

CHAPTER-1

1. INTRODUCTION

Male reproductive system comprises of Vas deferens, Bulbourethral glands, Seminal vesicles, Ejaculatory ducts, Epididymis, Prostate gland, Urethra, Scrotum, Testes, and Penis. All of them contribute to a healthy male reproductive system to achieve production, maturation, and transport of sperms. A walnut-sized and shaped prostate gland between urinary bladder and rectum is an accessory gland that nourishes sperm ejaculation fluid. The prostatic fluid provides balanced alkalinity that protects sperm and increases its longevity after ejaculation in the vaginal environment. The average weight of the prostate gland in an adult man is around 20 grams at the age of 20-30. Since the gland grows with aging, the average weight also increases around 30-40 grams in the middle to late age.

1.1 Prostate gland

The initial part of urethra (~3cms) forms a urinary bladder, which is covered by the Prostate gland. Dorsally ejaculatory ducts join the urethra within the gland at any side of the prostatic utricle. The walls of the prostatic urethra are highly intricated and lined with transitional epithelium. Normally, the ureter has a longitudinal ridge (urethral crest) running the length of the gland. Prostatic fluid secreted by ductal glands is collected into longitudinal grooves (urethral sinuses). A short diverticulum in the urethral crest is formed near joints of ejaculatory tubes and urethra, which is the prostatic utricle, the male vestigial remnants of the female uterus and vagina. As a ductal-acinar gland, the prostate is divided into three zones: peripheral (upper surface of the gland), central zones (part of the gland surrounding ejaculatory duct) and, transition zone (part of the prostate surrounding the urethra) containing a thick-continuous fibromuscular stroma and covered by a thin vascularized fibrous sheath.¹⁻³ (*Figure 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, secretes via long ducts into the urethral sinuses. The anterior isthmus, an area of the gland ventral to the urethra, is relatively free of glands and rich in fibromuscular tissues.

Histologically, the human prostate encompasses two types of cells; epithelial cells and stromal cells. Within the acini and tubules, the epithelium forms complex folds and papillae supported by a thin highly vascularized loose connective tissue. Most of the secretory epithelium is pseudostratified which comprises tall columnar secretory epithelial cells and cuboidal basal cells 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 epithelium contains scattered neuroendocrine cells, which partly control the release and expulsion of prostatic secretions during ejaculation. A fibro-muscular stroma containing randomly orientated smooth muscle cells and fibroblast structurally supports the epithelium. In a healthy male, the ratio of stromal to epithelial cells is found to be around 2:1.^{3, 4} (Figure 1.2)

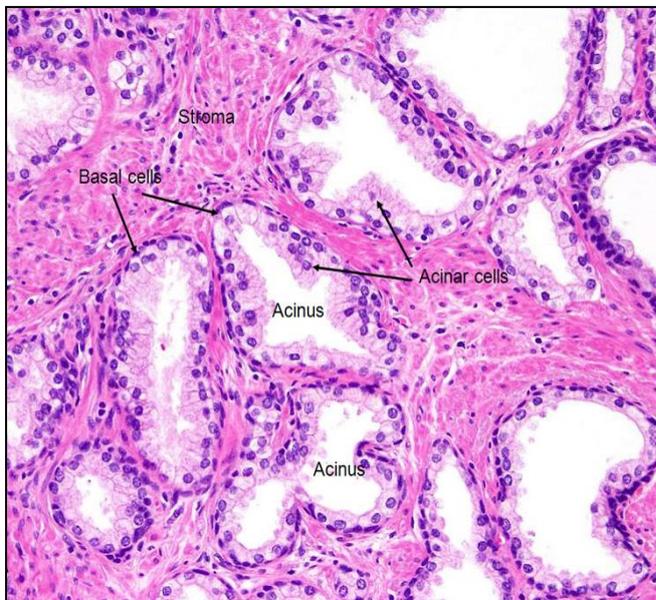


Figure 1. 1: Histology of the Prostate gland. Well defined acini of the gland surrounded by a single layer of secretory acinar (Luminal) cells; A thin layer of Basal stem cells beneath the Luminal cells; and stroma surrounds entire acini as supporting cells. (Adapted-American Urological Association)

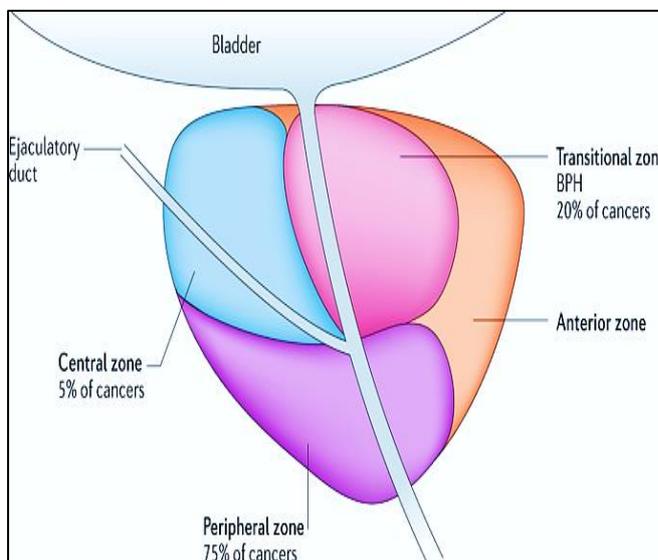


Figure 1. 2: Hypothetical zones of the Prostate. Zone-specific prevalence of prostatic diseases; The peripheral zone (75% of the PCa of the gland); transitional zone (most BPH and 20% of PCa of the gland); Central zone (5% of PCa of the gland. (Adapted⁵)

1.2 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 33% percent of the semen volume that contains various enzymes, zinc, citric acid, and complex proteolytic solution. (Figure 1.3) 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 phosphatase and fibrinolysin.⁶ Prostate-specific antigen (PSA) is also a constituent found in prostatic secretion. PSA is a proteolytic enzyme that liquefies semen for the motility of the sperm to fertilize the ovum (egg). (Table-1.1) Interestingly, only humans and dogs experience atypical growth of the prostate while other mammals are spared.⁷

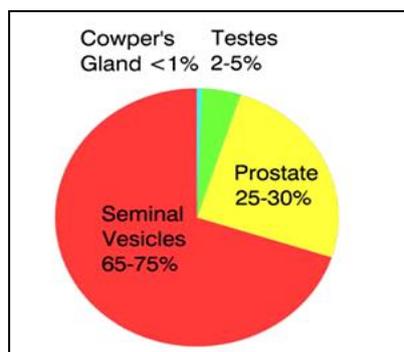


Figure 1. 3: Volume distribution in total semen. Prostatic secretions contribute to 25-30% of the total seminal fluid to augment the sperm capacity for fertilization. (Adapted)

Table 1. 1 Composition and significance of Prostatic fluid.

| 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 (PAP) | Hydrolyze a broad variety of small organic phosphomonoesters under acidic conditions |
| Seminal plasmin | An antibiotic that destroys bacteria |
| Prostaglandins | Smooth Muscle contraction for sperm transport |

1.3 Prostate Gland Development

The most detailed description of prostatic development has been reported for the mouse and rat, while prostatic development in the human is especially incomplete and under-represented in the literature. Prostatic development can be subdivided into several stages⁸ (Table 1.2):

Table 1. 2 Timeline of human prostatic development.⁸

| Developmental event | Fetal Age |
|--|-----------------------|
| Pre-bud stage | 8–9 weeks |
| Initial budding | 10–11 weeks |
| Bud elongation & branching morphogenesis | 11 weeks & thereafter |
| Ductal Formation | 11 weeks & thereafter |

Prostate gland development initiates from an endodermal structure within urogenital sinus (UGS), which begins approximately in the third trimester of fetal age and completed by the time of birth. During this stage, the formation of epithelial buds begins from urogenital sinus epithelium (UGE) and extends to the adjacent urogenital sinus mesenchyme (UGM) which initiates the budding followed by its extension and elongation leading to branching and ductal formations which persists until complete prostate formation. Post-birth, the growth of the prostate remains on hold until puberty when testicular androgens drive the proliferation of prostatic cells and maturation of the gland through the adulthood.⁹ (Figure 1.4)

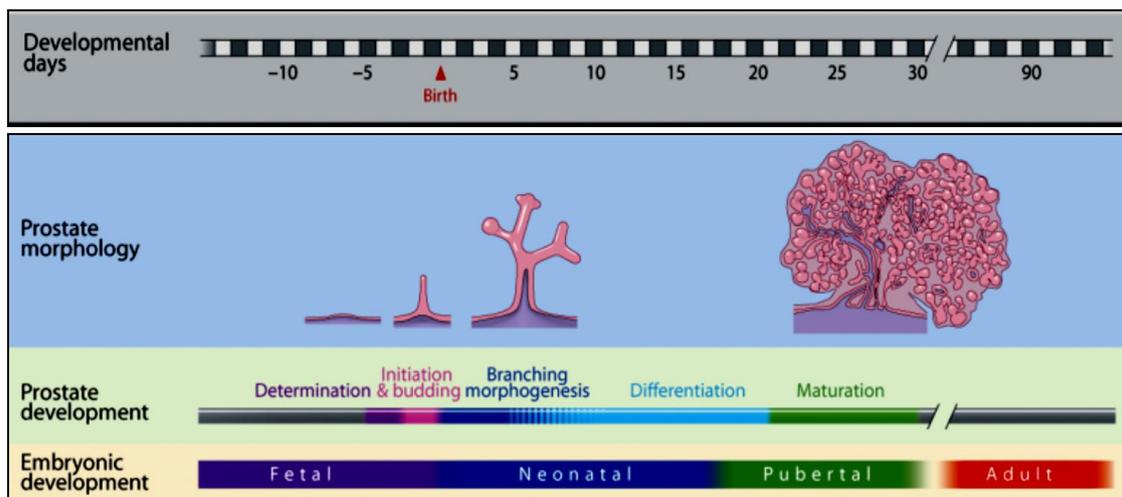


Figure 1. 4: Development of the prostate. Prenatal budding, branching, and morphogenesis of the prostate gland followed by the differentiation of the prostatic cells to form a mature prostate gland during puberty. (Adapted¹⁰)

Role of Androgens in the prostate development

One of the most important factors for prostate development is androgen mediated activation of AR signaling during the fetal stage. However, as Testosterone levels decrease

during the third trimester, the gland enters a quiescent state which persists until puberty. During puberty, 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 again decline and the prostate undergoes a period of involution. (Figure 1.5)

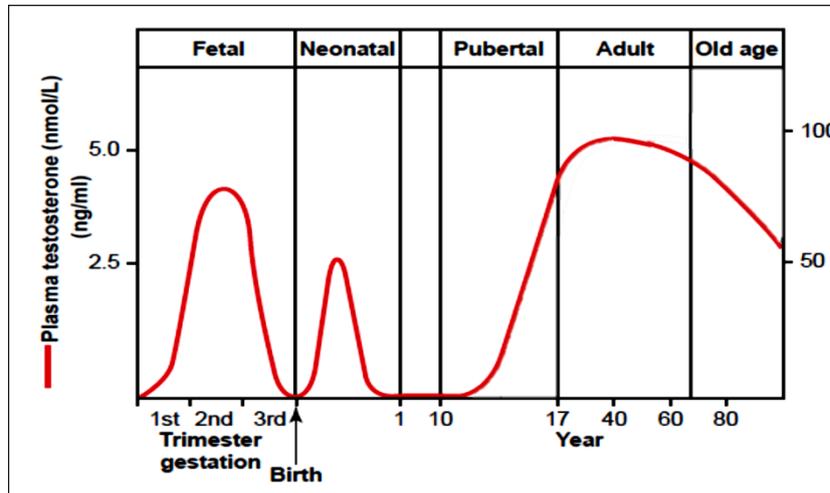


Figure 1. 5: Testosterone levels. Age dependant Plasma Testosterone levels in males. (Adapted¹¹)

Testicular androgens also play an important role in the maintenance of its structural and functional integrity. The rise in serum Testosterone levels induces exponential growth of the prostate gland from childhood (~2 g) to adult (~20 g) in males. The human prostate reaches its normal adult size by 18–20 years of age and subsequently halts growth despite sustained circulating levels of androgen. This transition from prostatic growth to a steady-state phase is maintained by a balance of cell proliferation/death and is controlled by AR signaling and its specific co-factors in both the stroma and epithelium.¹² (Figure 1.6)

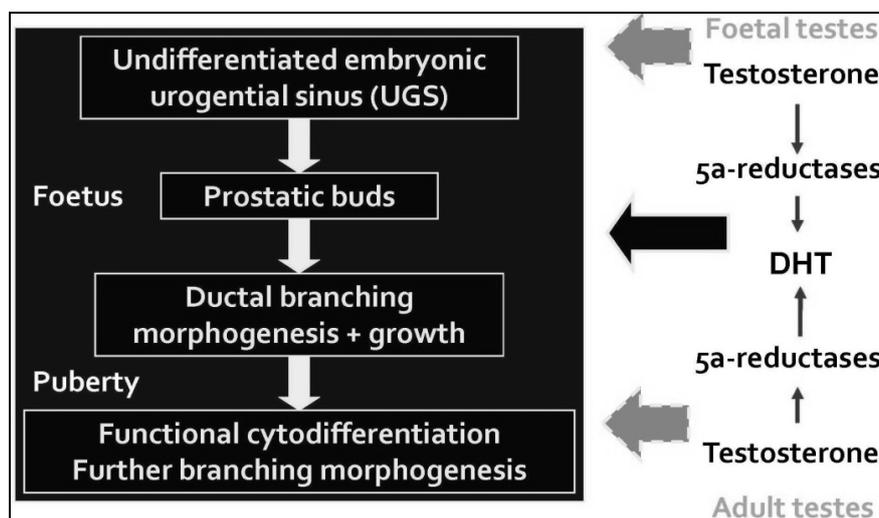


Figure 1. 6: Steroid hormone actions. Androgens are crucially required from the gestational stage to puberty that form a fully mature and functional prostate gland. (Adapted ^{Akhilesh Prajapati Ph.D. Thesis})

Furthermore, during branching morphogenesis of the gland, the formation of branched vascular bed ascends along with neovascularization in the stromal compartment and capillary beds reaching to the ductal basement membrane.¹³ For early prostatic development via androgens, activation of AR signaling begins at early gestational and young stages in males.¹⁴ During the murine fetal stage, it has been observed that mesenchyme of the UGS expresses high levels of AR that encourages the formation of prostatic buds in the UGE. Thus, AR expression in the UGM dominates than in UGE during fetal development in mice.¹⁵ After the completion of bud formation, AR signaling dominates in UGE for epithelial bud extension.^{16, 17} (*Figure 1.7*) Similar pattern has been observed in humans where expression of stromal-AR dominate the expression of epithelial-AR.¹⁸ Concurrent with gland development, the Basal Stem Cells (BSCs) differentiate into secretory luminal and basal phenotypes. The differentiation of epithelial cells from stem/progenitor cells into luminal cells happens with alterations in cellular cytokeratin and AR expression.^{19, 20} Additionally, AR expression is vital for the production of secretory proteins by the luminal epithelium and mesenchymal differentiation into smooth muscle cells.²¹ A structurally well-defined, fully functional prostate gland appears at the maturation during puberty.

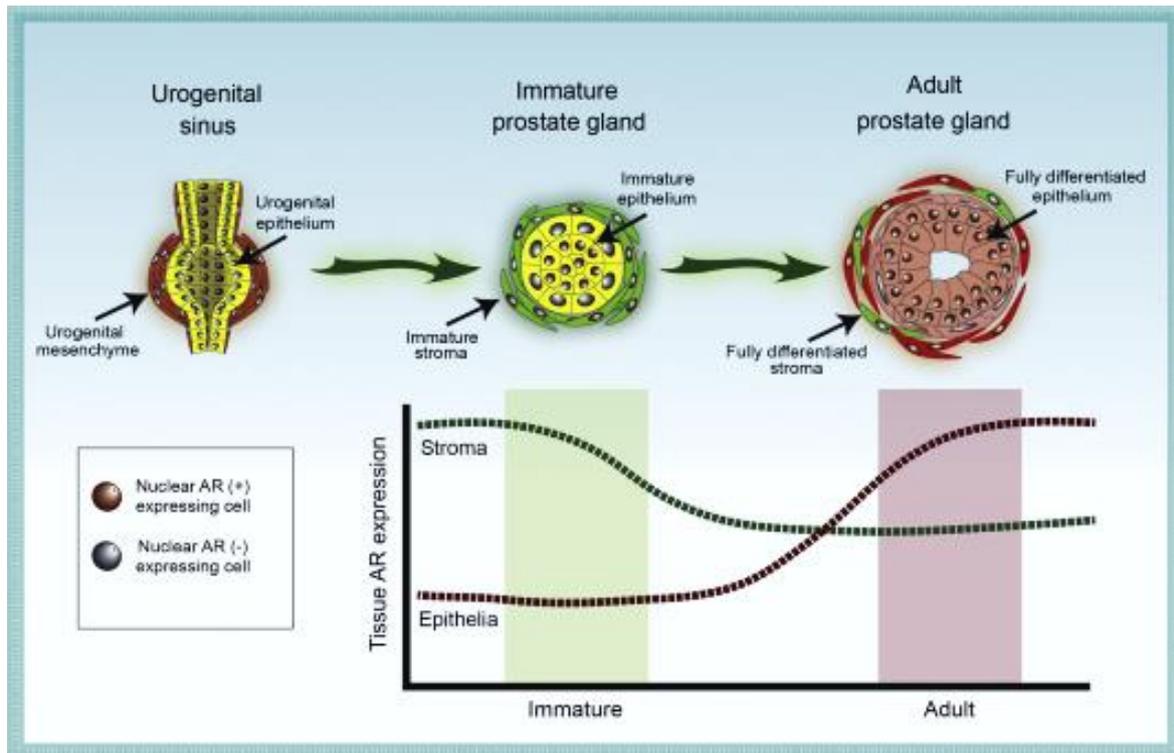


Figure 1. 7: AR expression in cell types of the developing prostate. AR expression stimulates a specific cell proportion of the UGM for the differentiation of the prostate gland in the gestational period. Post-birth, the gain of AR expression has been observed in prostate epithelial cells (especially luminal cells) and small a subset of stromal cells during adult age form a fully functional prostate gland. (Modified²²)

1.4 Epithelial-Stromal compartmentalization of Prostate: Cellular perspective

The developmental organization of the gland is achieved by a uniform compartmental organization into the Epithelial compartment and Stromal compartment. A uniform layer of secretory luminal epithelial cells surrounds the acinar duct. Stromal cells provide a supportive muscular matrix to epithelial cells layer with an intermediate basement membrane composed of Lamins.

The prostate epithelial cell layer comprises four cell types known as BSCs, neuroendocrine (NE), transit-amplifying (TA), and secretory luminal cells that are identified by their morphology, location and distinct marker expression (*Figure 1.8*). The BSCs form a layer of flattened to cuboidal shaped cells above the basement membrane and express p63 (a tumor suppressor gene - p53 homolog), Bcl-2 (a pro-apoptotic factor), cluster designation (CD)133, CD44, CD49b, CD49f, and the high molecular weight cytokeratin (CK)5 and 14.

The columnar shaped luminal cells are the dominating epithelial cell type in the prostate that performs exocrine functions via prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) secretion into the lumen of the acini. The terminally differentiated luminal cells are largely quiescent and androgen-dependent that specifically express CK8/CK18, CD57, p27Kip1 (a cell cycle inhibitor), and high AR expression.²³⁻²⁵ Low frequencies of NE cells have been found that are scattered between basal and luminal layers within the epithelial compartment. NE cells are also terminally differentiated, but unlike luminal cells, NE cells are androgen-insensitive and specifically express chromogranin-A, synaptophysin, neuron-specific enolase, and neuropeptides like bombesin, calcitonin, and neurotensin.^{23, 26-30} Additionally, a small group of intermediate cells referred to as luminal progenitors (LPs) cells are bipotent cells characteristics of basal cells (CK5, CK14, P63, CD49b) as well as luminal cells (CK8, CK18, AR, and PSA).³¹⁻³⁴ The cell types present in the stromal cell layer (smooth muscle cells, fibroblasts, and myofibroblasts) can be identified by specific mesenchymal markers; CD34, vimentin, α -Smooth Muscle Actin, CD44, CD117, and CD90.³⁵ The specific cell-pools are tightly regulated by a specific molecular stimulus, like hormones, growth factors, cytokines, signaling proteins, etc.

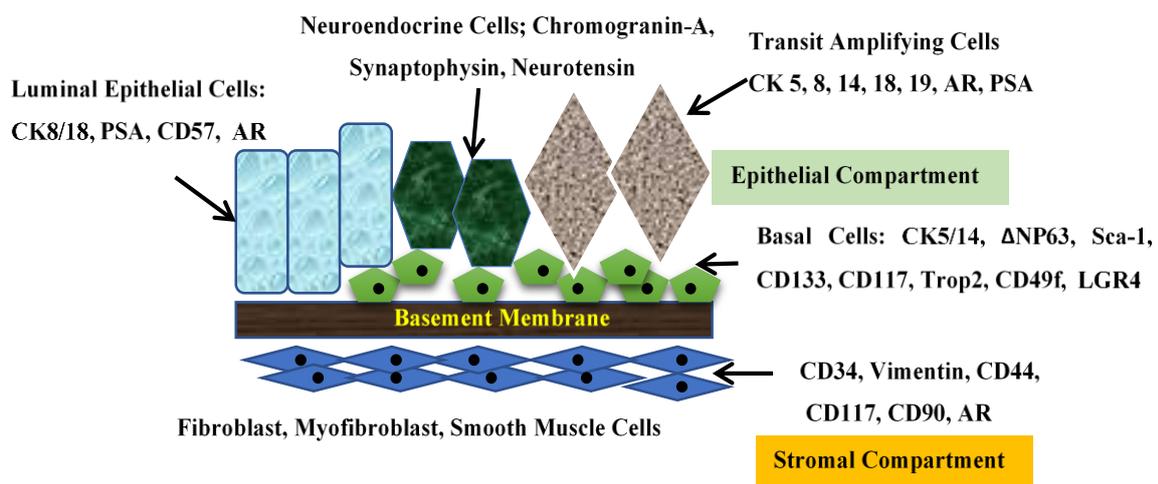


Figure 1. 8: Prostate gland cells organization and their identity markers. Prostate cells in stromal and epithelial compartments have been specifically characterized via expression of peculiar protein expression. (Adapted³⁶)

1.5 Prostate Endocrinology

As a hormonally regulated organ, the actions of androgens on stromal and epithelial cells accelerate the prostatic growth at sexual maturity.^{37, 38} The homeostasis of the gland, the

constant support of an adequate level of circulating androgens is vital.³⁹ The testes secrete several male sex hormones, which are collectively called androgens, including Testosterone, dihydroTestosterone (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 the 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 and converted by a membrane-bound enzymes, 5 α -reductase type -I and II, that catalyzes the irreversible conversion into DHT, with NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) as a cofactor,⁴⁰ especially in the adult prostate gland and external genitalia of the male fetus. (Figure 1.9) The Testosterone that does not bind to the receptors in tissues is rapidly converted into androsterone and dehydroepiandrosterone (DHEA) by the liver and simultaneously conjugated as either glucuronides or sulphates. These are excreted either into the gut by way of the liver bile or into the urine through the kidneys.

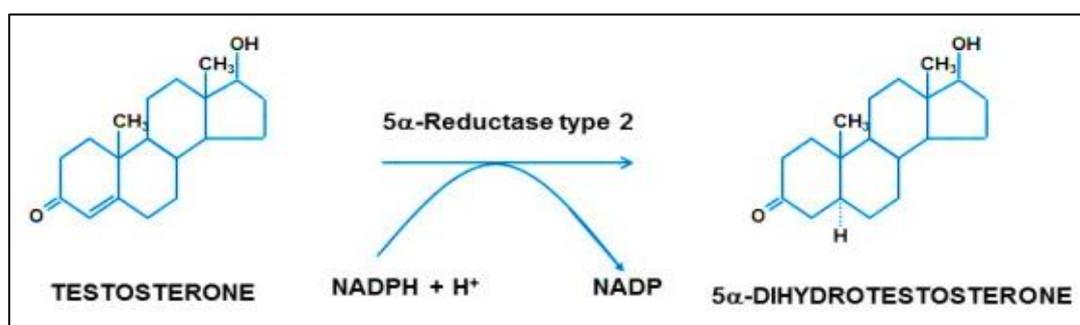


Figure 1. 9: Conversion of Testosterone into DHT. Formation of DHT is achieved in the stromal cells of the prostate where 5 α -Reductase-I & II enzyme converts Testosterone into a more potent androgen, DHT. (Adapted⁴¹)

Rodent studies have demonstrated that castration during fetal stages impair prostate development, which depends on the bud initiation stage during castration.⁴²⁻⁴⁶ Wilson *et al* determined that DHT is a reduced metabolite of Testosterone and the primary form of

androgen required in prostatic development.⁴⁷ The formation of DHT is derived from Testosterone by the action of the 5 α -reductase enzyme in stromal cells, which has a higher affinity to AR as compared with the parent Testosterone.⁴⁸ DHT induced AR is crucially required in the UGS mesenchyme during the fetal stages of prostate morphogenesis. However after completion of budding and branching of UGS, AR expression in the epithelial cells remain dominant.^{19, 49-51}

Prins *et al* showed that AR induction in prostate epithelium begins as early as postnatal days 1–2 (before cytodifferentiation of the epithelium and mesenchyme),¹⁹ possibly to drive morphogenesis and differentiation of adjacent mesenchymal cells is regulated by that androgen signals in the epithelial cells. However, the proximal-distal axis of the developing and adult prostate did not show a substantial difference in AR expression pattern suggesting that factors other than androgens control differential gene expression in this axis.^{19, 51} Other steroid hormones also play a crucial role during prostate development including estrogens and retinoids.^{37, 52, 53} Specific steroid hormone receptors have been identified to understand prostate morphogenesis in rats, which vary in time and cell-specific manner.²⁵

1.6 Prostatic disorders

There are mainly three types of pathological conditions in the prostate gland: Prostatitis, BPH, and PCa.

1.6.1 Prostatitis

Prostatitis is an inflammation of the prostate gland due to infection that can prevail in men of all ages. It is further divided into two categories; *Chronic bacterial prostatitis* is characterized by chronic bacterial infections from the urinary tract that infect the prostate gland leading to the inflamed prostate gland. (*Figure 1.10*) This Can be treated with antibiotics over a specific time. The most common form of prostatitis, *chronic nonbacterial prostatitis* is characterized by inflammation of the prostate gland without bacteria in their urine. Symptomatically Patients have genital, abdominal, or pelvic pain, symptoms pertaining to urine flow/persistency/frequency, and often erectile dysfunction. (Adapted: <https://my.clevelandclinic.org/health/diseases/15319-prostatitis>)

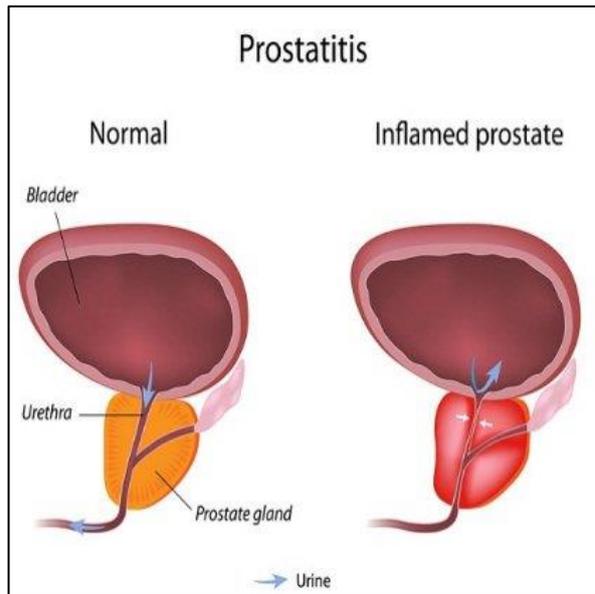


Figure 1. 10: Prostatitis. Inflammation in the prostate due to infections leading to urethral constriction. (Adapted-<https://www.University-urology.com/prostatitis-1/prostatitis-4/>)

1.6.2 Benign Prostate Hyperplasia (BPH)

The phase of the prostate growth persists for most of a man's life post adulthood. BPH most often occurs during late adulthood of life and greatly increases the burden to health in the elderly population. BPH is a non-malignant tumor formation with increased prostate volume, yet gland works normally. This overgrowth of the gland causes constriction of urethra and bladder, leading to blockage of urine flow. (Figure 1.11)

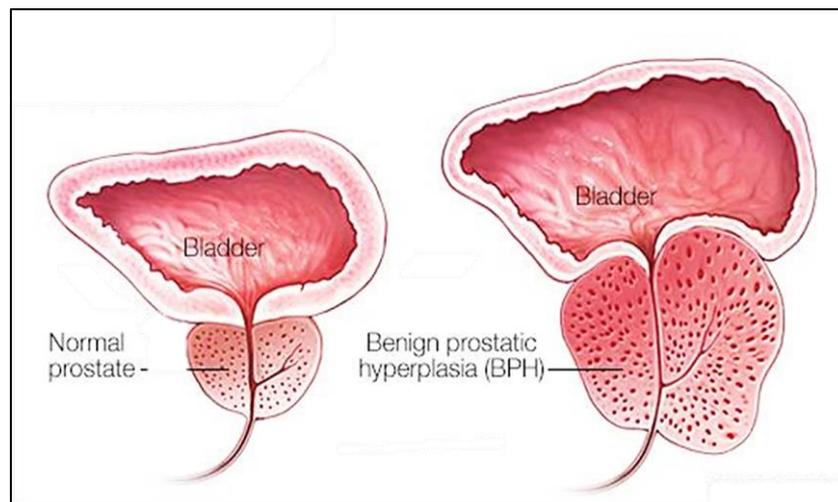


Figure 1. 11: Benign Prostate Hyperplasia. Non-malignant tumor formation in the transition zone of the prostate. (Adapted- <https://newsnetwork.mayoclinic.org/discussion/mayo-clinic-q-and-a-new-steam-treatment-for-benign-prostatic-hyperplasia/>)

1.6.3 Prostate Cancer (PCa)

PCa is a malignant tumor formation with loss of tissue morphology and functionality that can metastasize to other parts of the body. PCa is considered as the second most mortality related cancer in men after skin cancer globally. The tumor slowly diminishes the structural and functional integrity of the prostate gland. Untreated tumors then grow exponentially to develop castrate resistance PCa (CRPC) which metastasizes to grow in other body parts like lymph, bones, liver, intestine. (Figure 1.12) (Adapted: <https://www.cancer.org/cancer/prostate-cancer.html>)

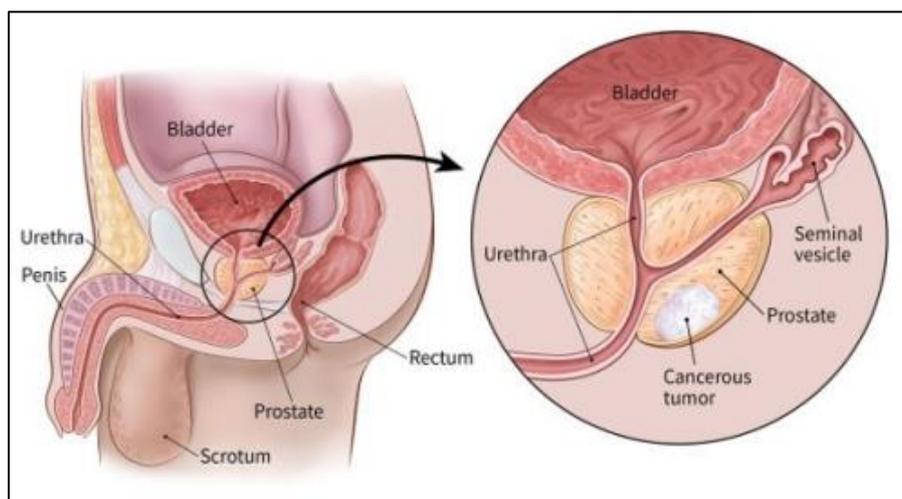


Figure 1. 12: Prostate Cancer. Malignant tumor formation in the peripheral region of the gland that can metastasize to the different body parts. (Adapted-<https://www.cancer.org/cancer/prostate-cancer/about/what-is-prostate-cancer.html>)

1.7 Epidemiology of BPH and PCa

BPH affects ~70-80% of elderly men by 80 years of age with different severity of the disease.⁵⁴ Thus, Aging is considered a primary factor of BPH eruption as the prostate gland is a continuously growing tissue. The incidences of the BPH and PCa have been rising in the past 30 years. It has been reported that despite the Indian subjects have lower prostate volume than the western population, Indian men have higher International Prostate Symptom Score (prevalence of symptomatic BPH).⁵⁵ Racially, Asian men have increased BPH occurrence than in Caucasian or African-American populations. Whereas, PCa incidences are highest in the African-American population followed by Caucasians, and least in Asian men. But, the population data collected between 1998-2007 showed a doubled number of BPH incidences in American men.⁵⁶ The precise prevalence of BPH is largely unavailable in the Indian population. Further, BPH is not considered as the inducer

on the neoplastic transformation of the gland, and it does not correlate with PCa incidences. Yet, one of the largest studies of 27 years on Danish men discovered that men with clinical BPH have increased the risk of developing PCa by 2-3 folds and associated mortality.⁵⁷

The incidences of PCa are increasing globally, making the leading form of cancer and the second most cancer-associated mortality in men according to WHO data.⁵⁸ (<http://gco.iarc.fr/>) According to Globocan and Indian Council of Medical Research (ICMR) 2018 data, a total of 25,696 new cases of PCa were registered accounting for 2.45% of total cancer cases across India and 17,184 PCa related deaths which are 2.39% of total cancer-related deaths across India. The data suggests that almost every man dies once he acquires PCa in India. (<http://cancerindia.org.in/globocan-2018-india-factsheet/>) According to the American Cancer Society, 174,650 men were diagnosed with PCa of which 31,620 men died due to PCa in the year 2018. (American Cancer Society. Facts & Figures 2019. American Cancer Society. Atlanta, Ga. 2019) Hence, the increasing incidence rate and mortality have become a challenge to understand both diseases more profoundly.

1.8 Etiopathology of Prostatic diseases

BPH and PCa are major pathologies due to increased proliferation. Both of the diseases share many similarities yet many differences at the histopathological level.

1.8.1 Benign Prostate Hyperplasia

BPH is multinodular hyperplasia that erupts in the transitional and periurethral zones of the prostate to form adenomata.⁵⁹ BPH is described as the prostate enlargement and lower urinary tract symptoms (LUTS) with voiding dysfunction and bladder outlet obstruction (BOO) with symptomatic changes in routine life (urgency, frequency, nocturia, and urine intermittency).⁶⁰ During BPH, a compressive obstruction on the urethra caused by hyperproliferation of the prostatic cells around the urethral region is the key reason of the BOO.⁶¹ Thus, Clinically, BPH does not affect mortality but it surely deteriorates the quality of life in elderly men.

The cell proliferation of the clinical BPH showed higher inconsistencies in the occurrence of stromal and epithelial hyperplasia. The alterations in the cellular composition of the specific cell types revealed very varying percentages of the cells like connective tissue

(16.1-56.1%), smooth muscle (20.2-59.3%), epithelium (4.3-24.8%), and epithelial lumen (5.3-21.9%) during BPH.⁶⁰ Due to the increase in cell mass, the expression of cell-specific signature markers also enhances. For instance, with increased connective tissue and smooth muscle, the collagen network becomes dense and the number of fibers increases.⁶² Additionally due to increased secretory luminal epithelial cell number, there is elevated serum levels of prostatic specific antigen (PSA), which correlate with increased prostate volume during BPH condition.⁶³ The function and growth of the cells in the prostate gland are regulated by hormones, especially by Hypothalamus-Pituitary-Gonadotropin (HPG) axis/Testosterone which plays a vital role in the BPH pathogenesis.

1.8.2 Prostate Cancer

PCa is primarily developed in the posterior region of the prostate gland forming a nodule protruding towards the extracapsular portion of the gland due to rapidly multiplying tumor cells. The development of the PCa occurs in multiple steps like the initiation of prostatic intraepithelial neoplasia (PIN) to localized PCa followed by metastasis to other organs.⁶⁴ The most common sites of PCa metastasis are the bones (pelvis) and lymph nodes (iliac nodes). To understand the severity of the PCa pathogenesis, the Gleason grading system was developed using histological patterns of the cancerous tissue. Symptomatically, PCa is not as profound as BPH and can remain incognito, as it may not affect the urethra or urine flow and other normal functions of the gland during the initial development stage. Yet, back pain, hip or pelvic pain, loss of libido, blood in the urine or semen, and sudden weight loss are few general symptoms of PCa.

According to the American Cancer Society, there are several types of PCa depending on the type of cells involved in tumorigenesis, adenocarcinoma (Luminal epithelial cells), small cell carcinoma (epithelial cells), neuroendocrine tumors (neuroendocrine cells), and sarcoma (smooth muscle and fibroblast cells). Among these types, adenocarcinoma (luminal epithelial cell) is determined as the most common type of PCa of the prostate gland. During adenocarcinoma, secretory luminal cells form highly proliferative tumor nodules leading to increased expression of PSA, Prostatic Acid Phosphatase (PAP), and NKX3.1. Hence, increasing serum PSA levels is considered as a primary marker of PCa detection.⁶⁵ The regulation of the secretory function of the gland is under the control of HPG axis/Testosterone actions in healthy and diseased conditions. Hence, the growth and progression of PCa is also dependant upon hormones as in BPH condition.

1.9 Risk Factors of prostate pathologies

Prostatitis, BPH, and PCa substantially impact public health globally. There are several factors responsible for the development of prostatitis, BPH, and PCa. And increasing the exposure of these factors made treatment more challenging and immensely contributed to increased global incidences. The precise molecular etiopathology of the prostate is very scanty but factors like the embryonic reawakening, aging, androgens, stem cells, and inflammation are hypothesized to be responsible for the BPH and PCa development.

1.9.1 Diet and Lifestyle

BPH and PCa are generally linked to the consumption of a specific diet. The food intake rich in trans fat, saturated fat, or carbohydrate intake has been linked to PCa. Also, Higher levels of Vitamin E showed an increased risk of BPH and PCa development. Higher intake of Calcium, Whole milk, red meat has been linked to the BPH/LUTS and PCa development in studies. On the contrary, increased consumption of Vegetables, Fruits, Fish, Vitamin A, and Vitamin D depicted decreased risk of disease development. Further, reports have also shown a positive association of diabetes and obesity with BPH and PCa both.^{55, 66}

1.9.2 Genetic Factors

The occurrence of prostate pathologies is genetically predisposed linked with race and family history. A study by Roberts *et al* demonstrated the increased the risk for development of symptomatic BPH in men with a family history of an enlarged prostate.⁶⁷ Similar observation were depicted in PCa also, where a man has twice the risk on acquiring PCa with the family history on PCa in a first-degree relative (father or brother). Racially, African-Americans depicted increased prevalence and severity of PCa than in white men.⁵⁵ Genetic mutations in Glutathione S-transferase enzyme genes and the number of CAG repeats in the androgen receptor gene were substantially associated with an increased risk of symptomatic BPH in North Indian men.^{68,69} Additionally, mutations in *BRCA1* and *BRCA2* have been also associated with PCa along with breast cancer. Further, mutations in *p53*, *AR*, *BRIP1*, *CHEK2*, *TMPRSS2-ETS* gene family genes (and many more genes) have depicted to promote PCa cell growth.⁵⁵

1.9.3 Hormones

The development, growth, and function of the prostate gland are hormonally regulated. The entire Hypothalamus-Pituitary-Gonadotropin axis synergizes the dynamics of the

gland, and they are not considered as the initiators of the prostate pathologies. However, they significantly contribute to the BPH and PCa progression, especially Testosterone and DHT. A study by Parsons *et al* showed three times increased risk of BPH with higher levels of DHT in midlife compared with the lower levels.⁷⁰ Similar pieces of evidence were also found in PCa patients with increased intra-tumoral Testosterone and DHT levels.⁷¹ Increased tissue androgen levels lead to AR-mediated hyperproliferation of the cells, that contribute to aggressive tumor growth.

1.9.4 Activation of Stem cells

The resident stem cells of the tissue/organ exhibit capacity to differentiate into tissue-specific functional cells and repair of the damaged tissue. These adult stem cells are mostly quiescent and reside in specialized locations of the gland.⁷² Reactivation of the stem cell niche of the prostate gland has been considered as one of the key contributing factors of abnormal cell proliferation that eventually contribute to BPH and PCa pathogenesis.³⁶ The role of the stem cell markers, Sca-1, CD44, CD117, CD49f, LGR4^{73, 74} in the BPH and PCa development have been discussed in the following sections.

1.10 AR-dependent therapeutics of BPH and PCa

There are plenty of medical and surgical treatment options available for BPH and PCA. α -Blockers (5 α -Reductase inhibitors) are used for muscle relaxation of the prostate and bladder to improve urine flow by decrease urethral blockage. The use of 5- α -Reductase causes inhibition of the conversion of Testosterone to DHT hormone. This will reduce hormone-mediated cell proliferation and prostate growth. Anti-androgen therapies, like Nilutamide and Enzalutamide, inhibits the activation of AR to decrease cell growth and BPH symptoms. Surgically, BPH is treated with many methods like Transurethral Incision of the Prostate (TUIP), Photo-selective Vaporization of the Prostate (PVP), Transurethral Resection of the Prostate (TURP), Holmium Laser Enucleation of the Prostate (HoLEP), Thulium Laser Enucleation of the Prostate (ThuLEP), Transurethral Electro-vaporization of the Prostate (TUVP) and Transurethral Water-Jet Ablation. (Adapted: [https://www.urologyhealth.org/urologic-conditions/benign-prostatic-hyperplasia-\(bph\)#Treatment](https://www.urologyhealth.org/urologic-conditions/benign-prostatic-hyperplasia-(bph)#Treatment))

During PCa, with tumor growth and metastasis, treatments become more challenging and increase the mortality rate. In most cases, tumors are surgically removed followed by

medications like androgen synthesis inhibitors or anti-androgen therapies against tumor relapse. However, PCa therapeutics always depend upon tumor stage, tumor grade, and its molecular profile. For instance, CRPC does not respond to many of conventional treatments during metastasis into other organs leading to tumor relapse in the patient. Thus, radiation therapies are given to kill metastasized cancer cells to prevent its further spread and relapse. However, recent evidence explicitly exhibited that the key to comprehending the complete conjecture of AR lies within AR-NTD. And, small molecule EPI-506 is one such drug under clinical trial that specifically targets NTD of AR and AR-V7 in metastatic CRPC.⁷⁵

1.11 Endocrinology of BPH and PCa development

The endocrine axis of the prostate plays a pivotal role to support the growth of both BPH and PCa. The key hormones of the endocrine axis are Gonadotropin-releasing Hormone (GnRH), Luteinising Hormone (LH), Follicle Stimulatory Hormone (FSH) and Testosterone. Pulsatile secretion of GnRH from the hypothalamus induces the secretion of the LH and FSH from the pituitary gland. Sequentially, LH positively stimulates the synthesis of Testosterone in the testes, and upon reaching plateau concentration, Testosterone inhibits the signaling of GnRH and LH. Further, FSH stimulates prostatic inhibin peptide to inhibit FSH release from the pituitary gland.⁷⁶ Recent therapeutics have started to explore the drugs target in the HPG axis to attain rapid androgen deprivation in the BPH and PCa patients.

During the pathogenesis of BPH and PCa, the serum levels of LH show inconsistent alterations from very low to significantly higher than the normal range. Moreover, LH levels were not significantly different between BPH and PCa. Serum FSH levels were not increased or decreased beyond the normal range. Yet, a study showed higher serum FSH levels in elderly men with PCa as compare to BPH.⁷⁷ Testosterone directly acts on the prostate cells upon stimulus and it has been known that serum Testosterone levels decline in men with aging. Studies reported Serum Total and Free Testosterone levels remained at the lower limit of the normal range and their levels do not significantly differ among BPH and PCa.^{77, 78} Furthermore, the increasing trend of intraprostatic DihydroTestosterone (DHT) levels were found in BPH and PCa condition. And PCa patients depicted very high levels of intraprostatic DHT levels than in BPH patients. But due to immense variations

in these levels reported in many studies, the intra-prostatic DHT levels were not considered as the causal factor of the development.⁷¹ (Table 1.3)

Table 1. 3: Serum level of hormones in men with BPH and PCa condition

| Hormone | Normal range | BPH | PCa |
|------------------------------------|---|---|---|
| LH | 2-12 mIU/mL | 0.2–20.5 mIU/ml | 0.07–34.89 mIU/ml |
| FSH | 1-12 mIU/ml | 6.06 mIU/ml ⁷⁷ | 7.56 mIU/ml ⁷⁷ |
| Total Testosterone | 5-23 nmol/L | 4.5 ng/ml ⁷⁷ 4.38 ng/ml ⁷⁸ | 4.05 ng/ml ⁷⁷ 5.16 ng/ml ⁷⁸ 3.4-4 ng/ml ⁷⁹ 0.9–1.4 ng/ml ^{*79} |
| Free Testosterone | 0.24-0.36 nmol/L | 24.37 nmol/L 0.05-0.1 ng/gm ^{*80} | 23.09 nmol/L |
| Dihydro Testosterone | 14-77 ng/dL 0.7-2.1 ng/gm ^{*71} | 3-6 ng/gm ^{*80} 1.0-8.15 ng/gm ^{*71} | 3.3 ng/ml ⁷⁹ 8.6–11.6 ng/gm ^{*79} |
| * = intraprostatic (tissue) levels | | | |

A. Gonadotropin-Releasing Hormone (GnRH)

GnRH hormone acts via GnRH Receptor (GnRHR), which are expressed on several tissues like the ovaries, endometrium, placenta, breast, and prostate.⁸¹ The presence of GnRHR has been detected in the prostate gland, yet role in the development of BPH and PCa is still unknown. Its role in cell proliferation was determined in Pancreatic cancer where the expression of GnRH was significantly associated with the cell proliferation via activation of the Bcl-2/Bax/caspase pathway.⁸² The inhibition of GnRHR has been explored in the PCa condition as a blocker of Androgen production. The treatment with the most potent GnRH antagonist, Degarelix, blocks GnRHRs and LH and FSH secretion to decline the Testosterone production within 1–3 days of the administration, inhibiting its mitogenic effect in tumor cells.^{81, 83} However, due to the risk of Histamine releasing an initial Testosterone surging properties of Degarelix, the drug is not widely used for the treatment of BPH and PCa.⁸⁴

B. Luteinizing Hormone (LH)

Although the levels of LH do not markedly increase in prostate pathologies, their contribution in the progression is more evident. A study on Chinese men with BPH, that serum LH, and FSH levels are significantly correlated with increased age and prostate volume.⁸⁵ LH concentrations were not significantly different between the BPH

and PCa patients with different stages. Further, LH receptor mRNA and proteins were expressed in androgen-dependent and independent PCa cell-lines and inhibition of LH Receptor reduced the growth of the PCa cells.⁸⁶ Hence, inhibition of LH and LH receptor provides a viable target to explore therapeutic possibilities.

C. Follicle Stimulating Hormone (FSH)

It has been observed that the FSH receptor (FSHR) expression is not detected in the normal tissue, but higher expression of FSHR has been detected in the malignant tissue of a PCa patient. Further, FSH directly stimulated PCa tumor growth and metastasis via promoting angiogenesis within the tumor leading to hormone-refractory PCa development.⁸⁷ Inhibition of FSH secretion by Degarelix (GnRH inhibitor) decreased the tumor growth in PCa patients.⁸⁸

D. Testosterone

Production and secretion of Testosterone from testes are stimulated by GnRH-LH. Testosterone initiates the sperm cell differentiation in the testes and prostatic fluid production in the prostate gland. Upon its stimulus in the prostate gland, Testosterone is converted into its more potent form, DHT by a 5α -Reductase enzyme present in the stromal cells. DHT is then secreted from the stromal cells and acts on epithelial cells in a paracrine fashion to activate AR.⁸⁹ Despite the decreased total and free Testosterone levels in serum, increased levels of intraprostatic DHT and AR were observed particularly during BPH and PCa conditions.^{71,79,80} Regardless of no association between serum Testosterone with BPH and PCa development, Testosterone plays the most substantial role in the development of BPH and PCa. However, there is no clear evidence on the correlation between circulating androgens and prostate volume in elderly men with BPH.⁸⁹

It has been discovered that both BPH and PCa tumor cells largely depend on the androgen/AR axis for the growth and progression of the tumor. Hence, most of the therapeutic drugs available to treat BPH and PCa are the inhibitors of Testosterone synthesis pathway or anti-androgens in patients. GnRH antagonists, LH/FSH antagonists were used in patients to inhibit the production of Testosterone. Subsequently, tissue DHT levels were significantly involved in the disease progression, 5α -reductase inhibitors (finasteride and dutasteride) were used to inhibit

Testosterone to DHT conversion as a treatment. To inhibit the action of Testosterone/DHT, anti-androgens (Nilutamide, Bicalutamide, Enzalutamide, etc.) were used that inhibits actions of AR in the tumor cells.

1.12 Androgen Receptor

In the nuclear receptor superfamily, AR is the fourth member of group C of “Estrogen Receptor-like” sub-family (NR3C4). It is a ligand inducible nuclear receptor present in the cell cytoplasm that acts as a transcription factor (TF) to regulate gene expression. The prostate gland is one of the tissues with abundant AR expression acting as a core molecule in prostate homeostasis.

1.12.1 Structure of AR

AR is made up of three main domains N-terminal domain (NTD), DNA Binding Domain (DBD), Ligand Binding Domain (LBD), and a hinge region. (*Figure 1.13*)

1. NTD (Exon-1) is responsible for the ligand-activated transcriptional activity and the constitutive activity without bound ligand.⁹⁰ The activation of the AR NTD, which spans the first 538 amino acids of the molecule, is extremely important for the gene regulation function of AR and its participation in protein-protein interactions. The NTD of AR plays a central role in cancer development and progression, making it an ideal target for novel anti-cancer drugs.

NTD is made up of 3 motifs- the dimerization surface that spans amino acids 1 through 36 and 370 through 494, the activation function 1 (AF1) spanning residues 101 to 370 responsible for ligand-activated transcriptional activity, and activation function 5 (AF5) spanning residues from 360 to 485 which is responsible for activity without bound ligand. The AF-1 motif is made up of two units: the ligand-dependent TAU-1 (amino acids 101–307) and ligand-independent TAU-5 (amino acids 360–528).⁹¹

2. DBD (Exon-2 and 3) contains two zinc finger domains, which functions as a DBD binding site for receptor homodimer formation.⁹² Each zinc finger facilitates the interaction with the androgen-response element (ARE) half-site and AR dimerization.⁹³

3. Hinge region (Exon-4) is a connecting bridge between DBD with the LBD; It also contains a ligand-dependent nuclear localization signal (NLS) for AR nuclear translocation by interacting with Filamin-A (FlnA).⁹⁴
4. LBD (Exon-5, 6, 7, 8) interacts with AF-2 and facilitates the binding of androgens, hence represents the primary key of AR signaling.⁹⁵

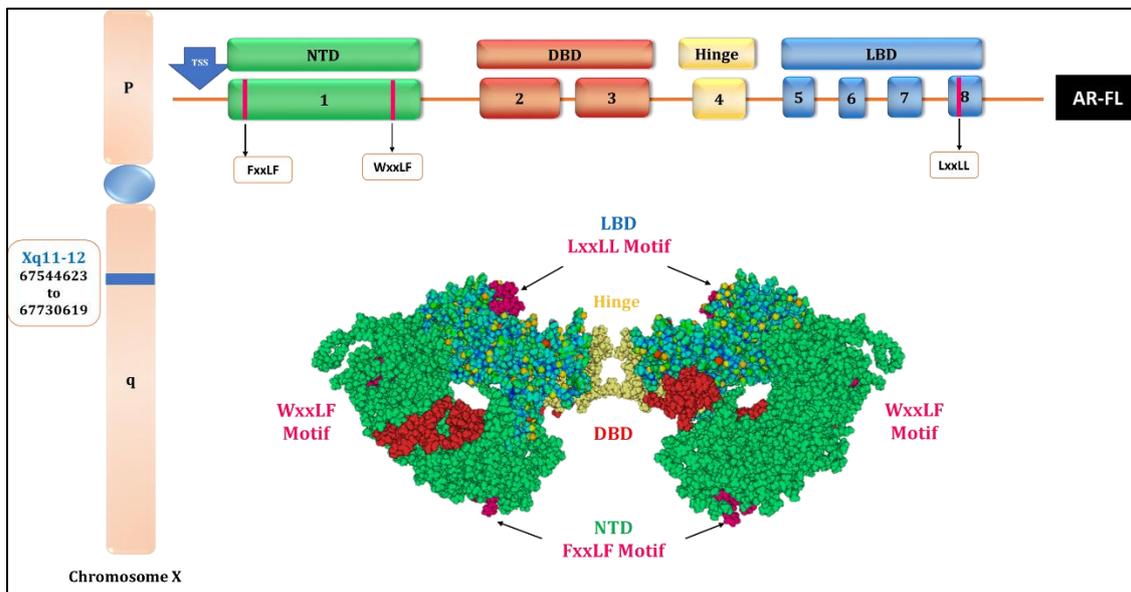


Figure 1.13: Protein structure of AR representing motifs and phospho-sites. AR gene belongs to chromosome X–q arm. It consists of 8 exons containing vital domains: Interacting NTD (exon-1: Green) with FxxLF and WxxLF motifs, Transcription controlling DBD (exon2-3: Red), Dimer forming Hinge region (exon-4: Yellow), Androgen binding LBD (Exon 5-8: Blue) containing LxxLL motif to identify ARE sites on DNA. The exons and motifs of AR are also illustrated in the 3D-image with color-coding prepared on PyMol™ Software.

1.12.2 AR Signaling

The interaction of Androgens or cell signaling proteins with AR enhances its nuclear import. Binding of androgen leads to AR-Heat shock proteins (HSP90/HSP70) complex dissociation in the cytoplasm and stabilized by phospho-activation of AR.⁹⁶ Later, AR forms a homodimer and translocates inside the nucleus from the cytoplasm and binds to the Androgen Response Elements (AREs) in the gene promoter or enhancer region, resulting in up- or down-regulation of target gene transcription.⁹⁷ The process also includes various chaperone proteins, undergo post-translational modifications including phosphorylation, co-activation, and co-repression.^{98,99} (Figure 1.14A) The interaction with Androgen induces the highest phosphorylation at serine-81 (S81) residue of AR which stabilizes the protein after Hsp70-90 detachment and increases its nuclear localization.

Nevertheless, the activity of AR is regulated by multiple phosphorylation sites that are intriguingly regulated by signal transduction proteins.

Apart from the activation via Testosterone/DHT, AR activation is also regulated several signal transduction pathways like Akt, MAPK, Jak/Stat etc.¹⁰⁰ These signaling proteins induce phosphorylation of AR in an androgen-independent manner and causes its nuclear localization followed by gene regulation via ARE sites. (Figure 1.14B)

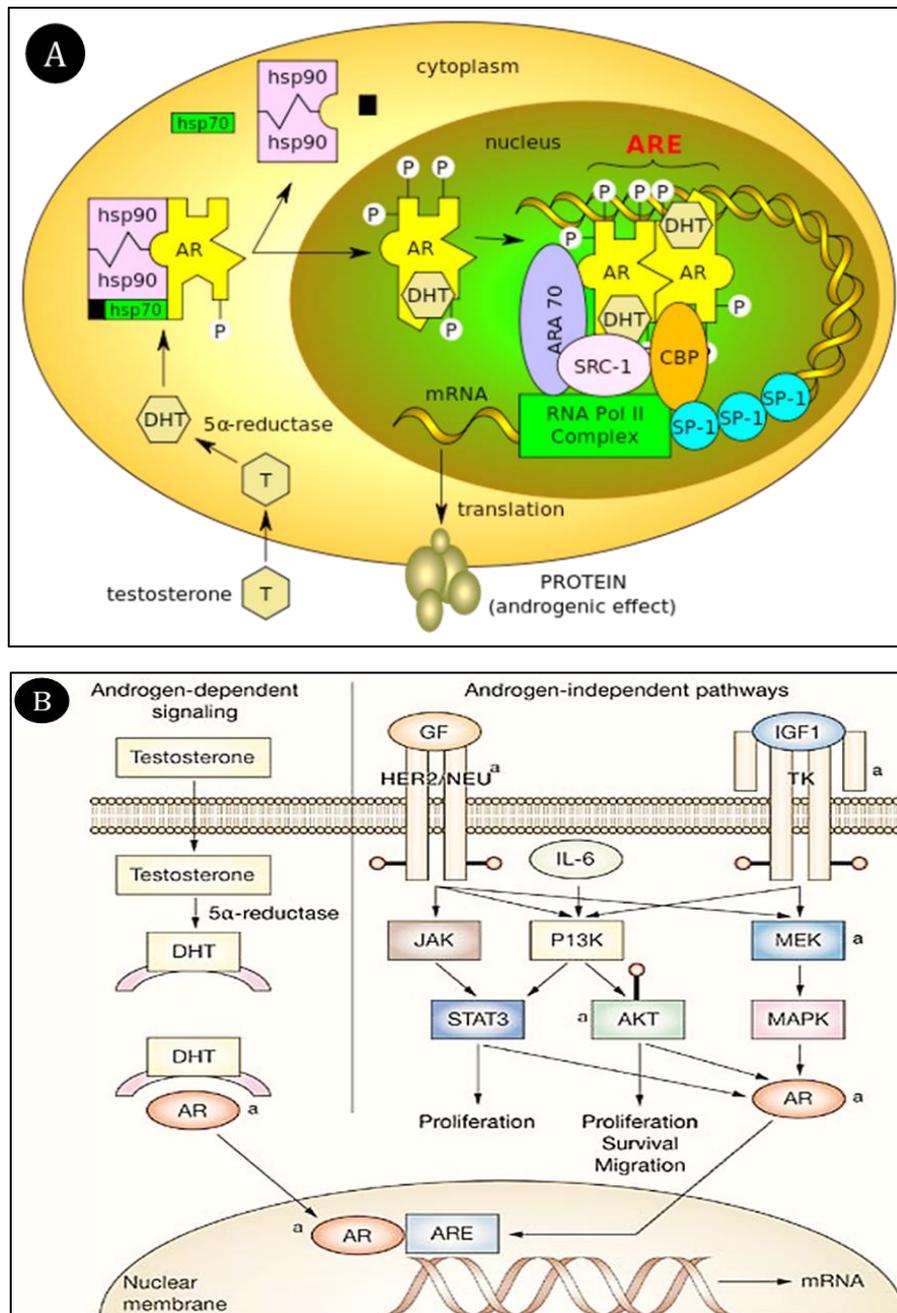


Figure 1. 14: AR signaling in a prostate cell. (A) Activation of AR by DHT leads to its dissociation from Hsp90/70 and nuclear translocation. Interaction of AR with various coregulators leads to its binding to ARE site of the target gene to be transcribed.

(Adapted¹⁰¹) **(B)** Activation of AR by Androgen dependent (Androgen Mediated) and Androgen independent (Growth factor and signal transduction pathway mediated activation).(Adapted¹⁰⁰)

AR is the key TFs in the prostate gland that extensively regulates the gene transcription in normal and diseased conditions. During the proliferative conditions of the tumor development, an increased number of AR binding sites has been demonstrated. Cheng *et al* demonstrated 4143 and 2380 DNA binding sites with the coverage of 2796 and 1854 genes in androgen-dependent and independent PCa cell-lines respectively.¹⁰² A patient study demonstrated a far higher number of DNA binding sites in patient-derived tumors. ChIP-Seq data published by Pomerantz *et al* discovered 9179 DNA binding sites of AR in tumor samples of prostate cancer (PCa) patients against 2690 sites in normal prostate tissue samples.¹⁰³ Further, These sites are regulated by AR with coordination of coactivators and corepressors interactions and perform diverse functions.¹⁰⁴ Thus, AR can completely hijack the transcriptional process of a tumor cell during PCa.

1.12.3 AR Variants

Posttranslational modifications of AR regulates its functionality stability and cellular localization.¹⁰⁵ These Posttranslational changes cause the formation of shorter AR protein structures as compare to full length-AR (FL-AR), of which most of the structures lack CTD-LBD, known as AR-Vs. Due to the absence of LBD, anti-androgens class drugs can not inhibit the functionality of AR. In absence of LBD/AF-2 activity in AR-Vs, constitutive activity of AF-1 shifts from the TAU1 region to TAU5 to exerts transcriptional activity of AR-Vs similar to androgens mediated activation FL-AR.⁹¹

Approximately 20 variants have been discovered and all of them have intact NTD (Exon-1) and cryptic exons (CE1/2/3/4). CEs are formed due to stop codons present in the transcribed “intronic” sequences between exons, and present in the first seven variants, AR-V1 to AR-V7. (Figure 1.15) 19 amino acids containing AR-V1 are truncated at the end of exon 3 and an additional CE1 whereas, AR-V7 (also known as AR3) contains CE3 at the end of exon-3.⁹¹ Both, AR-V1 and AR-V7 have been found as predominant AR variants expressed with ~20-fold higher expression in CRPC.¹⁰⁶ Moreover, AR-V7 is the most predominant and active isoform found in advanced PCa conditions.¹⁰⁷

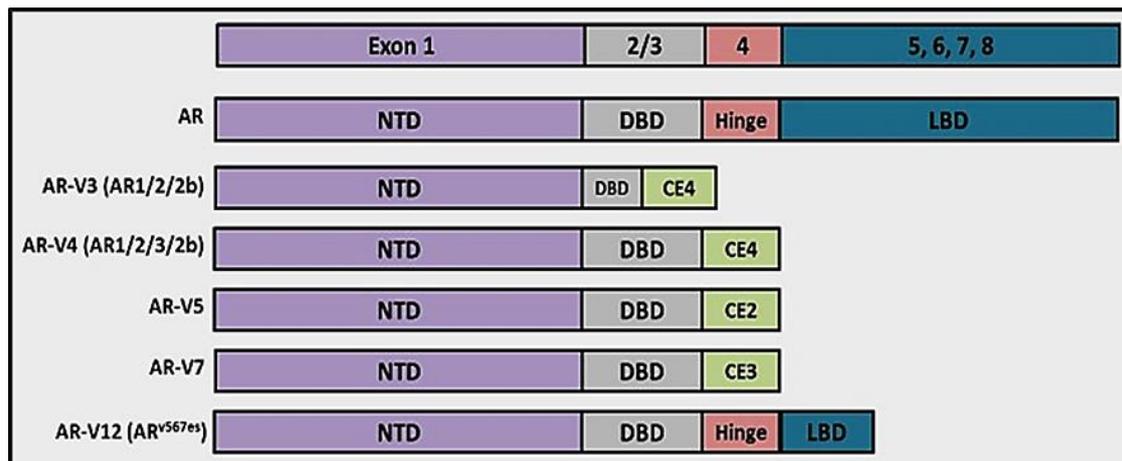


Figure 1. 15: AR Splice variants. Truncated DBD or LBD containing protein variants of AR. NTD: N-terminal domain, HR: hinge region, DBD: DNA-Binding domain, LBD: Ligand Binding Domain, CE: cryptic exon. (Adapted¹⁰⁸)

Reports suggested co-expression of AR-V1 and AR-V7 variants resulted in AR-V1 dependant inhibition of AR-V7 in CRPC, suggesting AR-V1 as a negative regulator of AR-V7.^{109, 110} Hillebrand *et al* assessed the disease-specific expression of AR variants and discovered increased AR-V4 levels (AR-FL and AR-V7 activator) in BPH tumors, AR-V1 levels (AR-FL activation and AR-V7 inhibition) in primary PCa tumors, and AR-V7 (castration-resistant cell growth driver) in CRPC.¹¹¹ The expression of AR-FL along with AR-V567es and AR-V7 variants was significantly elevated in CRPCs and AR-V7 was found to be associated with drug resistance, poorer prognosis, poor patient survival in abiraterone treated CRPC xenografts.¹¹² Hence, the presence of AR-V7 in tumor samples has become a significant predictor of CRPC.¹¹³ The presence of AR-FL and AR-Vs defines the transcriptome of the cells that are correlated with the progression and severity of PCa.

1.12.4 AR Phosphorylation

Several posttranslational modifications like phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation can regulate the transcriptional activity of AR.¹¹⁴ Cellular localization, stability and transcriptional activity of AR are regulated by its phosphorylation at multiple phosphorylation sites within AR. Modifications in AR phosphorylation affects normal prostate functioning and disease progression. There are 18 important phospho-sites present on serine, threonine, and tyrosine residues of AR protein. 14 out of 18 sites are in the AF1 region of NTD encompassing the phosphorylation of serine (S16, S81, S94, S213, S256, S293, S308, S424, S515), threonine (T282), and tyrosine (Y223, Y267, Y363, Y534) residues.¹¹⁵ The remaining four phosphor-sites are in

DBD (S578), hinge region (S650), and AF2 region of LBD (S791 and T850). (*Figure 1.16A*) Each phosphorylation site impacts the activation and degradation of the AR protein in the cell. Most important phosphorylation sites within AR-NTD are regulated by androgens and kinases like PIM-1, Akt, CDKs, etc. Among the multiple phosphorylation sites in the AR, phosphorylation of Serine-81 (S81) and Serine-213 (S213) in the NTD is the key phospho-sites that control most of the AR functions.

In response to androgen-mediated activation, AR is phosphorylated on S81 residue, that stabilizes the protein after HSP70/90 detachment, and induces its nuclear localization. The phosphorylation of S81 residue is also regulated by CDK1, CDK5, and CDK9.¹¹⁶⁻¹¹⁹ Thus, during the rapid cell proliferation mode of the cells, AR can be activated in androgen-independent fashion. Further, phosphorylation at S81 caused enhanced chromatin binding and transcription abilities of AR.¹²⁰ A recent patient study by Russo *et al* depicted reactivation and nuclear localization of pARs81 to be associated with the development of CRPC.¹²¹ However, AR protein without S81 phosphorylation was also found to bind on ARE sites in the chromatin.¹¹⁵ Hence, other regulatory phosphorylation sites are also substantially involved that control AR actions. (*Figure 1.16B*)

Another major AR regulatory phosphorylation site is at S213 residue. The pARs213 is majorly regulated by several proteins like PIM-1, Akt, CXCL12/G protein-coupled receptor chemokine receptor 4 (CXCR4).¹¹⁵ During tumorigenesis, Akt is one of the most common overexpressed proteins that inhibit PTEN to drive cell survival and proliferation. Further, Akt induced phosphorylation of AR at S213 residue increases the transcriptional activity of AR during PCa.¹²²⁻¹²³ Additionally, Immunohistological localization of pARs213 expression appeared to be elevated in epithelial cells but absent in surrounding stromal cells of the PCa tumor.¹²² pARs213 exhibited unusual transcriptional activity of AR as it decreases transcription of endogenous PSA, NKX3.1, and IGFBP5, and instead transcribes IL6, cyclin A1, and caveolin to promote PCa. This novel aspect of pARs213 explains differential transcriptional outcomes in CRPC.¹²⁴⁻¹²⁶ Apart from S213 mediated mysterious transcriptional ability, reports also suggested that S213 phosphorylation induces MDM2 mediated proteolysis of AR.^{124, 127, 128} In addition to these two major AR regulatory sites, there are also key phosphorylation sites regulated by signaling proteins.¹¹⁵ (*Figure 1.16B*) Among these sites, S308 and S215/S792 sites were also discovered as the

inhibitors of AR transcriptional activity regulated by Cyclin D3/CDK1p58 and Akt respectively.¹¹⁴

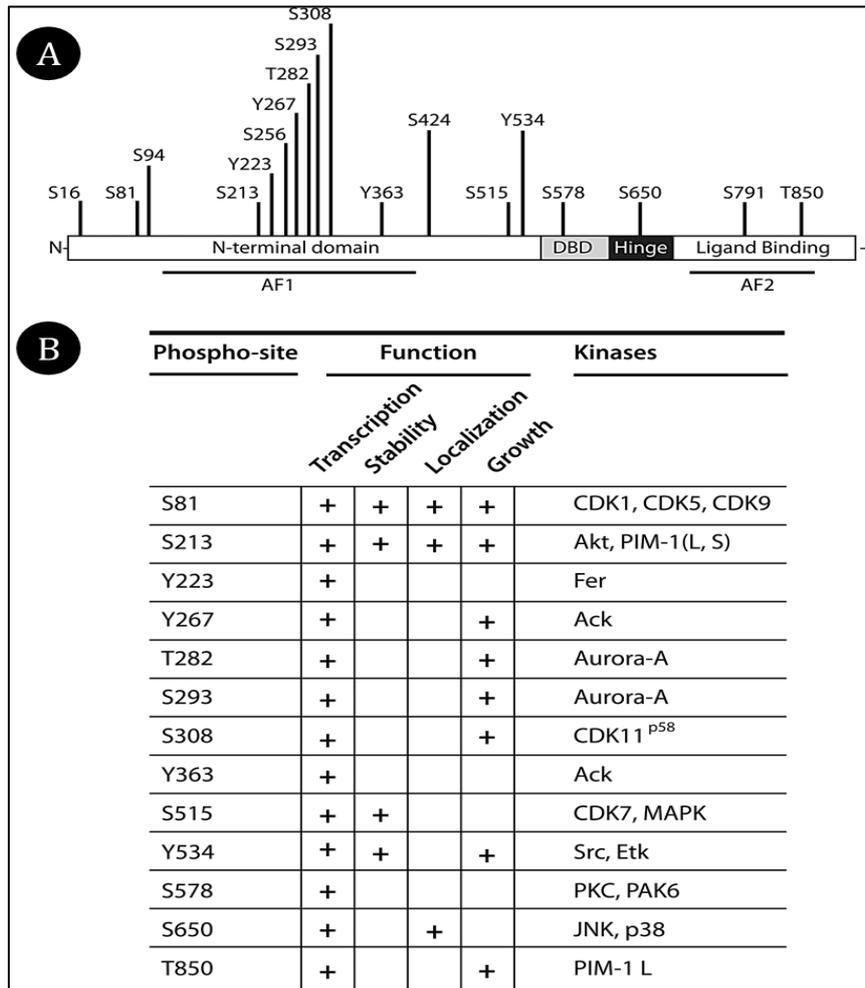


Figure 1.16: AR phosphorylations. (A) Region-specific phospho-sites on AR protein, **(B)** Role of phospho-sites of AR functions and their regulating kinases. (Adapted¹¹⁵)

1.13 Distribution and role of AR in cellular compartments of the prostate

AR is the key driver of prostatic growth and differentiation and shows complex expression patterns in cells. In 1991, a study demonstrated that a few AR-positive fibroblasts were present within the ventral and dorsal lobes of the rat prostate, where the majority of the cells were AR-negative.¹²⁹ Later, the expression of AR in different types of cells in dorsal, ventral, and anterior lobes of the human prostate has been discovered. At the cellular level, the epithelial compartment has stem/progenitor cells and secretory luminal cells whereas the stromal compartment majorly consist of fibroblasts, myofibroblasts, smooth muscle cells (SMC), endothelial cells, and immune cells. These cells exhibit expression of specific proteins that provide them an individual identity as described earlier. Both the cell compartments have extensive cellular crosstalk due to their paracrine actions during

healthy and diseased conditions. In the prostate, AR is abundantly present in the nuclei of luminal epithelial and stromal cells, whereas, AR expression is absent or low in basal stem cells (BSCs).^{130, 131} Further, AR upon DHT stimulation, also increased the production of several growth factors and cytokines from stromal cells that drive cellular proliferation in the human prostate.¹³² Hence, activation of AR with Testosterone/DHT in all the cell types of the prostate can support the growth of the cells.

1.13.1 Epithelial compartment

The epithelial compartment of the prostate encompasses three cell types: Stem cells, Neuroendocrine cells, and Luminal cells. The stem cells can be further categorized into two subtypes: BSCs and Luminal progenitors (Transit-amplifying cells). Each of these cells has been studied in terms of the expression and function of AR

A. Stem/Progenitor cell Population

Several studies have investigated the expression of AR in BSC populations of the prostate. It has been found previously that androgen deprivation in a regressed normal prostate showed unaffected BSCs and apoptotic luminal cells in rodents. Upon androgen replenishment, the regressed prostate regained its normal size.¹³³ The major function of regaining the prostate volume is due to the potentiation of AR-mediated BSC differentiation into luminal cells in the normal prostate. The mouse lineage tracing experiments by Xie *et al* discovered the specific subpopulation CARN progenitor cells; with NKX3.1⁺/AR^{low} expression and NKX3.1⁺/AR⁻. The BSCs subtype with low AR levels depicted the vital role of autonomous AR expression within progenitors for their complete differentiation into luminal cells as AR knockout cells fail to differentiate into luminal cells post regressions. Further, conditional AR knockout in BSCs revealed the presence of ~20% AR^{low} BSC population suggesting the presence of specific subpopulation in the BSCs. However, the percentage of AR^{low} BSC population remained constant after multiple prostate regressions and does not alter with Testosterone induction.¹³⁴ Thus, the presence of low AR in a certain subpopulation of BSCs is dispensable for maintaining homeostasis of the gland.

BSCs typically express CD133, CD49b, CD49f, CD117, P63, Sca-1, Trop2, CK5, and CK14.³⁶ In 2004, Richardson *et al* identified CD133⁺ (Prom1) stem cells population in normal human prostate.¹³⁵ Subsequent investigations in mice found additional subtypes of

BSCs that were directly associated with the development of PCa. A single cell with Lin^{-ve}/Sca-1⁺/CD133⁺/CD44⁺/CD117⁺ signature generated a wild-type acini in the mouse prostate.¹³⁶ Moreover, in the PTEN-null mice model, it has been observed that a very small subset of cells in the BSC layer has high nuclear AR expression.¹³⁴ Williamson *et al* demonstrated CD133⁺ cells in the basal layer with a low level of AR against luminal cells in prostatic tissue.¹³⁷ Also, low AR levels were proved to increase the expression of CD133⁺ CSCs in endometrial cancer and ovarian cancer cell lines.¹³⁸ AR expression pattern in human BSCs is widely explored that depicts absence or low levels of AR in BSCs. Undetectable or very low amount of AR has been observed in BSCs of the LNCaP and C4-2 PCa cells xenografts.¹³⁹ CD133 was determined as a potent CSC marker and found to be associated with loss of AR in tumor cells of PCa patients.¹⁴⁰ Moreover, a study in endometrial cancer and ovarian cancer cell lines proved that CD133⁺ CSCs are under the regulation of AR.¹³⁸ On the contrary, the presence of AR imposes an adverse effect on CD44⁺ and CD34⁺ stem cells. Knocking out AR in mice leads to increased fibroblast cells with a higher CD44⁺/CD34⁺ population than wild-type in bone marrow fibroblasts.¹⁴¹ Though, no evidence on the regulatory role of AR over CD49f⁺/CD117⁺ BSCs in the human prostate has been discovered yet.

Schroeder *et al* experimentally proved AR suppression increases CD44⁺ population in murine epithelial cells of PCa.¹⁴² In mouse lineage tracing experiments, luminal progenitors (~1%) were able to generate organoids containing both basal and luminal cells.¹⁴³ Further, increased luminal progenitor populations with the expression of Trop2 and C45 were found to be associated with aging and BPH development in BPH tissues.¹⁴⁴ Additionally, significant involvement of luminal progenitors in the development of PCa and castration resistance (CRPC) has been observed.¹⁴⁵ Very recently, characterization of luminal progenitors exhibited a unique expression pattern with high levels of Cytokeratin(CK)8⁺/CK18⁺/NKX3.1⁺ with low levels of Basal markers AR⁺/p63⁺/CK5⁺ along with low androgen dependency.¹⁴⁶ Further, two new stem cell subsets, AR⁺/PSA^{low} and AR⁻/PSA⁻, have been studied by Qin *et al* and found that both possess high survival and tumorigenic potency with resistance to androgen deprivation and drugs.¹⁴⁷ Hence, the above evidences lay the foundation of novel functions of AR in maintaining stemness in the epithelial compartment during the undifferentiated state as well.

In contrast to BSCs, bipotent LPs do express AR and other luminal cell markers along with BSC markers. Recently, the characterization of LPs from mice exhibited a unique expression pattern with high levels of CK8⁺/CK18⁺/NKX3.1⁺ with low levels of Basal markers AR⁺/p63⁺/CK5⁺ along with low androgen dependency.¹⁴⁶ Two specific stem cell subsets in LNCaP cells, AR⁺/PSA^{low} and AR⁻/PSA⁻, have been studied by Qin *et al* and found that both possess high survival and tumorigenic potency with resistance to androgen deprivation and drugs.¹⁴⁷ Further, Kwon *et al* also depicted another type of mouse luminal progenitor subpopulation during mouse regeneration experiments which expressed Sca1⁺/CD49f^{low} that highly expressed AR but devoid of NKX3.1. The organoids produced by these cells were CK5⁺/P63⁺/CK8⁺/AR⁺, suggesting bidirectional differentiation ability of certain cell populations within the gland. The organoids with the LP phenotype also show prostate regeneration potentials *in vivo*.¹⁴⁸ Previously, it has been demonstrated that the intermediate, transit-amplifying cells with CD133⁻/CD49b⁺/CK18⁺ signature do not express AR protein until complete luminal differentiation in the human prostate epithelium.^{149, 150} But, recent investigations by Karthaus *et al* discovered the presence of LPs in the human prostate also. *In vitro* organoid generation experiments from a single cell-derived from the human prostate depicted the generation of functional organoids from both CD26⁺ luminal and CD49f⁺ basal cell types. Surprisingly both types of organoids expressed CK8⁺/AR⁺ of luminal phenotype and p63⁺/CK5⁺ of basal phenotype, suggesting the presence of bipotent LPs in the human prostate.¹⁴³ Karthaus *et al* also elucidated human CD26⁺ luminal cells generated CK5 expressing basal organoids with profound nuclear AR upon androgen induction. Moreover, the study also outlines the vital role of Leucine-rich repeat G-protein coupled receptor 4 (LGR4) in the organoids, derived from both human basal and luminal cells.¹⁴³ LGR4 is the key protein to drive basal to luminal differentiation in mice and its overexpression drives metastatic PCa in men.^{73, 151} The presence of LGR4 in both, BSCs and Luminal cells suggests its critical role in the cellular functions of both types of cells, however, its status in LPs is yet unknown.

Significant involvement of CD49^{hi}/CD133⁺ tumor-initiating cells produced castrate-resistant adenocarcinoma and adenosquamous tumors with CK5⁺/P63⁺/AR⁺ LP phenotype in Pten/P53 knockout (KO) mice. Moreover, the aberrant proliferation of such progenitors is more evident in Pten/P53 KO mice than in the Wild type, suggesting a substantial role of tumor suppressors in the homeostasis of LPs.¹⁴⁵ Further, a study with human Neuroendocrine PCa xenograft with LP phenotype exhibited low AR levels that mediated

the single-cell origin of CRPC.⁸³ These evidences direct the presence of subpopulations within basal and luminal cell types, including the LP population in the human prostate become more evident. Some of the certain bipotent subpopulations derived from luminal lineage are AR-positive. Several regulator roles of AR over stem/progenitor cells have been elucidated in *Table-1.4*.

Table 1. 4: Regulatory role of AR over stem/progenitor cells in the prostate.

| Cancer Stem Cell marker | | Relation between Stem-marker and AR variants | Cancer/Model Type |
|--------------------------------|--------|---|--|
| Stem cell Surface Receptors | CD133 | AR directly increases expression and progenitor phenotype. ¹³⁸ | Endometrial cancer cells |
| | CD44 | AR Represses the expression of CD44. ¹⁵² | PCa cell line Breast cancer |
| | LGR4 | Overexpression of LGR4 increases AR levels. ¹⁵³ | LNCaP Cells and PC3 cells |
| Stemness Transcription Factors | NKX3.1 | Direct upregulation by Androgen receptor (AR) binding to the 11-kb region of NKX3.1 3' UTR. ¹⁵⁴ | Luminal cells and CARNs in mice |
| | SOX2 | Repress expression in PC3 cells. ¹⁵² Activation of AR decreases SOX2 Levels | PCa cell line Prostate Epithelial Cells, Human Embryonic Stem Cells |
| | OCT4 | Degradation of AR by MDM2 increases Oct4 expression. ¹⁵⁵ | HPET and HuSLC Cells |
| | NANOG | Direct upregulation of NANOG by promoter binding. ^{156, 157} Degradation of AR by MDM2 increases Nanog expression. ¹⁵⁵ | PCa and Hepatocellular Cancer HPET and HuSLC Cells |
| | cMYC | Direct upregulation by promoter binding ^{158, 159} | CRPC Patient samples |
| | Klf4 | Direct upregulation of KLF4 by promoter binding of AR. ¹⁶⁰ | PCa cell lines |

| | | | |
|--|-----|---|-------------------------------|
| | P63 | TAP63 α and Δ NP63 were decreased after AR activation. ¹⁵⁰ | PC3-AR and RWPE-AR cell lines |
|--|-----|---|-------------------------------|

B. Secretory Luminal cell population

Activation of AR signaling in BSCs leads to cellular differentiation into Secretory luminal cells. A study by Frank *et al* depicted that overexpression of AR induced growth arrest in the cells and coordinate with β -CATEININ/TCF-4 for the differentiation in the normal prostate BSCs.¹⁶¹ Hence among all the cell types, the luminal cells of the prostate have the highest expression of AR that regulates the secretory functions in the adult prostate.⁸² In response to androgens, AR regulates the secretion of the luminal cells via controlling the gene expression of PSA, PAP, Spermine Binding Protein (SBP).^{162,163} Further, AR has been found as a vital protein for lipid biosynthesis within the prostate gland via regulation of Sterol Response Element Binding Protein 1 (SREBP1) TF that controls the expression of key lipid biosynthesis enzymes (ASN, ACLY, ACC).¹⁶⁴

1.13.2 Stromal Compartment

In the stromal compartment, AR is expressed in all cell-types like myofibroblast, fibroblast, and SMC. The predominant cell-type present in the tumor stroma is myofibroblast that depicts physiological and molecular responses to androgens *in vivo*. Upon AR knockdown in stromal cells, the normal development of the prostate gland is hindered.¹⁶⁵ A patient-based PCa study reviewed by Leach and Buchanan suggests that increased disease progression is associated with decreased AR. Hence, stromal AR plays a defensive role during PCa via controlling the secretion of stromal-derived growth factors like FGF, IGF, HGF, PDGF, IL-6, TGF α & β , Wnt, and CXCL12 during disease progression.¹⁶⁶ Recently, rat derived SMCs showed AR-mediated hyperproliferative activity upon Testosterone stimulation.¹⁶⁷ A BPH and PCa based study depicted higher expression of AR in the stroma of BPH patients as compared to PCa patients, and low-level expression of stromal AR was also associated with the mortality of PCa patients.¹⁶⁸

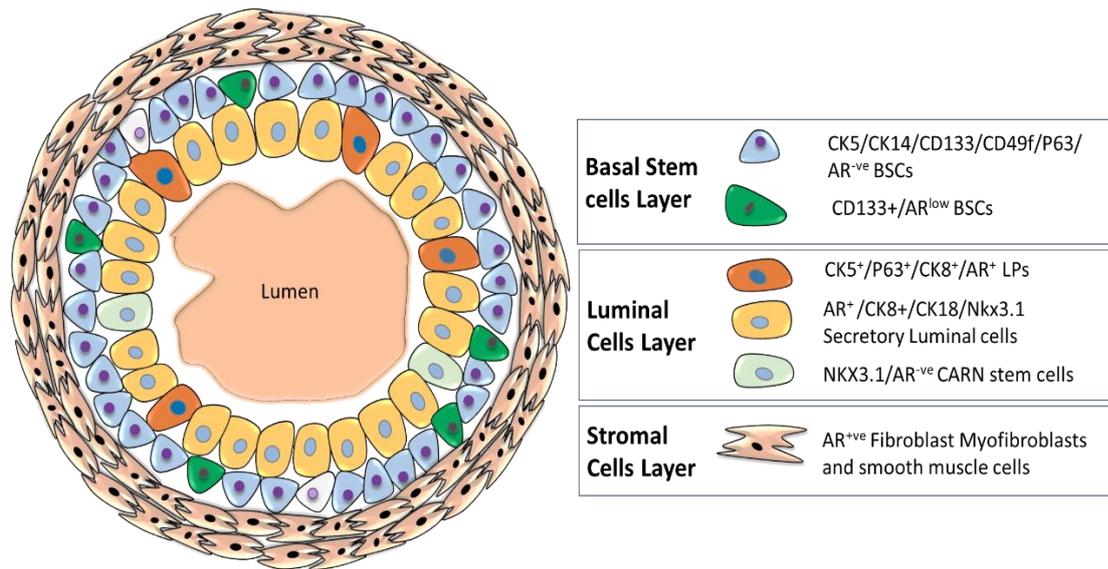


Figure 1. 17: A model of typical prostatic acini to represent the presence of AR in various cell types. Cells present in Basal, Luminal, and stromal compartments are shown with the degree of AR expression in these cells. AR⁺: Highly expressed AR; AR^{lo}: minimal expression of AR; AR⁻: No AR expression.

On the contrary, cell-lines based co-culturing of AR suppressed mouse cancerous fibroblasts with human PCa cell-lines have decreased cell proliferation and colony formation.¹⁶⁹ It has been discovered that prostatic epithelia could be playing a supportive role to increase the stromal population via Epithelial to Mesenchymal Transition (EMT) process, which in turn enhances AR signaling within the tissue.¹⁷⁰ This infers that Stromal AR also plays a substantial role in the prostate intra-compartmental interactions, influencing both normal and diseased condition of the gland. However, the precise crosstalk between stromal-epithelial cell types in terms of AR signaling in prostatic diseases is not yet attained for a better understanding of the diseases. (*Figure 1.17*)

1.13.3 Role of AR in stromal-epithelial crosstalk in disease condition

As both stromal and epithelial cell types cumulatively contribute to the progression of BPH and PCa, the cellular crosstalk via their paracrine action on each other plays a vital role.¹⁷¹ It has been discovered that the transcriptional activity of AR was elevated in normal stromal cells when co-cultured with epithelial cell-conditioned media. But, co-culture between cancerous epithelial cells with normal stromal cells depicted decreased AR levels in stromal cells.¹⁷² Further, activation of AR in stromal cells profoundly controls the expression and secretion of andromendins containing (cytokines and growth factors) that influence the growth and severity of prostate pathologies.^{170, 173} These secreted factors

have a substantial effect on the maintenance of epithelial stem cell pool. Additionally, loss of stromal-AR expression was associated with the progressive stage of the PCa.¹⁶⁶ However, the role of AR for mediating cellular crosstalk is not completely known, especially during BPH conditions. (Figure 1.18)

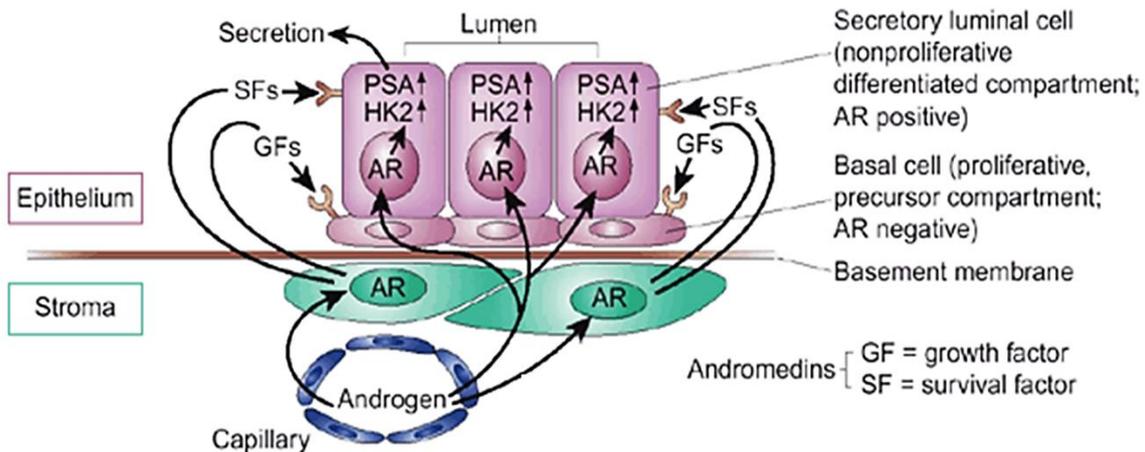


Figure 1. 18: The crosstalk between epithelial and stromal cells. The secretion of andromedins - GFs and survival factors (SF) from stromal cells regulates the secretion of PSA and human kallikrein-2 (HK2) during prostate disease progression. The production of Andromedins is under the control of AR activation and signaling in stromal cells. (Adapted¹⁷⁴)

1.14 Transcriptional role of AR in prostate stem cells during BPH and PCa development.

The expression of BSC markers and their populations are greatly altered in BPH and PCa conditions. It has been observed that CSCs have conserved transcriptional profile as in normal BSCs during PCa.¹⁷⁵ Further, AR has been proved to be present at low or undetectable levels in the prostate BSCs.^{23, 25, 139, 176} Conditional AR knockout in BSCs revealed the presence of an AR^{+ve} (~20%) population which remained constant even after multiple prostate regressions. These cells failed to differentiate into luminal cells post regressions.¹³⁴ The low level of autonomous AR expression within BSCs is vital for their complete differentiation into luminal cells. An androgen deprivation on a regressed normal prostate showed unaffected BSCs and apoptotic luminal cells in rodents. Upon androgen replenishment, the regressed prostate regained its normal size.¹³³ Thus, the presence of AR in BSCs is vital for the homeostasis of the gland. A small subset of cells in the BSC layer has high nuclear AR expression, which if lost, forms PCa.¹³⁴ It has also been observed that AR^{+ve} prostate CSCs cause the formation of the initial androgen-sensitive cancer.¹⁷⁷ And

later, they turn into rapidly expanding CSCs instigating androgen insensitive PCa. These cells with transcriptionally active AR can regenerate/relapse the tumor due to their higher self-renewal and proliferative potential.¹⁷⁸

Due to substantial chromatin remodeling in the cancer cells, stemness specific TFs (Oct4, Sox2, Nanog, Δ NP63 $\alpha/\beta/\gamma$) were also highly expressed in PCa.¹⁷⁹⁻¹⁸² Oct4 expression was absent in BPH patients while it was mildly expressed in higher Gleason score (>6) PCa patients.¹⁸³ Differing with this report, high nuclear localization of Oct4 has been observed in benign and malignant tumors.¹⁸⁴ It has been observed that AR plays a repressive role in Oct4 transcription by binding to their promoter region.^{183, 185, 186} Unlike Oct4, Sox2, and Nanog are widely studied transcriptional factors in both BPH and PCa. Immunohistological studies depicted the mild expression of Sox2 and Nanog during BPH and highly expressed in PCa tumors driving lineage plasticity and drug resistance.¹⁸⁷⁻¹⁹⁰ Kregel *et al* proved the suppressive role of AR over the Sox2 gene.¹⁸⁵ Unlike Oct3/4 and Sox2, AR positively controls Nanog by direct binding to its promoter to strengthen stemness in PCa.¹⁵⁷ On the contrary, AR also suppressed the expression of Nanog in prostate stem cells.¹⁹¹ Similarly, cMYC is also one of the core stem cell regulatory TFs essential to maintain stemness.¹⁹² Overexpression of cMYC gene has been reported in both, BPH and PCa conditions.¹⁹³ cMYC gene also has an AR binding site on its enhancer sequence that supports AR/cMYC mediated CRPC cell growth.¹⁵⁸ Expression of P63 (especially NTD truncated P63 protein variants- Δ NP63 $\alpha/\beta/\gamma$) is the most apparent TF specifically expressed in the prostate BSCs.¹⁸⁰⁻¹⁸² Tan *et al* demonstrated strong nuclear expression of AR in P63(4A4) positive cells in benign luminal cells.¹⁹⁴ Previously, we have demonstrated the presence of CD49b, CD44, CD117, CD34, and P63 stem/progenitor markers along with the coexpression of AR in epithelial cells isolated from BPH patient tissue.¹⁹⁵ Further, malignant transformation of these cells depicted the alterations in the stem progenitor population with decreased expression of Sox2, Nanog, and P63, but increased expression of AR, PSA, and MYC oncogene. (Unpublished) Thus, loss of stem/progenitor phenotype and divergence toward luminal cells was observed upon the malignant transformation of stem/progenitor cells. However, precise co-expression of AR and stem/progenitor proteins during BPH is not well understood.

In recent years, a plethora of evidences have been discovered to unravel the regulatory role of AR over CSCs during PCa condition. Yet, the presence and role of AR in

stem/progenitor cells during BPH have remained intangible. In the present study, we aimed to investigate the correlation of AR with stem/progenitor cells and its transcriptional regulatory role during BPH condition. We also aimed to investigate the role of Stromal-AR in controlling epithelial stemness during the BPH condition.

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