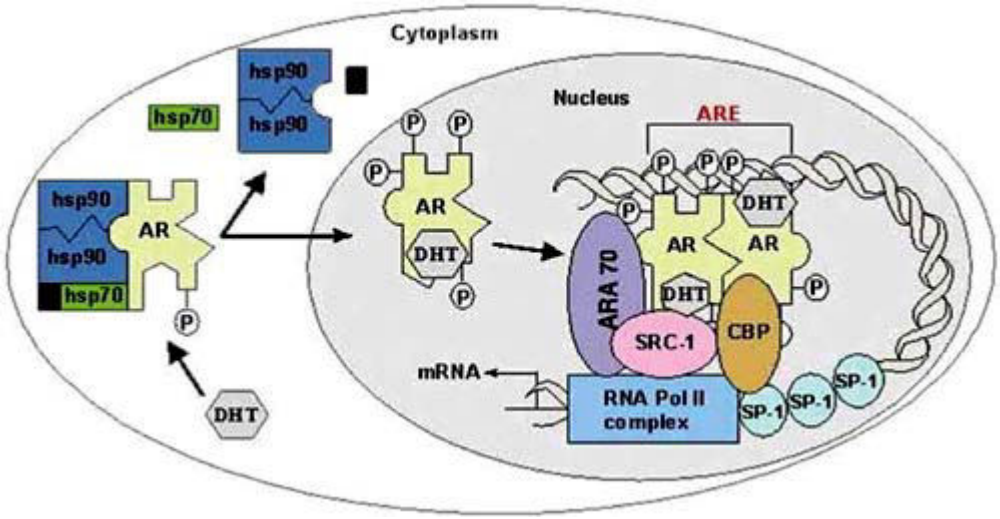

Figure 1. 10: *AR signaling in cell*

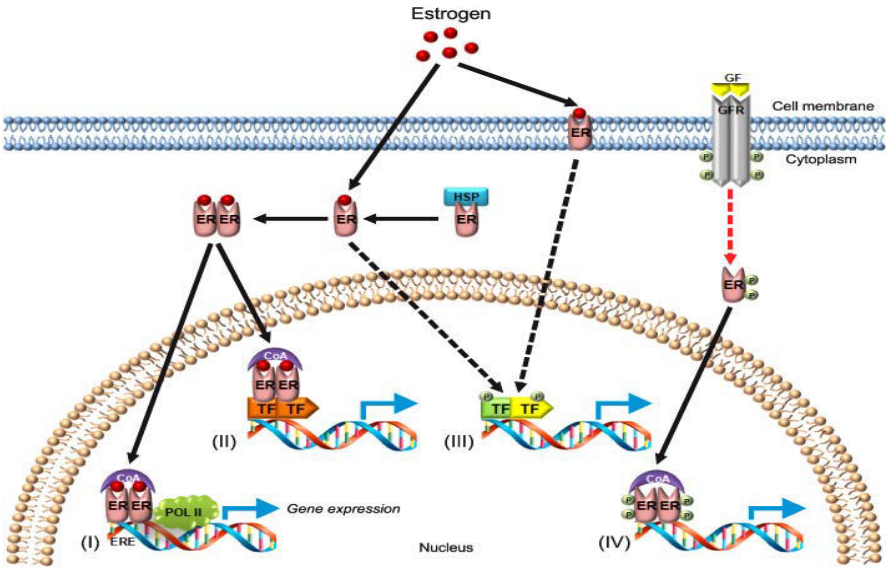

Figure 1. 11: *ER signalling in cell: Growth factor (GF), Heat Shock Protein (HSP), RNA polymerase II (POL II) and co-activator (CoA)*

1.6.2 Estrogens

In addition to testosterone, small amounts of estrogens are formed in the male (about one fifth the amount in the non-pregnant female), and a reasonable quantity of estrogens can be recovered from man's urine. The exact source of estrogens in the male is unclear, but the following are known: (1) the concentration of estrogens in the fluid of the seminiferous tubules is quite high and probably plays an important role in spermatogenesis. This estrogen is believed to be formed by the sertoli cells by converting testosterone to estradiol. (2) Much larger amounts of estrogens are formed from testosterone and androstanediol in other tissues of the body, especially the liver, probably accounting for as much as 80 percent of the total male estrogen production. Estrogens exert inhibitory and/or stimulatory effects on prostate gland. They may act by inhibiting the hypothalamic/pituitary testicular axis or directly at the

prostate through receptor mediated effect. Estrogen receptor (ER) has different subtypes, ERα and ERβ (Jin et al. 1996; Chang and Prins 1999).

ERα and ERβ genes locate on the chromosome 6 and chromosome 14 respectively, demonstrating that they are encoded by separate genes and are distinct from each other. ERα and ERβ share high amino acid homology (DNA binding domain, 95 percent; ligand binding domain, 55 percent), have the same affinity for estradiol and can heterodimerize or homodimerize to form a signaling dimeric complex. Both prostatic stroma and epithelium express ERs and estrogens, which are clearly implicated in the growth of prostate. ERβ locates primarily in the epithelium but ERα is in the stroma.

There are four mechanisms of ER signalling have been reported: I) Classical ligand-dependent II) Non-classical ligand-dependent III) Non-Genomic Ligand-dependent IV) ligand-independent *(Fig. 1.11)*. The classical ligand-dependent mechanism of ER begins with the activation of conformational changes in ER and promoting homodimerization, followed by binding to specific DNA sequence called Estrogen response elements (EREs) and activation of transcription cofactor proteins (McKENNA and O'MALLEY 2001). Non-classical ligand-dependent: genomic target with indirect binding actions; estrogen-bound ER dimers interact with transcription factors, followed by regulations of target genes. Non-genomic ligand-dependent targeted actions through estrogen-ER complex formation and phosphorylation (P) leads to activation of transcription factors (TF) and target proteins in nucleus (Kushner et al. 2000). In ligand-independent genomic target, polypeptide growth factors, such as epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) etc., phosphorylates ER and modulates the expression of ER genes via various cellular pathways (Smith 1998; Kushner et al. 2000).

1.7 Prostatic disorders

There are mainly three types of pathological conditions in the prostate gland: *(Fig 1.12)*

1) Inflammation of the prostate gland (Prostatitis)
2) Prostate Enlargement
    a) Benign Prostatic Hyperplasia (BPH)
    b) Prostate Cancer (PCa)

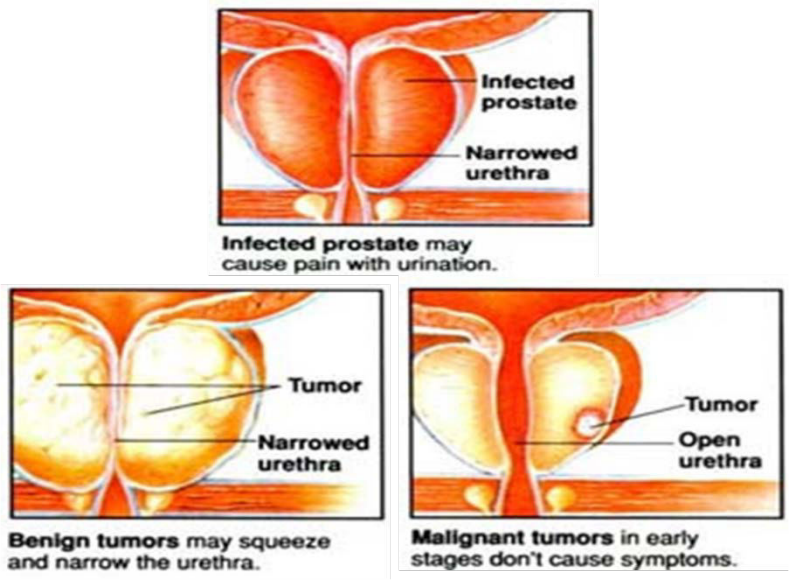

Figure 1. 12: *Prostatic disorders*

1.7.1 Prostatitis:

Prostatitis is an acute or chronic bacterial infection of the gland, occasionally progressing to a debilitating illness. It is a frequently painful condition that involves inflammation of the prostate and sometimes the areas around the prostate. Clinical symptoms are dribbling, testicular pain, urethral burning etc.

Four types of Prostatitis have been identified:

- Chronic prostatitis/chronic pelvic pain syndrome
- Acute bacterial prostatitis
- Chronic bacterial prostatitis
- Asymptomatic inflammatory prostatitis

1.7.2 Prostate enlargement:

Prostate gland enlargement is a common condition in elderly men. It is also called as Benign Prostate Hyperplasia (BPH) (increase in cell number), prostatic hypertrophy (increase in cell volume) and neoplasia (abnormal growth of tissue leading to carcinoma) *(Fig 1.13)*. Prostate gland enlargement can cause bothersome urinary symptoms such as Lower Urinary Track Symptoms (LUTS).

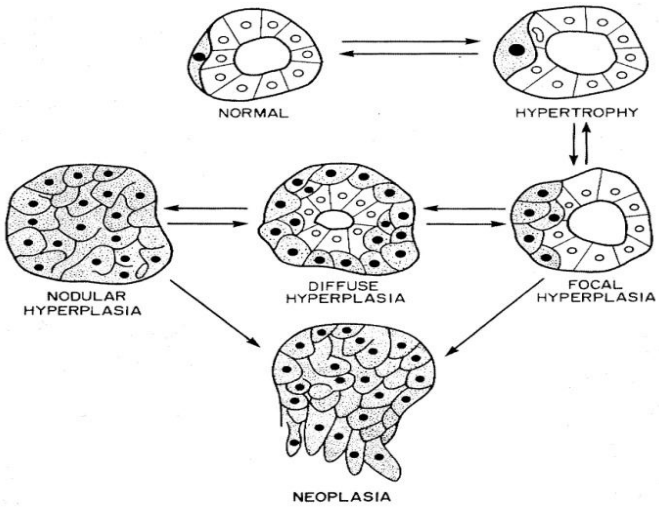

Figure 1. 13: *Pathological conditions of prostate*

1.7.2.A: Benign Prostatic Hyperplasia (BPH)

BPH is a slow progressive enlargement of the prostate gland which can lead to LUTS in elderly men. It is characterized by hyper-proliferation of epithelial and stromal cells in the transition zone of the prostate gland, which can be observed histo-pathologically (Schuster and Schuster 1999) *(Fig 1.14).*

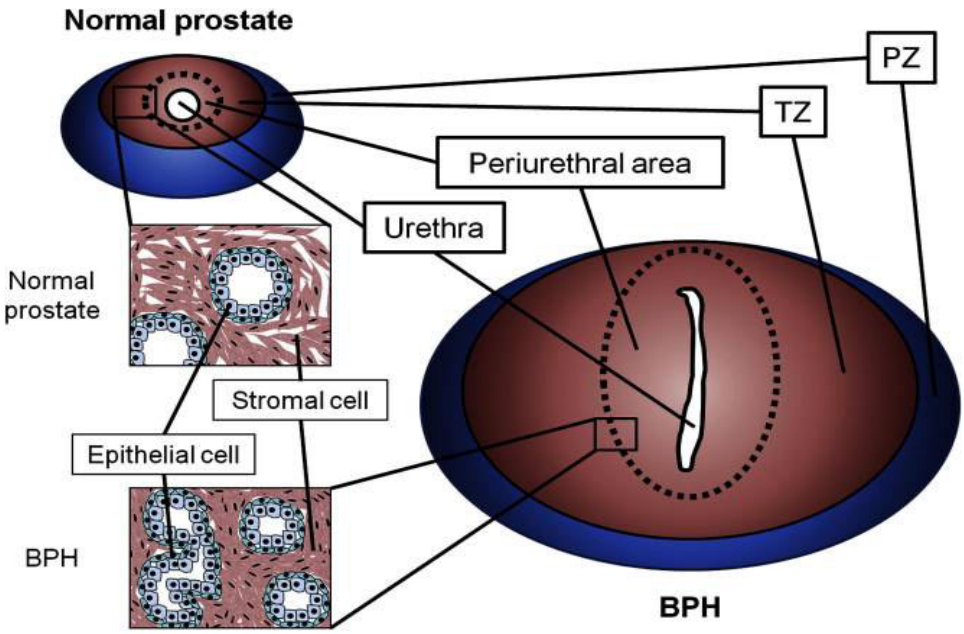

Figure 1. 14: *Benign prostate hyperplasia*

1.7.2.1: Epidemiology

The prevalence of BPH increases markedly with increasing age. Autopsy studies have observed a histological prevalence of 8%, 50% and 80% in the 4^{th}, 6^{th} and 9^{th} decades of life, respectively (Patel and Parsons 2014). Multiple observational studies from Europe, the US and Asia have demonstrated older age to be a risk factor for BPH onset and clinical

progression by several different metrics. Studies have also demonstrated geographic heterogeneity in prostate volume and LUTS prevalence. Significantly lower prostate volumes have been observed in men from South east Asia compared to western populations (Jin et al. 1999).

1.7.2.2: Pathophysiology

Despite of its obvious importance as a major health problem, little is known in terms of biological processes that contribute to the development of BPH. To explain the etiology behind the pathogenesis of BPH, several theories, including stem cell, hormonal imbalance, apoptosis, epithelial-mesenchymal transition, embryonic awakening and inflammation, have been proposed in recent years, all of them seem to contribute together to some extent in the pathogenesis of BPH (Schenk et al. 2011; Notara and Ahmed 2012). According to stem cell theory, the stem cell population residing in the prostate gland is increased due to abnormal proliferation and down regulated apoptosis of stem cells, which may eventually contribute to BPH pathogenesis. Earlier, it was reported by Berry et al. that stem cell population is responsible for prostate gland maintenance (Berry et al. 1986). Changes in tissue consistency and cellular hyperplasia are accompanied by down regulation of apoptotic factors and increased level of anti-apoptotic factors that decrease the rate of prostatic cell death and thus, contributing to hyper-proliferation of prostatic tissue (Zhu and Kyprianou 2008). It has been reported that stromal to epithelial ratio is altered in BPH, where the ratio increases from 2:1 in normal glands to 5:1 in BPH (Shapiro et al. 1992). Stromal hyper-proliferative activity is thought to promote the development of BPH as the existence of adult stem cells in the prostate stromal compartment is speculated to expand the stroma in response to stimuli during the pathogenesis of BPH (Cramer et al. 2003).

1.7.2.3: Role of Hormonal imbalance in BPH pathogenesis:

Androgens play an important role in normal and abnormal prostate development (Bosland 2000). Both testosterone and DHT have been shown to induce prostate adenocarcinomas in experimental rat models (Noble 1977). It is known that AR signaling plays a crucial role in BPH development, and that blockade of this signaling decreases BPH volume and can relieve lower urinary tract symptoms, but the mechanisms of AR signaling in BPH development remains unclear, and the effectiveness of current drugs for treating BPH is still limited.

Tang et al found no difference in AR expression between the peripheral zone (PZ) and the transitional zone (TZ) in BPH (Tang et al. 2007). In other studies, although nucleic AR expression was detected in both epithelial and stromal cells of hyperplastic nodules, higher

expression was detected in prostate epithelial cells than in stromal cells (Kyprianou and Davies 1986; Peters and Barrack 1987). Others found higher 5-α reductase activity in stromal cells than in epithelial cells, with AR distributed evenly between epithelial and stromal cells (Krieg et al. 1983). Importantly, the primitive BPH nodules found in the periurethral area of the TZ had higher concentrations of androgens and higher nuclear AR expression than those in other prostate regions (Izumi et al. 2013). Increased prostatic androgen levels may increase the chance for prostate enlargement. Recently, O'malley *et al.,* 2009 succeeded in quantifying the expression of four different androgen-responsive genes—ELL associated factor 2 (*EAF2*, also known as *U19*), elongation factor, RNA polymerase II, 2 (*ELL2*), FK506 binding protein 5 (*FKBP5*), and phosphoserine aminotransferase 1 (*PSAT1*, also known as *PSA*)—in either BPH or normal tissue (O'Malley et al. 2009). They demonstrated that all of the assayed genes displayed increased expression in BPH as compared with the adjacent normal glandular tissue. Data from several mouse models with selective knockout of AR in stromal smooth-muscle cells and/or fibroblasts indicate that the AR in stromal cells can also promote BPH development.

These findings suggest that androgen may play important roles in promoting the proliferation of epithelial and stromal cells in the periurethral area of the TZ, thus leading to development of BPH with urinary obstruction. The detailed mechanisms of androgen/AR signaling need to be clarified and new therapies are needed for better treatment of BPH patients.

The prostate is also a target tissue of estrogens. Estrogens exert their effects on target cells and tissues through interaction with estrogen receptors (ERs), namely ERα and ERβ. Estrogens have been implicated as a cause of benign prostatic hyperplasia (Ho et al. 2008). Excessive estrogenization during prostatic development may contribute to the high incidence of benign prostatic hyperplasia and prostatic carcinoma currently observed in the aging male population (Santti et al. 1994). An animal model of BPH showed that estradiol exerted a synergistic effect with androgens in inducing glandular prostatic hyperplasia in castrated dogs , whereas castrated dogs treated with estradiol alone developed squamous prostate epithelial metaplasia (Ho et al. 2008). Studies also reported associations between aromatase polymorphisms and altered risks of prostatic hyperplasia (Azzouzi et al. 2002; Roberts et al. 2006). Long term treatment of adult animals with estrogen or androgen leads to prostatic neoplasia (Messina et al. 1994).

The expression of ERα and ERβ in malignant prostate tissue remains unresolved (Enmark et al. 1997; Griffiths et al. 1998). In general, ERα stimulation in the prostate results in

hyperplasia, inflammation and dysplasia (Ellem and Risbridger 2009). Induction of squamous metaplasia of the prostate depends on ERα, not ERβ (Ricke et al. 2006). Estrogens have also been postulated to mediate prostatic cancer progression via ERα, as prostatic intraepithelial neoplasia does not occur in ERαKO mice and Selective ER Modulators (SERMs) that bind and inhibit ERα prevent PCa progression in mice and men.

In mice, whose ERβ gene has been inactivated (Beta Estrogen Receptor Knockout (BERKO) mice) epithelial cells in the ventral prostate are not at G0 but are always in the cell cycle. As BERKO mice age, there is a progressive hyperplasia and at 2 years of age Prostatic intraepithelial neoplasia (PIN) lesions are present. One possible mechanism for this prostate phenotype is that ERβ is a pro-apoptotic gene and that its inactivation results in accumulation of cells normally destined for death. If this is the role for ERβ in the prostate, BERKO mice will be very useful for the *in-vivo* study of estrogen effects on the cell cycle and for characterization of the cellular changes which occur as epithelium progresses from hyperplasia to dysplasia and carcinoma (Dupont et al. 2000). If ERβ does have a regulatory function in the prostate, ligands for the receptor would be useful in controlling proliferative diseases of the prostate. The major estrogen in mouse ventral prostate extracts is not estradiol, but 5α-androstane-3β, 17β- diol (3βAdiol). If 3βAdiol is the ligand for ERβ in the prostate, inhibition of 5α-reductase would be expected to have adverse effects on the prostate. This may explain the apparent increase in PCa in men treated with the 5α-reductase inhibitor finasteride. ERβ is the dominant estrogen receptor in prostatic epithelium and loss of ERβ results in proliferation of prostatic epithelium. It is anticipated by several reports that ERβ ligands will have a role in controlling proliferative diseases of the prostate.

1.7.2.4: Role of stem cells in BPH pathogenesis

Prostate is, structurally and functionally, a highly complex organ composed of multiple differentiated cell types, including basal, luminal and neuroendocrine cells, along with small population of relatively undifferentiated cells generally known as “stem cells” that are endowed with self-renewal and differentiation capacities (Mahapokai et al. 2000).

As the adult prostate is relatively slow growing organ with limited cycles of cell proliferation and apoptosis, the possible existence of adult prostate stem cells (PSCs) was controversial for many years. Several investigations based on stem cell models have elegantly defined role of stem cells in cellular turnover and morphogenesis of normal prostate (Isaacs and Coffey 1989; Bonkhoff and Remberger 1996) Lin et al. showed that primary culture of prostate cells from BPH patients possessed many common stem cell markers, including CD30, CD44,

CD54, neuron-specific enolase (NSE), CD34, vascular endothelial growth factor receptor-1 (Flt-1) and stem cell factor (SCF, also known as KIT ligand or steel factor) (Cramer et al. 2003; Lin et al. 2007). Although the origin of these stem cells is not known, the CD49(+) CD54(+) NSE(+) SCF(+) cell marker profile of these cells suggest that they are in a lineage closely related to MSCs. They possessed ability to differentiate into myogenic, adipogenic, and osteogenic lineages (Cramer et al. 2003; Ceder et al. 2008).

This stromal population expressed vimentin (a mesenchymal marker), CD133, c-Kit and SCF, with expression profiles similar to those observed in the Cajal cells of gastrointestinal tract, which represent a subset of stem cell-like cells. Altered patterns of c-Kit expression have been reported in benign lesions of prostate and breast tissues (Simak et al. 2000; Kondi-Pafiti et al. 2010). It has been suggested that c-Kit regulates cell proliferation in prostate and plays a crucial role in the pathophysiology of BPH via altering the expression of JAK2 and STAT1pathways (Imura et al. 2012).

Expression of pluripotency markers such as Oct4A, Sox2, c-Myc and Klf4 might represent a stemness specific gene signature. A very recent study have demonstrated a relatively high expression of stemness associated genes, including Oct4A, Sox2, c-Myc, Nanog, and Klf4, in BPH as compared to normal prostate tissue (Le Magnen et al. 2013). Thus, several studies have revealed the presence of stem cells that express pluripotency-associated markers and are hyper-proliferative and capable of differentiation into different cell lineages within the hyperplastic prostate tissue. The presence of these high proliferative and plastic stem cells in the BPH tissue samples suggest that BPH could occur as a result of changes in the stem cell properties that could ultimately give rise to a clonal expansion of cell populations.

1.7.2.5: Stromal – Epithelial interaction:

Prostatic epithelial proliferation, differentiation and cell death are all subjected to controlling factors derived from adjacent stromal cells (Sensibar et al. 1991; Nemeth et al. 1997). Two important growth factors, keratinocyte growth factor (KGF) and transforming growth factor-β (TGF-β), are produced by prostatic stromal cells (Li et al. 2005). Prostatic epithelial cells contain receptor proteins for these growth factors (Watson et al. 2010); KGF is growth promoting (Li et al. 2005), and TGF-β is inhibitory to these cells (Sutkowski et al. 1992). Paracrine signals originating from the adjacent prostatic stromal cells, and resulting in growth stimulation or inhibition, are thought to be the fundamental step in the sequence of events resulting in abnormal growth in the prostate. *(Fig 1.15)*

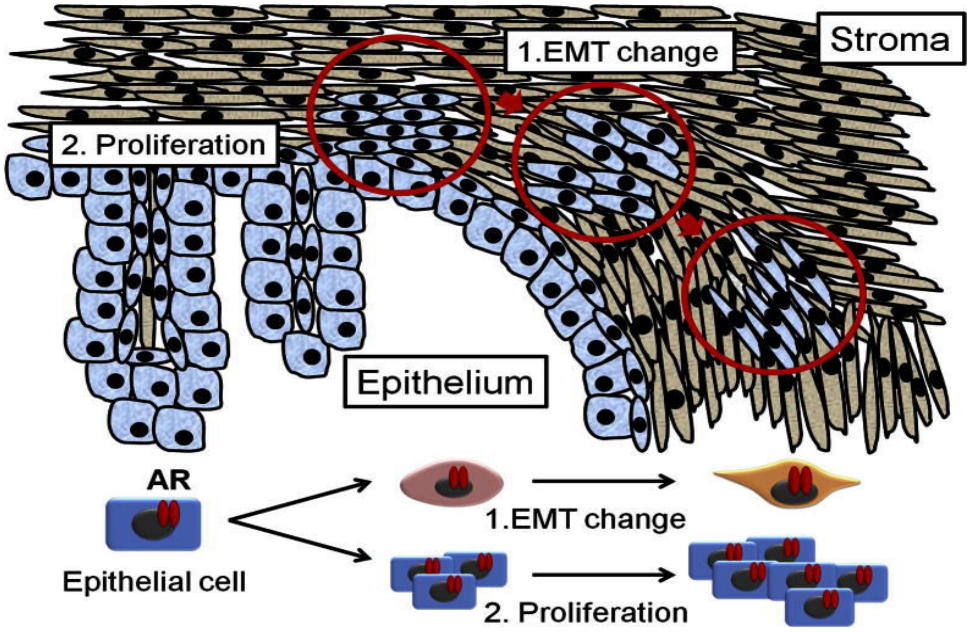

Figure 1. 15: *Epithelial- stromal interaction in the prostate*

1.7.2.6: Hereditary and Genetic polymorphism:

Clinical BPH appears to run in families. If one or more first degree relatives are affected, an individual is at greater risk of being afflicted by the disorder (Rhodes et al. 1999). Roberts *et al.,* described data from the Olmsted County Study, regarding the association between family history of BPH and moderate to severe LUTS in the month prior to interview (Roberts et al. 2004). Men with a first degree male relative with prostatic enlargement had a 30 percent increase in risk compared to men without such family members. The increase in risk was greatest among men with relatives diagnosed before the age of 60. Men with a positive family history were also 1.3 times more likely to have an impaired peak urinary flow rate (<15ml/sec).

Different genetic polymorphism are associated with prostatic disorders, out of which various single nucleotide polymorphism (SNP/Snips) have been found in the hormonal regulatory genes and are also found to be associated with BPH, PCa or with both. These Snips occurs at every 1000 base pair in the human genome. Reports suggest an association between AR gene polymorphisms and BPH (Roberts et al. 2004). Several polymorphisms in the PSA promoter have been reported which can influence serum levels of PSA. A recent study mentioned these SNPs as a predictive marker for BPH (Thompson et al. 1997). Similarly *Cramer et al.,* found number of polymorphisms in the *PSA* gene which are associated with changes in serum PSA levels (Cramer et al. 2003). Xue *et. al.,* has reported that GG genotype of AR with AG genotype of PSA may lead to rapid and early disease progression (Xue et al. 1998). The AG genotype frequency of PSA was found to be 55.5 % (OR 0.73, 95% CI 0.16-3.4).

1.7.2.7: Risk Factors

BPH is an age dependent pathology of elderly men. BPH does not develop in men castrated before the age of forty (Moore 1944). Apart from the steroid hormones and their receptors, several other factors also play a crucial role in prostate pathology.

1.7.2.7.a: Age:

Both androgens and estrogens stimulate prostate growth. Adipose tissue, which accumulates with age, aromatizes circulating testosterone into estrogen (Shibata et al. 2000), and it has been hypothesized that alterations in the balance between testosterone and estrogen levels in prostate tissue with age may contribute to benign prostatic hyperplasia (Shibata et al. 2000; De Nunzio et al. 2012).

Epidemiological studies reported that the prevalence of BPH rises greatly with increased age. It has been reported that 70% of US men between age of 60-69 years are diagnosed as BPH patients, however it increased to 80% in those 70 years or older age (Wei et al. 2005; Parsons 2010). Population-based studies of other research teams have also demonstrated similar trends (Lee and Jeong 2014). Literature suggests that there are strong genetic components to BPH pathogenesis (Parsons 2010). Case-control analysis of lineage individual having ≤64 years of age were found to be four to six fold higher age-specific risks of BPH surgery among all primary male relatives. Similar group of researchers have demonstrated that 50% of men undergoing surgery for BPH of ≤60 years of age had a genetic lineage (Pandya et al. 2013). Several other findings have also reported higher risk of BPH at young age with genetic lineage (Sanda et al. 1997; Pearson et al. 2003; Rohrmann et al. 2006).

1.7.2.7.b: Diet and Metabolic Factors:

Diet has been reported as a risk factor for the development of BPH. In a study where men eating red meat at least daily had a 38 percent increased BPH risk ($p = 0.044$) compared with vegetarians (Schenk et al. 2011). Thus, large amounts of vegetables and soy products in the diet are associated with the lower rate of BPH, as they are said to be high in phyto-oestrogens, such as genestin, that have anti-androgenic effects (Scarpa 2001). In another study done in Greece, Lagiou et. al., reported that butter and margarine may increase the risk of BPH, whereas fruit intake may reduce this risk (Lagiou et al. 1999).

Studies on BMI (Body Mass Index) have reported a positive association with prostate volume: for each 1 kg/m^2 increase in BMI, prostate volume increased by 0.41 cc (95% CI, 0.15 to 0.84; p=0.06). Compared with men with normal range BMI, overweight and obese

men had an increased odds of prostate enlargement (p=0.01); the risk for very obese men was particularly high (De Nunzio et al. 2012).

1.7.2.7.c: Environmental pollutants:

Constantly increasing environmental pollutants due to rapid industrialization, urbanization, use of diesel generators/diesel exhaust and through the scientific and technical advancement has stimulated interest in studying toxic substances and its effects on biological system (Gopalkrishnan 1998). Cadmium (Cd) and Lead (Pb) are well known endocrine disruptors that act as estrogenic or androgenic that disturbs the normal reproductive system (Stoica et al. 2000b; Martin et al. 2002a). International Agency for Research on Cancer (IARC) considered Cd and Pb as a potent human carcinogen which are of great interest to study their role in pathogenesis of BPH and PCa (Waalkes 2003; Bertin and Averbeck 2006). It has been reported that both BPH and PCa develops due to the DNA damage and mutations, which could arise through various reasons including environmental contaminants such as Cd and Pb exposure (Burcham 1999; Fenech and Ferguson 2001; De Bont and Van Larebeke 2004). Recently, Guzel et al., showed association of Cd and Pb to BPH development and progression through the altered pro-oxidant–antioxidant balance in blood and/or tissue of 25 patients of BPH (Guzel et al. 2012). Similarly, studies carried out with the trace metals like Cr, Mn, Fe, Ni, Zn and Cu also suggest the importance of metal ions either promoting or inhibiting prostatic disorders (Guntupalli et al. 2007). Studies from our lab also showed that Cd and Pb plays critical role in pathogenesis of BPH (Chirayu Pandya thesis 2009) (Pandya et al. 2013).

The metal binds with high affinity to the hormone-binding domain of both Androgen and Estrogen receptors, and activates them (Stoica et al. 2000a). Martin *et al* 2002 reported presence of epithelial proliferation and infolding in animals treated with cadmium (Martin et al. 2002b). Similarly results from our lab with BPH patients' data also demonstrated possible association of Cd content, smoking, maximum urinary flow rate (Qmax) and PAP level with severity of BPH (Pandya et al. 2013). Other agents like DDT, PCBs, insecticides like dieldrin, endosulfan, toxaphene are also the cause of BPH and PCa. Apart from environmental influence, cigarette smoking is also recognized as one of the strongest epidemiologic risk factor responsible for manifestation of BPH and cancer (Parsons 2010).

Several pesticides, chlorpyrifos, fonofos, coumaphos, phorate, permethrin, and butylate showed correlation with exposure and increased pathological conditions in the prostate with a familial history, suggesting gene–environment interactions (Alavanja et al. 2003; Mahajan et

al. 2006). However, extensive studies with DES in rodent models predict marked abnormalities in the adult prostate including increased susceptibility to adult-onset carcinogenesis following early diethylsorbitol exposures (Rajfer and Coffey 1978; Arai et al. 1983; Prins et al. 2001; Huang et al. 2004). Short exposure of bisphenol A (10 μg/kg BW/day) on neonatal rat model showed significantly increased incidence and grade of prostatic intraepithelial neoplasia (Kaufman and Vermeulen 2005). A more extensive epidemiologic study of capacitor manufacturing plant workers highly exposed to PCBs revealed a strong exposure–response relationship for PCa mortality (Prince et al. 2006).

1.7.3 *In-vivo* and *in-vitro* models for studying BPH pathogenesis:

Cellular and animal models of BPH have been studied extensively in order to find satisfactory and cost-effective therapeutic options for the patients, in which surgical intervention carries a high risk. The difficulties in obtaining long-term follow-up data as well as follow-up biopsy tissues motivated to develop animal and cell line models. The study of BPH pathogenesis is also hampered because of lack of suitable models and unavailability of commercial cell line.

Men develop spontaneous BPH and it has only been described in the dog and chimpanzees (Steiner et al. 1999). Differences in anatomic structures, limitations of transgenic technology, ethical issues and high cost have made the use of dogs and chimpanzees in BPH research less ideal. Alternatives to the study of spontaneous disease in other species are in-vivo induced BPH models by steroid hormones, xenografts and in-vitro models *(Table 1.2)* (Mahapokai et al. 2000).

MODEL	BENEFIT	DRAWBACK
Xenograft	Human cells, BPH types	Immune function, genetics
Tissue recombination	Human cells, SEI, in-vitro and in-vivo	Immune function
Human BPH cell line	Human cells, in-vitro`	Immune Function
Chimpanzee	Anatomy, spontaneous, in situ	Genetics, SH, cost, anatomy
Dog	Literature, spontaneous, in situ	Genetics, SH, cost, anatomy
Rat	In vivo, cost and time	Genetics, anatomy
Mouse (Transgenics)	Pathway analysis, in situ	Lacks multifactorial initiation, anatomy
Key: stromal–epithelial interactions (SEI); special housing (SH)		

Table 1. 2*: Different models to study BPH pathogenesis: Benefits and drawbacks*

1.7.3.1: Human Cells

Epithelial and stromal cells cultured from normal human prostates and from prostatectomy specimens from patients with BPH as an alternative for researchers to understand BPH pathogenesis. Most of the available human prostate cell lines are derived from malignancies and hence are not suitable for studying BPH.

1.7.3.2: Hormone Induced Model

As the age advances in man, estrogen to androgen ratio alters which leads to the (Belanger et al. 1994) enlargement of the prostate. This concept has led to hormone induction models of BPH. Like men, dogs and rodents have hormone responsive prostates making them particularly important in BPH research. The administration of androgens and estrogens to recreate a hormonal milieu in dog and rodents as similar to men as they age (Walsh and Wilson 1976; DeKlerk et al. 1979; Aumuller et al. 1982; Bartsch et al. 1987; Juniewicz et al. 1989; el Etreby et al. 1991; Takezawa et al. 1992; Winter et al. 1995; Constantinou and Omata 1996; Mahapokai et al. 2000; Zhou et al. 2009). Due to less ethical, similar anatomic and genetic makeup and low costs, rodents become best workable organism for BPH research. Transgenic technology has further opened the rodent model for BPH research. Other models of prostatic hyperplasia include gene over-expression of FGF3 (Muller et al. 1990), FGF7 (Kitsberg and Leder 1996) and p27Kip1-gene knockout mice (Cordon-Cardo et al. 1998). Interestingly, LXR null mice develop prostatic stromal hyperplasia and provide a unique model for this type of prostatic hyperplasia (Kim et al. 2009).

1.7.4 Treatment options for BPH

1) Medical therapy:

a) Alpha blockers
b) 5α-reductase inhibitors
c) Phytotherapy

2) Surgery:

a) Transurethral resection of the prostate(TURP)
b) Open prostatectomy
c) Minimally Invasive Surgical Therapies
d) Transurethral incision of the prostate (TUIP)
e) Laser prostatectomy

1.7.4.1a: Alpha blockers:

Prostatic tissue contains high levels of both α_1 and α_2 adrenoceptors – 98 percent of the α_1 adrenoceptors are associated with stromal elements of the prostate (Kobayashi et al. 1993). Thus α_1-receptor blockers relax smooth muscle, resulting in relief of bladder outlet obstruction that enhances urine flow (Jepson and Bruskewitz 2000). It was demonstrated in

1978 that phenoxybenzamine, a nonselective α_1/α_2 blocker, was effective in relieving the symptoms of BPH (Caine et al. 1978). Thus, α_1 selective antagonists such as terazosin, doxazosin and prazosin were developed that had fewer side effects than phenoxybenzamine (Lepor 1995). Doxazosin, alfuzosisn and terazosin have gained favour in clinical practice because they are longer acting than prazosin. Due to side effects, many $\alpha_1$selective antagonists need to be titrated and are often started at the lowest dose and built up over time to the maximal dose or a dose where clinical effects are satisfactory.

1.7.4.1b: 5α-reductase inhibitors

The enzyme 5-α reductase is crucial for the conversion of testosterone to DHT (Bartsch et al. 2002). DHT once created, enters the nuclei initiating transcription of messenger RNA (mRNA) which then synthesis proteins such as enzymes in the cytoplasm. Inhibitors of 5α reductase, finasteride and dutasteride potentially decrease serum and intra-prostatic dihydrotestosterone levels, thus reducing prostatic tissue growth (Boyle et al. 2004).

1.7.4.1c: Phytotherapy:

Phytotherapy, or the use of plant extracts, is becoming widely used in the management of many medical conditions including BPH (Wilt et al. 1998). The action mechanisms of phytotherapeutic agents are poorly understood but have been proposed to be (i) anti-inflammatory, (ii) inhibitors of 5α-reductase, and more recently (iii) through alteration in growth factors (Lowe and Fagelman 1999). The most popular phytotherapeutic agents are extracted from the seeds, barks and fruits of plants which are as follows:

1. Saw Palmetto Berry (*Serenoa repens*)
2. African plum tree (*Pygeum africanum*)
3. Pumpkin Seed (*Cucurbita pepo*)
4. Rye Pollen (*Secale cereale*)
5. Other extracts: South African Star Grass (*Hypoxis rooperi*), Opuntia (Cactus flower), stinging nettle and Pinus (Pine flower).

1.7.4.2: Surgical treatments:

The oldest and most invasive therapy for BPH is open prostectomy (Serretta et al. 2002b), commonly done through a transvesical approach, but may be done retropubically. Early complications of this operation include haemorrhage, sepsis and urinary retention with the late complication being bladder neck stricture (2-3%) (Han et al. 2002). Transurethral resection of the prostate (TURP) has lower morbidity but open prostatectomy produces

equivalent, if not superior improvement with a similar or lower re-operation rate (Serretta et al. 2002a).

Approximately 90% of prostatectomies are done by TURP, most common surgical treatment for BPH (Han et al. 2002). TURP involves surgically debulking the periurethral and transitional zones of the prostate to relieve obstruction. Debulking is done by electrocautery in the standard TURP through endoscopic instruments introduced into the urethra and bladder. Tissue is resected in small pieces until the adenoma is removed and a new channel for passage in the prostatic urethra created, much like fashioning a pumpkin for halloween with the capsule left behind.

Transurethral incision of the prostate (TUIP), a similar approach to a TURP, is used except that no surgical debulking is undertaken. Between one and three incisions are made into the prostate at the level of the bladder neck back almost to the insertion of the ejaculatory ducts. This releases the "ring" of BPH tissue at the bladder neck, creating a larger opening (Aho and Gilling 2003). There is a reduced risk of morbidity such as haemorrhage. In some instances, ejaculation may be preserved in younger men, especially if one incision is made. The procedure only works if the tissue in the periurethral area is not too bulky, otherwise a "ball-valve" mechanism of adenoma may develop.

Laser energy may be used to produce coagulation necrosis, vaporisation of tissue or resection of tissue (Chen et al. 2010). Laser as an energy source has an advantage of standard electrocautery by being relatively bloodless and does not carry the risk of hyponatraemia, which may occur via absorption of irrigant in a standard TURP (Yip et al. 2011). There are several evolving therapies for BPH involving lasers including Holmium laser resection of the prostate (HoLRP), Holmium laser enucleation of the prostate (HoLEP), Holmium laser ablation of the prostate (HoLAP), Neodymium:yttrium-aluminium-garnet (Nd:YAG) and Potassium-titanyl-phosphate (KTP) (Bouchier-Hayes et al. 2006).

1.7.5 Future Treatments

Evidence from the use of botulinum toxin in other lower urinary tract disorders of muscle spasticity (e.g. detrusor hyperreflexia, detrusor sphincter dyssynergia) has led to its expanded application in non-neurogenic conditions such as idiopathic detrusor overactivity, detrusor underactivity (Kuo 2004). Although its mechanism of action in the prostate is unclear, botulinum toxin has been shown to block the release of neurotransmitters such as acetylcholine at the neuromuscular junction as well as in autonomic neurons (Maria et al.

2003). There may also be significant inhibitory effects on urethral norepinephrine release (Brisinda et al. 2009) and intraprostatic injection of botulinum toxin induces selective denervation and subsequent atrophy of the glands via apoptosis (Kuo and Liu 2009). Thus due to the hyperplasia of connective and smooth muscular tissue in BPH, the prostate would seem to be an ideal location for botulinum toxin to have a pharmacological and clinical effect (Leippold et al. 2003).

Recently, it has been reported that Vitamin D has astonishing potential as a therapeutic agent of BPH (Manchanda et al. 2014). Vitamin D is produced in the skin by the enzymatic modification of cholesterol after exposure to ultraviolet B radiation. 1α,25 dihydroxyvitamin D3 (1α,25(OH)2D3), the active form of vitamin D, is produced in the kidney by hydroxylation of its precursor, 25-hydroxyvitamin D3 (25(OH)D3), and plays a central role in calcium homeostasis and bone remodeling (Roberts et al. 2004). It is also a potent regulator of cell growth and differentiation of prostatic cells, having an antiproliferative action on prostate cells (Peehl et al. 1994). The prostate cells can convert Vitamin-D in its active form, and are also known to express Vitamin D receptor (VDR) thus showing a potential role of Vitamin-D in prostate gland maintenance (Crescioli et al. 2005). Apart from that, many Vitamin-D analogs are known to inhibit cellular pathways, thereby inhibiting proliferation and inflammatory response (Adorini et al. 2010).

1.7.2.B: Prostate Cancer (PCa)

PCa is the most prevalent and is the second most frequently diagnosed cancer and sixth leading cause of cancer-related deaths among men in the world (Jemal et al. 2009). Its etiology, although not clear, is partly attributed to multigenic and epigenetic mechanisms (Maitland and Collins 2005; Shen and Abate-Shen 2010; Maitland et al. 2011; Oldridge et al. 2011). Gleason and others described that when the transition of normal gland into adenocarcinoma of prostate takes place, its normal histological structure is disrupted resulting in abnormal proliferation of the glandular structure, destruction of basement membrane and progressive loss of basal cells (<1%) (Gleason 1966; Maitland et al. 2011). In addition, AR(+) luminal cells increase and contribute in bulk of prostate mass (>99%) in PCa (Grisanzio and Signoretti 2008).

1.8 Link between BPH and PCa:

An association between BPH and PCa was recognized first time by Sommers , 1957 in autopsy studies of prostate gland (Sommers 1957). Since then, several studies confirmed these findings by examining the tissue histology, biochemistry, genetics and lesser extend to

epidemiology of the two diseases (Orsted et al. 2011). Histologically, BPH is defined as microscopic or macroscopic nodules with hyperplasia of stromal cells and epithelial cells and arises in the transitional zone of the prostate. Despite of its prevalence, the exact etiology of BPH is unknown. Similarly, the etiology of PCa is also not fully understood. Although, it is widely accepted that PCa is preceded by prostatic intraepithelial neoplasia (PIN) (Merrimen et al. 2009; Davidsson et al. 2011; Montironi et al. 2011), it is unclear whether PIN stems directly from normal prostatic tissue or originates from hyperplasia (De Marzo et al. 2010). *(Fig 1.16)* Despite of differences, BPH and PCa share many common features, including hormone-dependent growth and response to anti-androgen therapy (Andriole et al. 2010). Moreover, studies have indicated another common risk factors association with both the diseases such as chronic inflammation, metabolic disruption, and genetic variation (Alcaraz et al. 2009; De Nunzio et al. 2011; De Nunzio et al. 2012). Although, both diseases are affected by several common pathophysiological mechanisms *(Fig 1.17)*. It is not obvious whether, BPH is the first step in the pathway to PCa. Epidemiological study by Simons *et al* 1993 demonstrated null association between PCa and BPH, and also reported PCa related mortality in men with BPH (Simons et al. 1993). In support of the null associations, Prostate Cancer Prevention Trial (PCPT) reports also suggested that BPH is not associated with PCa incidence (Thompson et al. 1997; Schenk et al. 2011).

Contrary to the findings from these landmark trials, combined analysis of a prospective study of 340 men with BPH and a retrospective study of 290 men with PCa showed a fourfold increased risk of disease coexistence when compared with a similar number of age-matched controls (Armenian et al. 1974). Recently, autopsy studies reported similar results (Orsted et al. 2011; Orsted and Bojesen 2013).

Although the precise mechanism of tumourigenesis in prostate is still in debate, it is widely accepted that cancers can arise from normal cells which may accumulate mutation, genetic changes, and molecular pathway alterations that disrupt self-renewal control capacity. *(Table1.3)*

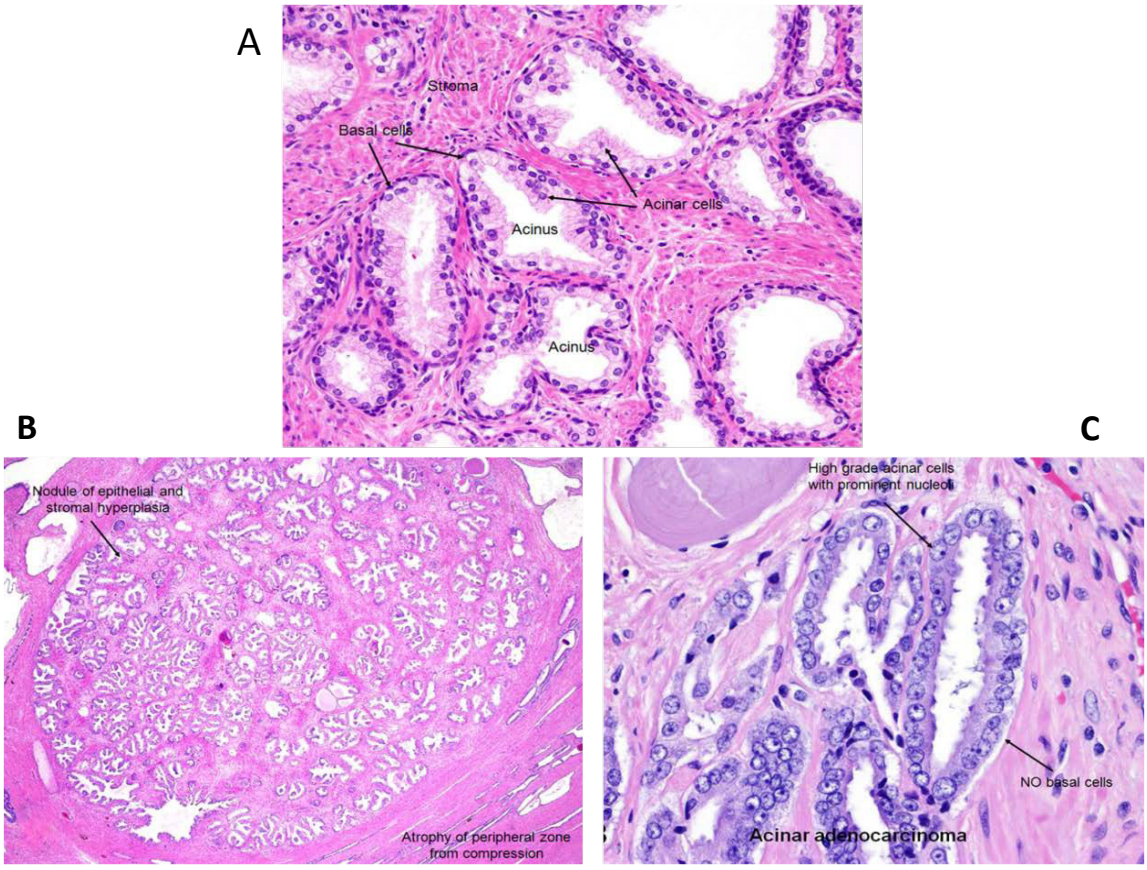

Figure 1. 16: *Comparative histology of a) Normal, b) Hyperplasic and c) malignant prostate gland*

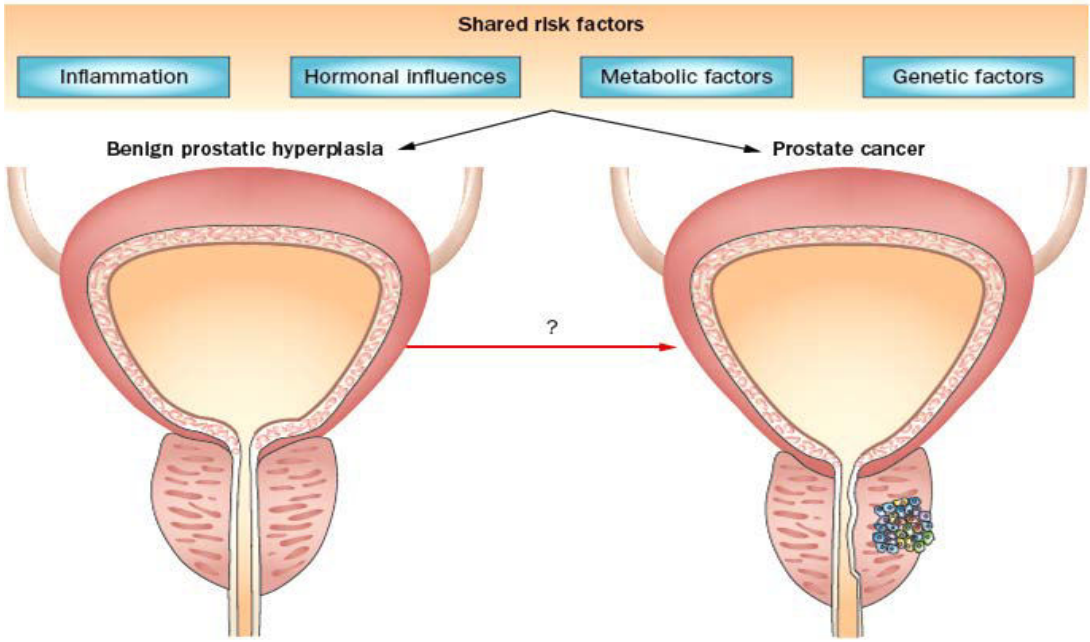

Figure 1. 17*: Shared risk factors between benign prostatic hyperplasia (BPH) and prostate cancer (depicted by black arrows). It remains unclear whether BPH is a premalignant lesion and intermediary stages (depicted by the red arrow) exist.*

Factors	Normal Prostate	BPH	PCa
Prostate specific factors			
5α-reductase	Normal	Up-regulated	Up-regulated
Androgen Receptor (AR)	Normal	Up-regulated	Up-regulated
AR co-activator	Normal	Up-regulated	Up-regulated
Androgen co-repressor	Normal	Up-regulated	Up-regulated
PSA level in serum	(0-4ng/ml)	**(2-8ng/ml)**	(4-10ng/ml)
Growth factors	FGF-2,7,9	FGF 1,2,9	FGF-1,2,**6,8**
	IGF 1,2	**IGF-2 high**	IGF-1 high
	IGFBP-2	IGFBP-3	**IGFBP-2 high** IGFBP-3 high
NE cells	Normal	**Number decease**	Number increase
Luminal cell factors	Vimentin	Vimentin increase	Vimentin over exp
	Intracellular space normal	**Intracellular space increase**	Intracellular space decrease
	PSMA normal	**PSMA decease**	PSMA increase
Basal cells	Present	**Present**	Absent
Stromal cell factor	Fibroblast content normal	Fibroblast content increase	Fibroblast content increase
	NMMHC	**NMMHC increase**	NMMHC
	Elastin	**Elastin decrease**	Elastin increase
	SMMHC	SMMHC decrease	SMMHC decrease
Stem cell markers	CD44, P63, Sca-1, CD133, CD117, Trop2, CD49f, $p27^{Kip1}$, CK 5(+), 8(-), PSCA	CD44, **p63**, Sca-1, CD133, **$p27^{Kip1}$**, CD117, Trop2, CD49f, AR, **CK5(+)**, **8(-),** PSCA high	CD44, Sca-1, CD133, CD117, Trop2, CD49f, CK5(-), PSCA high, AR

Table 1. 3: *Molecular alterations in BPH and PCa, NMMHC: non-muscle myosin heavy chain, SMMHC: Smooth Muscle myosin heavy chain, FGF: Fibroblast Growth factor, IGF: Insulin-like growth Factor, IGFBP: IGF Binding Protein, PSMA: Prostate-specific membrane antigen*

1.9 References

Abate-Shen C, Shen MM. 2000. Molecular genetics of prostate cancer. *Genes Dev* **14**: 2410-2434.

Abrahamsson PA. 1999. Neuroendocrine differentiation in prostatic carcinoma. *Prostate* **39**: 135-148.

Adorini L, Penna G, Fibbi B, Maggi M. 2010. Vitamin D receptor agonists target static, dynamic, and inflammatory components of benign prostatic hyperplasia. *Ann N Y Acad Sci* **1193**: 146-152.

Aho TF, Gilling PJ. 2003. Laser therapy for benign prostatic hyperplasia: a review of recent developments. *Current opinion in urology* **13**: 39-44.

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, Knott C, Thomas K, Hoppin JA, Barker J et al. 2003. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol* **157**: 800-814.

Alcaraz A, Hammerer P, Tubaro A, Schroder FH, Castro R. 2009. Is there evidence of a relationship between benign prostatic hyperplasia and prostate cancer? Findings of a literature review. *Eur Urol* **55**: 864-873.

Amorino GP, Parsons SJ. 2004. Neuroendocrine cells in prostate cancer. *Crit Rev Eukaryot Gene Expr* **14**: 287-300.

Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ et al. 2010. Effect of dutasteride on the risk of prostate cancer. *N Engl J Med* **362**: 1192-1202.

Arai Y, Mori T, Suzuki Y, Bern HA. 1983. Long-term effects of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. *International review of cytology* **84**: 235-268.

Armenian HK, Lilienfeld AM, Diamond EL, Bross ID. 1974. Relation between benign prostatic hyperplasia and cancer of the prostate. A prospective and retrospective study. *Lancet* **2**: 115-117.

Aumuller G, Funke PJ, Hahn A, Hoffbauer G, Tunn U, Neumann F. 1982. Phenotypic modulation of the canine prostate after long-term treatment with androgens and estrogens. *Prostate* **3**: 361-373.

Azzouzi AR, Cochand-Priollet B, Mangin P, Fournier G, Berthon P, Latil A, Cussenot O. 2002. Impact of constitutional genetic variation in androgen/oestrogen-regulating genes on age-related changes in human prostate. *Eur J Endocrinol* **147**: 479-484.

Bartsch G, Rittmaster R, Klocker H. 2002. Dihydrotestosterone and the concept of 5α-reductase inhibition in human benign prostatic hyperplasia. *World journal of urology* **19**: 413-425.

Bartsch W, Klein H, Sturenburg HJ, Voigt KD. 1987. Metabolism of androgens in human benign prostatic hyperplasia: aromatase and its inhibition. *J Steroid Biochem* **27**: 557-564.

Belanger A, Candas B, Dupont A, Cusan L, Diamond P, Gomez JL, Labrie F. 1994. Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. *J Clin Endocrinol Metab* **79**: 1086-1090.

Bergerat JP, Ceraline J. 2009. Pleiotropic functional properties of androgen receptor mutants in prostate cancer. *Hum Mutat* **30**: 145-157.

Berry SJ, Coffey DS, Strandberg JD, Ewing LL. 1986. Effect of age, castration, and testosterone replacement on the development and restoration of canine benign prostatic hyperplasia. *Prostate* **9**: 295-302.

Bertin G, Averbeck D. 2006. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie* **88**: 1549-1559.

Bonkhoff H, Remberger K. 1993. Widespread distribution of nuclear androgen receptors in the basal cell layer of the normal and hyperplastic human prostate. *Virchows Arch A Pathol Anat Histopathol* **422**: 35-38.

Bonkhoff H, Remberger K .1996. Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate* **28**: 98-106.

Bonkhoff H, Stein U, Remberger K. 1994. Multidirectional differentiation in the normal, hyperplastic, and neoplastic human prostate: simultaneous demonstration of cell-specific epithelial markers. *Hum Pathol* **25**: 42-46.

Bonkhoff H, Stein U, Remberger K.1995. Endocrine-paracrine cell types in the prostate and prostatic adenocarcinoma are postmitotic cells. *Hum Pathol* **26**: 167-170.

Bosland MC. 2000. The role of steroid hormones in prostate carcinogenesis. *J Natl Cancer Inst Monogr*: 39-66.

Bouchier-Hayes DM, Anderson P, Van Appledorn S, Bugeja P, Costello AJ. 2006. KTP laser versus transurethral resection: early results of a randomized trial. *Journal of endourology / Endourological Society* **20**: 580-585.

Boyle P, Roehrborn C, Harkaway R, Logie J, de la Rosette J, Emberton M. 2004. 5-Alpha reductase inhibition provides superior benefits to alpha blockade by preventing AUR and BPH-related surgery. *European urology* **45**: 620-627.

Brisinda G, Cadeddu F, Vanella S, Mazzeo P, Marniga G, Maria G. 2009. Relief by botulinum toxin of lower urinary tract symptoms owing to benign prostatic hyperplasia: early and long-term results. *Urology* **73**: 90-94.

Bruchovsky N, Lesser B, Van Doorn E, Craven S. 1975. Hormonal effects on cell proliferation in rat prostate. *Vitam Horm* **33**: 61-102.

Burcham PC. 1999. Internal hazards: baseline DNA damage by endogenous products of normal metabolism. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **443**: 11-36.

Caine M, Perlberg S, Meretyk S. 1978. A Placebo-controlled Double-blind Study of the Effect of Phenoxybenzamine in Benign Prostatic Obstruction. *British journal of urology* **50**: 551-554.

Carson C, 3rd, Rittmaster R. 2003. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* **61**: 2-7.

Ceder JA, Jansson L, Ehrnstrom RA, Ronnstrand L, Abrahamsson PA. 2008. The characterization of epithelial and stromal subsets of candidate stem/progenitor cells in the human adult prostate. *Eur Urol* **53**: 524-531.

Chang WY, Prins GS. 1999. Estrogen receptor-beta: implications for the prostate gland. *Prostate* **40**: 115-124.

Chen Q, Zhang L, Fan QL, Zhou J, Peng YB, Wang Z. 2010. Bipolar transurethral resection in saline vs traditional monopolar resection of the prostate: results of a randomized trial with a 2-year follow-up. *BJU Int* **106**: 1339-1343.

Chen W, Zouboulis CC, Fritsch M, Blume-Peytavi U, Kodelja V, Goerdt S, Luu-The V, Orfanos CE. 1998. Evidence of heterogeneity and quantitative differences of the type 1 5alpha-reductase expression in cultured human skin cells--evidence of its presence in melanocytes. *J Invest Dermatol* **110**: 84-89.

Chodak GW, Kranc DM, Puy LA, Takeda H, Johnson K, Chang C. 1992. Nuclear localization of androgen receptor in heterogeneous samples of normal, hyperplastic and neoplastic human prostate. *J Urol* **147**: 798-803.

Claessens F, Denayer S, Van Tilborgh N, Kerkhofs S, Helsen C, Haelens A. 2008. Diverse roles of androgen receptor (AR) domains in AR-mediated signaling. *Nucl Recept Signal* **6**: e008.

Constantinou CE, Omata S. 1996. Analysis of the relative biomechanical effects of alpha 1 and alpha 2 antagonists in modifying the compliance of the prostate and micturition parameters of the hormonally manipulated male rat. *Neurourol Urodyn* **15**: 85-101.

Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, Gaudin PB, Fazzari M, Zhang ZF, Massague J et al. 1998. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. *J Natl Cancer Inst* **90**: 1284-1291.

Cramer SD, Chang BL, Rao A, Hawkins GA, Zheng SL, Wade WN, Cooke RT, Thomas LN, Bleecker ER, Catalona WJ et al. 2003. Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. *J Natl Cancer Inst* **95**: 1044-1053.

Crescioli C, Morelli A, Adorini L, Ferruzzi P, Luconi M, Vannelli GB, Marini M, Gelmini S, Fibbi B, Donati S et al. 2005. Human bladder as a novel target for vitamin D receptor ligands. *J Clin Endocrinol Metab* **90**: 962-972.

Cunha GR. 1973. The role of androgens in the epithelio-mesenchymal interactions involved in prostatic morphogenesis in embryonic mice. *Anat Rec* **175**: 87-96.

Davidsson S, Fiorentino M, Andren O, Fang F, Mucci LA, Varenhorst E, Fall K, Rider JR. 2011. Inflammation, focal atrophic lesions, and prostatic intraepithelial neoplasia with respect to risk of lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev* **20**: 2280-2287.

De Bont R, Van Larebeke N. 2004. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis* **19**: 169-185.

De Marzo AM, Nelson WG, Bieberich CJ, Yegnasubramanian S. 2010. Prostate cancer: New answers prompt new questions regarding cell of origin. *Nat Rev Urol* **7**: 650-652.

De Nunzio C, Aronson W, Freedland SJ, Giovannucci E, Parsons JK. 2012. The correlation between metabolic syndrome and prostatic diseases. *Eur Urol* **61**: 560-570.

De Nunzio C, Kramer G, Marberger M, Montironi R, Nelson W, Schroder F, Sciarra A, Tubaro A. 2011. The controversial relationship between benign prostatic hyperplasia and prostate cancer: the role of inflammation. *Eur Urol* **60**: 106-117.

Dehm SM, Tindall DJ. 2006. Molecular regulation of androgen action in prostate cancer. *J Cell Biochem* **99**: 333-344.

DeKlerk DP, Coffey DS, Ewing LL, McDermott IR, Reiner WG, Robinson CH, Scott WW, Strandberg JD, Talalay P, Walsh PC et al. 1979. Comparison of spontaneous and experimentally induced canine prostatic hyperplasia. *J Clin Invest* **64**: 842-849.

Donkervoort T, Sterling A, van Ness J, Donker PJ. 1977. A clinical and urodynamic study of tadenan in the treatment of benign prostatic hypertrophy. *Eur Urol* **3**: 218-225.

Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. 2000. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development* **127**: 4277-4291.

el Etreby MF, Nishino Y, Habenicht UF, Henderson D. 1991. Atamestane, a new aromatase inhibitor for the management of benign prostatic hyperplasia. *J Androl* **12**: 403-414.

Ellem SJ, Risbridger GP. 2009. The dual, opposing roles of estrogen in the prostate. *Ann N Y Acad Sci* **1155**: 174-186.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjold M, Gustafsson JA. 1997. Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab* **82**: 4258-4265.

Fang S, Anderson KM, and Liao S. 1969. Receptor proteins for androgens. *J Biochem* **244**: 6584–6595.

Fenech M, Ferguson LR. 2001. Vitamins/minerals and genomic stability in humans. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **475**: 1-6.

Gleason DF. 1966. Classification of prostatic carcinomas. *Cancer Chemother Rep* **50**: 125-128.

Gopalkrishnan K. 1998. Characteristics of semen parameters in a selected population of Indian men over a period of 10 years. *CURRENT SCIENCE-BANGALORE-* **75**: 939-941.

Griffiths K, Cockett A, Coffey DS. 1998. *Regulation of Prostate Growth. In: Denis L, Griffiths K, Khoury S, Cockett ATK, McConnell J, chatelain C, et al, eds. International Consultation of Benign Prostatic Hyperplasia, 4th ed.,Paris: SCI*

Grisanzio C, Signoretti S. 2008. p63 in prostate biology and pathology. *J Cell Biochem* **103**: 1354-1368.

Guntupalli JNR, Padala S, Gummuluri ARM, Muktineni RK, Byreddy SR, Sreerama L, Kedarisetti PC, Angalakuduru DP, Satti BR, Venkatathri V. 2007. Trace elemental analysis of normal, benign hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique. *European journal of cancer prevention* **16**: 108-115.

Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, Chen H, Kong X, Melamed J, Tepper CG et al. 2009. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* **69**: 2305-2313.

Guzel S, Kiziler L, Aydemir B, Alici B, Ataus S, Aksu A, Durak H. 2012. Association of Pb, Cd, and Se concentrations and oxidative damage-related markers in different grades of prostate carcinoma. *Biological trace element research* **145**: 23-32.

Han M, Alfert H, Partin A. 2002. Retropubic and suprapubic open prostatectomy. In, Walsh PC, ed. Campbell's Urology. Philadelphia, Saundres. pp. 1423-1434.

Hayashi N, Sugimura Y, Kawamura J, Donjacour AA, Cunha GR. 1991. Morphological and functional heterogeneity in the rat prostatic gland. *Biol Reprod* **45**: 308-321.

Hayward SW, Baskin LS, Haughney PC, Cunha AR, Foster BA, Dahiya R, Prins GS, Cunha GR. 1996a. Epithelial development in the rat ventral prostate, anterior prostate and seminal vesicle. *Acta Anat (Basel)* **155**: 81-93.

Hayward SW, Baskin LS, Haughney PC, Foster BA, Cunha AR, Dahiya R, Prins GS, Cunha GR. 1996b. Stromal development in the ventral prostate, anterior prostate and seminal vesicle of the rat. *Acta Anat (Basel)* **155**: 94-103.

Heemers HV, Tindall DJ. 2007. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocr Rev* **28**: 778-808.

Ho CK, Nanda J, Chapman KE, Habib FK. 2008. Oestrogen and benign prostatic hyperplasia: effects on stromal cell proliferation and local formation from androgen. *J Endocrinol* **197**: 483-491.

Huang L, Pu Y, Alam S, Birch L, Prins GS. 2004. Estrogenic regulation of signaling pathways and homeobox genes during rat prostate development. *J Androl* **25**: 330-337.

Hudson DL, O'Hare M, Watt FM, Masters JR. 2000. Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. *Lab Invest* **80**: 1243-1250.

Husmann DA, McPhaul MJ, Wilson JD. 1991. Androgen receptor expression in the developing rat prostate is not altered by castration, flutamide, or suppression of the adrenal axis. *Endocrinology* **128**: 1902-1906.

Imura M, Kojima Y, Kubota Y, Hamakawa T, Yasui T, Sasaki S, Hayashi Y, Kohri K. 2012. Regulation of cell proliferation through a KIT-mediated mechanism in benign prostatic hyperplasia. *Prostate* **72**: 1506-1513.

Isaacs JT, Coffey DS. 1989. Etiology and disease process of benign prostatic hyperplasia. *Prostate Suppl* **2**: 33-50.

Izumi K, Mizokami A, Lin WJ, Lai KP, Chang C. 2013. Androgen receptor roles in the development of benign prostate hyperplasia. *The American journal of pathology* **182**: 1942-1949.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. 2009. Cancer statistics, 2009. *CA Cancer J Clin* **59**: 225-249.

Jepson J, Bruskewitz R. 2000. Clinical manifestations and indications for treatment. In: Lepor H, ed. Prostatic diseases . Philadelphia: WB Saunders; . 127-142.

Jin B, Turner L, Walters WA, Handelsman DJ. 1996. The effects of chronic high dose androgen or estrogen treatment on the human prostate [corrected]. *J Clin Endocrinol Metab* **81**: 4290-4295.

Jin B, Turner L, Zhou Z, Zhou EL, Handelsman DJ. 1999. Ethnicity and migration as determinants of human prostate size. *J Clin Endocrinol Metab* **84**: 3613-3619.

Jost A. 1953. Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Rec Prog Horm Res* **8**: 379–418.

Juniewicz PE, Lemp BM, Barbolt TA, LaBrie TK, Batzold FH, Reel JR. 1989. Dose-dependent hormonal induction of benign prostatic hyperplasia (BPH) in castrated dogs. *Prostate* **14**: 341-352.

Kaufman JM, Vermeulen A. 2005. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev* **26**: 833-876.

Kim GH, Park K, Yeom SY, Lee KJ, Kim G, Ko J, Rhee DK, Kim YH, Lee HK, Kim HW et al. 2009. Characterization of ASC-2 as an antiatherogenic transcriptional coactivator of liver X receptors in macrophages. *Mol Endocrinol* **23**: 966-974.

Kitsberg DI, Leder P. 1996. Keratinocyte growth factor induces mammary and prostatic hyperplasia and mammary adenocarcinoma in transgenic mice. *Oncogene* **13**: 2507-2515.

Kobayashi S, Tang R, Shapiro E, Lepor H. 1993. Characterization and localization of prostatic alpha 1 adrenoceptors using radioligand receptor binding on slide-mounted tissue section. *The Journal of urology* **150**: 2002-2006.

Kondi-Pafiti A, Arkadopoulos N, Gennatas C, Michalaki V, Frangou-Plegmenou M, Chatzipantelis P. 2010. Expression of c-kit in common benign and malignant breast lesions. *Tumori* **96**: 978-984.

Krieg M, Bartsch W, Thomsen M, Voigt KD. 1983. Androgens and estrogens: their interaction with stroma and epithelium of human benign prostatic hyperplasia and normal prostate. *J Steroid Biochem* **19**: 155-161.

Kuo H-C. 2004. Urodynamic evidence of effectiveness of botulinum A toxin injection in treatment of detrusor overactivity refractory to anticholinergic agents. *Urology* **63**: 868-872.

Kuo H-C, Liu H-T. 2009. Therapeutic effects of add-on botulinum toxin A on patients with large benign prostatic hyperplasia and unsatisfactory response to combined medical therapy. *Scandinavian journal of urology and nephrology* **43**: 206-211.

Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM, Webb P. 2000. Estrogen receptor pathways to AP-1. *The Journal of steroid biochemistry and molecular biology* **74**: 311-317.

Kyprianou N, Davies P. 1986. Association states of androgen receptors in nuclei of human benign hypertrophic prostate. *Prostate* **8**: 363-380.

Lagiou P, Wuu J, Trichopoulou A, Hsieh CC, Adami HO, Trichopoulos D. 1999. Diet and benign prostatic hyperplasia: a study in Greece. *Urology* **54**: 284-290.

Lasnitzki I, Mizuno T. 1977. Induction of the rat prostate gland by androgens in organ culture. *J Endocrinol* **74**: 47-55.

Le Magnen C, Bubendorf L, Ruiz C, Zlobec I, Bachmann A, Heberer M, Spagnoli GC, Wyler S, Mengus C. 2013. Klf4 transcription factor is expressed in the cytoplasm of prostate cancer cells. *Eur J Cancer* **49**: 955-963.

Lee H, Jeong SJ. 2014. Epidemiology of Lower Urinary Tract Symptoms: Emphasis on the Status in Korea. *Korean journal of urology* **55**: 300-308.

Leippold T, Reitz A, Schurch B. 2003. Botulinum toxin as a new therapy option for voiding disorders: current state of the art. *European urology* **44**: 165-174.

Lepor H. 1995. Alpha 1-adrenoceptor selectivity: clinical or theoretical benefit? *Br J Urol* **76 Suppl 1**: 57-61.

Li Y, Spataro BC, Yang J, Dai C, Liu Y. 2005. 1,25-dihydroxyvitamin D inhibits renal interstitial myofibroblast activation by inducing hepatocyte growth factor expression. *Kidney Int* **68**: 1500-1510.

Lin VK, Wang SY, Vazquez DV, C CX, Zhang S, Tang L. 2007. Prostatic stromal cells derived from benign prostatic hyperplasia specimens possess stem cell like property. *Prostate* **67**: 1265-1276.

Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, Ettinger SL, Gleave ME, Nelson CC. 2008. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res* **68**: 6407-6415.

Lonergan PE, Tindall DJ. 2011. Androgen receptor signaling in prostate cancer development and progression. *J Carcinog* **10**: 20.

Long RM, Morrissey C, Fitzpatrick JM, Watson RW. 2005. Prostate epithelial cell differentiation and its relevance to the understanding of prostate cancer therapies. *Clin Sci (Lond)* **108**: 1-11.

Lowe FC, Fagelman E. 1999. Phytotherapy in the treatment of benign prostatic hyperplasia: an update. *Urology* **53**: 671-678.

Lowsley OS. 1912. The development of the human prostate gland with reference to the development of other structures at the neck of the urinary bladder. *Am J Anat* **13**: 299–348.

Mahajan R, Bonner MR, Hoppin JA, Alavanja MC. 2006. Phorate exposure and incidence of cancer in the agricultural health study. *Environmental health perspectives* **114**: 1205-1209.

Mahapokai W, Van Sluijs FJ, Schalken JA. 2000. Models for studying benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis* **3**: 28-33.

Maitland NJ, Collins A. 2005. A tumour stem cell hypothesis for the origins of prostate cancer. *BJU Int* **96**: 1219-1223.

Maitland NJ, Frame FM, Polson ES, Lewis JL, Collins AT. 2011. Prostate cancer stem cells: do they have a basal or luminal phenotype? *Horm Cancer* **2**: 47-61.

Manchanda PK, Kibler AJ, Zhang M, Ravi J, Bid HK. 2014. Vitamin D receptor as a therapeutic target for benign prostatic hyperplasia. *Indian J Urol* **28**: 377-381.

Maria G, Brisinda G, Civello IM, Bentivoglio AR, Sganga G, Albanese A. 2003. Relief by botulinum toxin of voiding dysfunction due to benign prostatic hyperplasia: results of a randomized, placebo-controlled study. *Urology* **62**: 259-264; discussion 264-255.

Martin MB, Voeller HJ, Gelmann EP, Lu J, Stoica E-G, Hebert EJ, Reiter R, Singh B, Danielsen M, Pentecost E. 2002a. Role of cadmium in the regulation of AR gene expression and activity. *Endocrinology* **143**: 263-275.

Martin MB, Voeller HJ, Gelmann EP, Lu J, Stoica EG, Hebert EJ, Reiter R, Singh B, Danielsen M, Pentecost E et al. 2002b. Role of cadmium in the regulation of AR gene expression and activity. *Endocrinology* **143**: 263-275.

Mckenna n, O'malley BW. 2001. Nuclear Receptors, Coregulators, Ligands, and Selective Receptor Modulators. *Annals of the New York Academy of Sciences* **949**: 3-5.

McNeal JE. 1984. Anatomy of the prostate and morphogenesis of BPH. *Prog Clin Biol Res* **145**: 27-53.

McNeal JE, Bostwick DG. 1984. Anatomy of the prostatic urethra. *Jama* **251**: 890-891.

Merrimen JL, Jones G, Walker D, Leung CS, Kapusta LR, Srigley JR. 2009. Multifocal high grade prostatic intraepithelial neoplasia is a significant risk factor for prostatic adenocarcinoma. *J Urol* **182**: 485-490; discussion 490.

Messina MJ, Persky V, Setchell KD, Barnes S. 1994. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer* **21**: 113-131.

Montironi R, Mazzucchelli R, Lopez-Beltran A, Scarpelli M, Cheng L. 2011. Prostatic intraepithelial neoplasia: its morphological and molecular diagnosis and clinical significance. *BJU Int* **108**: 1394-1401.

Moore R. 1944. Benign Prostatic Hyperplasia and Cacinoma of the prostate. Occurrence and Experimental production in Animals. *Surgery* **16**: 152-167.

Muller WJ, Lee FS, Dickson C, Peters G, Pattengale P, Leder P. 1990. The int-2 gene product acts as an epithelial growth factor in transgenic mice. *Embo J* **9**: 907-913.

Nemeth JA, Sensibar JA, White RR, Zelner DJ, Kim IY, Lee C. 1997. Prostatic ductal system in rats: tissue-specific expression and regional variation in stromal distribution of transforming growth factor-beta 1. *Prostate* **33**: 64-71.

Nicholson TM, Ricke WA. 2011. Androgens and estrogens in benign prostatic hyperplasia: past, present and future. *Differentiation* **82**: 184-199.

Noble RL. 1977. The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration. *Cancer Res* **37**: 1929-1933.

Notara M, Ahmed A. 2012. Benign prostate hyperplasia and stem cells: a new therapeutic opportunity. *Cell Biol Toxicol* **28**: 435-442.

O'Malley KJ, Dhir R, Nelson JB, Bost J, Lin Y, Wang Z. 2009. The expression of androgen-responsive genes is up-regulated in the epithelia of benign prostatic hyperplasia. *Prostate* **69**: 1716-1723.

Oldridge EE, Pellacani D, Collins AT, Maitland NJ. 2011. Prostate cancer stem cells: are they androgen-responsive? *Mol Cell Endocrinol* **360**: 14-24.

Oliveira AG, Coelho PH, Guedes FD, Mahecha GA, Hess RA, Oliveira CA. 2007. 5alpha-Androstane-3beta,17beta-diol (3beta-diol), an estrogenic metabolite of 5alpha-dihydrotestosterone, is a potent modulator of estrogen receptor ERbeta expression in the ventral prostrate of adult rats. *Steroids* **72**: 914-922.

Orsted DD, Bojesen SE. 2013. The link between benign prostatic hyperplasia and prostate cancer. *Nat Rev Urol* **10**: 49-54.

Orsted DD, Bojesen SE, Nielsen SF, Nordestgaard BG. 2011. Association of clinical benign prostate hyperplasia with prostate cancer incidence and mortality revisited: a nationwide cohort study of 3,009,258 men. *Eur Urol* **60**: 691-698.

Pandya C, Gupta S, Pillai P, Bhandarkar A, Khan A, Bhan A, Prajapati A, Gupta S. 2013. Association of cadmium and lead with antioxidant status and incidence of benign prostatic hyperplasia in patients of Western India. *Biol Trace Elem Res* **152**: 316-326.

Parsons JK. 2010. Benign Prostatic Hyperplasia and Male Lower Urinary Tract Symptoms: Epidemiology and Risk Factors. *Current bladder dysfunction reports* **5**: 212-218.

Partin A, Rodriguez R. 2002. *The Molecular Biology, Endocrinology and physiology of the prostate and seminal vesicles. In, Walsh PC, ed. Campbell's Urology. Philadelphia, Saundres.*

Patel ND, Parsons JK. 2014. Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. *Indian J Urol* **30**: 170-176.

Pearson JD, Lei HH, Beaty TH, Wiley KE, Isaacs SD, Isaacs WB, Stoner E, Walsh PC. 2003. Familial aggregation of bothersome benign prostatic hyperplasia symptoms. *Urology* **61**: 781-785.

Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. 1994. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. *Cancer Res* **54**: 805-810.

Pelletier G. 2008. Expression of steroidogenic enzymes and sex-steroid receptors in human prostate. *Best Pract Res Clin Endocrinol Metab* **22**: 223-228.

Peters CA, Barrack ER. 1987. Androgen receptor localization in the human prostate: demonstration of heterogeneity using a new method of steroid receptor autoradiography. *J Steroid Biochem* **27**: 533-541.

Price D. 1936. Normal development of the prostate and seminal vesicles of the rat with a study of experimental postnatal modifications. *Am J Anat* **60**: 79–127.

Price D, and Williams-Ashman HG. 1961. The accessory reproductive glands of mammals. *Williams & Wilkens Co, Baltimore* **3rd edition**: 366–448.

Prince MM, Ruder AM, Hein MJ, Waters MA, Whelan EA, Nilsen N, Ward EM, Schnorr TM, Laber PA, Davis-King KE. 2006. Mortality and exposure response among 14,458 electrical capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). *Environmental health perspectives* **114**: 1508-1514.

Prins GS. 1993. Development of the prostate. In: Haseltine, F.,Paulsen, C. and Wang, C. ed. Reproductive issues and the aging male. *New York: Embryonic Inc,*: 101-112.

Prins GS, Birch L. 1995. The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen. *Endocrinology* **136**: 1303-1314.

Prins GS, Birch L, Habermann H, Chang WY, Tebeau C, Putz O, Bieberich C. 2001. Influence of neonatal estrogens on rat prostate development. *Reproduction, fertility, and development* **13**: 241-252.

Prins GS, Chang WY, Wang Y, van Breemen RB. 2002. Retinoic acid receptors and retinoids are up-regulated in the developing and adult rat prostate by neonatal estrogen exposure. *Endocrinology* **143**: 3628-3640.

Prins GS, Cooke PS, Birch L, Donjacour AA, Yalcinkaya TM, Siiteri PK, Cunha GR. 1992. Androgen receptor expression and 5 alpha-reductase activity along the proximal-distal axis of the rat prostatic duct. *Endocrinology* **130**: 3066-3073.

Prins GS, Korach KS. 2008. The role of estrogens and estrogen receptors in normal prostate growth and disease. *Steroids* **73**: 233-244.

Rajfer J, Coffey DS. 1978. Sex steroid imprinting of the immature prostate. Long-term effects. *Investigative urology* **16**: 186-190.

Rhodes T, Girman CJ, Jacobsen SJ, Roberts RO, Guess HA, Lieber MM. 1999. Longitudinal prostate growth rates during 5 years in randomly selected community men 40 to 79 years old. *J Urol* **161**: 1174-1179.

Ricke WA, Ishii K, Ricke EA, Simko J, Wang Y, Hayward SW, Cunha GR. 2006. Steroid hormones stimulate human prostate cancer progression and metastasis. *International journal of cancer Journal international du cancer* **118**: 2123-2131.

Roberts RO, Bergstralh EJ, Bass SE, Lieber MM, Jacobsen SJ. 2004. Prostatitis as a risk factor for prostate cancer. *Epidemiology* **15**: 93-99.

Roberts RO, Bergstralh EJ, Farmer SA, Jacobson DJ, Hebbring SJ, Cunningham JM, Thibodeau SN, Lieber MM, Jacobsen SJ. 2006. Polymorphisms in genes involved in sex hormone metabolism may increase risk of benign prostatic hyperplasia. *Prostate* **66**: 392-404.

Rohrmann S, Fallin MD, Page WF, Reed T, Partin AW, Walsh PC, Platz EA. 2006. Concordance rates and modifiable risk factors for lower urinary tract symptoms in twins. *Epidemiology* **17**: 419-427.

Sanda MG, Doehring CB, Binkowitz B, Beaty TH, Partin AW, Hale E, Stoner E, Walsh PC. 1997. Clinical and biological characteristics of familial benign prostatic hyperplasia. *J Urol* **157**: 876-879.

Santti R, Newbold RR, Makela S, Pylkkanen L, McLachlan JA. 1994. Developmental estrogenization and prostatic neoplasia. *Prostate* **24**: 67-78.

Scarpa RM. 2001. Lower urinary tract symptoms: what are the implications for the patients? *Eur Urol* **40 Suppl 4**: 12-20.

Schenk JM, Kristal AR, Arnold KB, Tangen CM, Neuhouser ML, Lin DW, White E, Thompson IM. 2011. Association of symptomatic benign prostatic hyperplasia and prostate cancer: results from the prostate cancer prevention trial. *Am J Epidemiol* **173**: 1419-1428.

Schuster GA, Schuster TG. 1999. The relative amount of epithelium, muscle, connective tissue and lumen in prostatic hyperplasia as a function of the mass of tissue resected. *J Urol* **161**: 1168-1173.

Sensibar JA, Griswold MD, Sylvester SR, Buttyan R, Bardin CW, Cheng CY, Dudek S, Lee C. 1991. Prostatic ductal system in rats: regional variation in localization of an androgen-repressed gene product, sulfated glycoprotein-2. *Endocrinology* **128**: 2091-2102.

Serretta V, Morgia G, Fondacaro L, Curto G, Lo bianco A, Pirritano D, Melloni D, Orestano F, Motta M, Pavone-Macaluso M et al. 2002a. Open prostatectomy for benign prostatic enlargement in southern Europe in the late 1990s: a contemporary series of 1800 interventions. *Urology* **60**: 623-627.

Serretta V, Morgia G, Fondacaro L, Curto G, Pirritano D, Melloni D, Orestano F, Motta M, Pavone-Macaluso M. 2002b. Open prostatectomy for benign prostatic enlargement in southern Europe in the late 1990s: a contemporary series of 1800 interventions. *Urology* **60**: 623-627.

Shabsigh A, Tanji N, D'Agati V, Burchardt T, Burchardt M, Hayek O, Shabsigh R, Buttyan R. 1999. Vascular anatomy of the rat ventral prostate. *Anat Rec* **256**: 403-411.

Shannon JM, Cunha GR. 1983. Autoradiographic localization of androgen binding in the developing mouse prostate. *Prostate* **4**: 367-373.

Shapiro E, Becich MJ, Hartanto V, Lepor H. 1992. The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic benign prostate hyperplasia. *J Urol* **147**: 1293-1297.

Shen MM, Abate-Shen C. 2010. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* **24**: 1967-2000.

Shibata Y, Ito K, Suzuki K, Nakano K, Fukabori Y, Suzuki R, Kawabe Y, Honma S, Yamanaka H. 2000. Changes in the endocrine environment of the human prostate transition zone with aging: simultaneous quantitative analysis of prostatic sex steroids and comparison with human prostatic histological composition. *Prostate* **42**: 45-55.

Simak R, Capodieci P, Cohen DW, Fair WR, Scher H, Melamed J, Drobnjak M, Heston WD, Stix U, Steiner G et al. 2000. Expression of c-kit and kit-ligand in benign and malignant prostatic tissues. *Histol Histopathol* **15**: 365-374.

Simons BD, Morrison AS, Young RH, Verhoek-Oftedahl W. 1993. The relation of surgery for prostatic hypertrophy to carcinoma of the prostate. *Am J Epidemiol* **138**: 294-300.

Smith CL. 1998. Cross-talk between peptide growth factor and estrogen receptor signaling pathways. *Biol Reprod* **58**: 627-632.

Sommers SC. 1957. Endocrine changes with prostatic carcinoma. *Cancer* **10**: 345-358.

Steiner MS, Couch RC, Raghow S, Stauffer D. 1999. The chimpanzee as a model of human benign prostatic hyperplasia. *J Urol* **162**: 1454-1461.

Stoica A, Katzenellenbogen BS, Martin MB. 2000a. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Mol Endocrinol* **14**: 545-553.

Stoica A, Katzenellenbogen BS, Martin MB. 2000b. Activation of estrogen receptor-α by the heavy metal cadmium. *Molecular Endocrinology* **14**: 545-553.

Sugimura Y, Cunha GR, Donjacour AA. 1986. Morphogenesis of ductal networks in the mouse prostate. *Biol Reprod* **34**: 961-971.

Sutkowski DM, Fong CJ, Sensibar JA, Rademaker AW, Sherwood ER, Kozlowski JM, Lee C. 1992. Interaction of epidermal growth factor and transforming growth factor beta in human prostatic epithelial cells in culture. *Prostate* **21**: 133-143.

Takao T, Tsujimura A. 2008. Prostate stem cells: the niche and cell markers. *Int J Urol* **15**: 289-294.

Takeda H, Mizuno T, Lasnitzki I. 1985. Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and postnatal prostate. *J Endocrinol* **104**: 87-92.

Takezawa Y, Fukabori Y, Yamanaka H, Mieda M, Honma S, Kushitani M, Hamataki N. 1992. Effects of the new steroidal antiandrogen TZP-4238 on hormone-induced canine prostatic hyperplasia. *Prostate* **21**: 315-329.

Tang J, Yang JC, Zhang Y, Liu X, Zhang L, Wang Z, Li J, Luo Y, Xu J, Shi H. 2007. Does benign prostatic hyperplasia originate from the peripheral zone of the prostate? A preliminary study. *BJU Int* **100**: 1091-1096.

Thompson IM, Coltman CA, Jr., Crowley J. 1997. Chemoprevention of prostate cancer: the Prostate Cancer Prevention Trial. *Prostate* **33**: 217-221.

Timms BG, Mohs TJ, Didio LJ. 1994. Ductal budding and branching patterns in the developing prostate. *J Urol* **151**: 1427-1432.

Umesono K, Evans RM. 1989. Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell* **57**: 1139-1146.

Waalkes MP. 2003. Cadmium carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **533**: 107-120.

Walsh PC, Wilson JD. 1976. The induction of prostatic hypertrophy in the dog with androstanediol. *J Clin Invest* **57**: 1093-1097.

Watson PA, Chen YF, Balbas MD, Wongvipat J, Socci ND, Viale A, Kim K, Sawyers CL. 2010. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc Natl Acad Sci U S A* **107**: 16759-16765.

Wei JT, Calhoun E, Jacobsen SJ. 2005. Urologic diseases in America project: benign prostatic hyperplasia. *J Urol* **173**: 1256-1261.

Weihua Z, Lathe R, Warner M, Gustafsson JA. 2002. An endocrine pathway in the prostate, ERbeta, AR, 5alpha-androstane-3beta,17beta-diol, and CYP7B1, regulates prostate growth. *Proc Natl Acad Sci U S A* **99**: 13589-13594.

Wilson CM, McPhaul MJ. 1996. A and B forms of the androgen receptor are expressed in a variety of human tissues. *Mol Cell Endocrinol* **120**: 51-57.

Wilson JD, Gloyna RE. 1970. The intranuclear metabolism of testosterone in the accessory organs of reproduction. *Recent Prog Horm Res* **26**: 309-336.

Wilt TJ, Ishani A, Stark G, MacDonald R, Lau J, Mulrow C. 1998. Saw palmetto extracts for treatment of benign prostatic hyperplasia: a systematic review. *Jama* **280**: 1604-1609.

Winter ML, Bosland MC, Wade DR, Falvo RE, Nagamani M, Liehr JG. 1995. Induction of benign prostatic hyperplasia in intact dogs by near-physiological levels of 5 alpha-dihydrotestosterone and 17 beta-estradiol. *Prostate* **26**: 325-333.

Xue Y, Smedts F, Debruyne FM, de la Rosette JJ, Schalken JA. 1998. Identification of intermediate cell types by keratin expression in the developing human prostate. *Prostate* **34**: 292-301.

Yip SK, Chan NH, Chiu P, Lee KW, Ng CF. 2011. A randomized controlled trial comparing the efficacy of hybrid bipolar transurethral vaporization and resection of the prostate with bipolar

transurethral resection of the prostate. *Journal of endourology / Endourological Society* **25**: 1889-1894.

Zhou Y, Xiao XQ, Chen LF, Yang R, Shi JD, Du XL, Klocker H, Park I, Lee C, Zhang J. 2009. Proliferation and phenotypic changes of stromal cells in response to varying estrogen/androgen levels in castrated rats. *Asian J Androl* **11**: 451-459.

Zhu ML, Kyprianou N. 2008. Androgen receptor and growth factor signaling cross-talk in prostate cancer cells. *Endocr Relat Cancer* **15**: 841-849.

Zoubeidi A, Zardan A, Beraldi E, Fazli L, Sowery R, Rennie P, Nelson C, Gleave M. 2007. Cooperative interactions between androgen receptor (AR) and heat-shock protein 27 facilitate AR transcriptional activity. *Cancer Res* **67**: 10455-10465.