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# **An Overview on Prostate Pathophysiology: New Insights into Prostate Cancer Clinical Diagnosis**

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Additional information is available at the end of the chapter

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## **Abstract**

The prostate is an accessory gland of the male reproductive tract, and its presence is universal in mammals. It is committed to the prostatic fluid production and storage, which is released with other semen components during ejaculation. Such fluid contributes to increasing motility and fertility of the spermatozoa, and the neutralization of the vagina, thus playing an important role in fertilization. Few pathological complications, often progressively aggravated with age, can affect this gland (i.e. benign and malignant proliferative changes; all to be described next in this chapter). Nowadays, the neoplastic expansion is the main motivator and contributor for studies on enlightening of growth regulation mechanisms and physiology of the prostate.

**Keywords:** physiology, pathology, benign prostatic hyperplasia, prostate cancer, biomarkers

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## **1. Prostate anatomy**

The human prostate is a pelvic gland located under the urinary bladder and in front of the rectum, and it is composed by glandular and non-glandular structures surrounded by one same capsule [1–3]. It consists mainly of muscular-fibrous tissue, which it is subdivided into about 50 tubule-alveolar glands [4], at the lateral and posterior segment of the urethra, which drain to 20–30 small prostatic ductules opening in the prostate, or close to the posterior wall of the prostatic urethra [5–8]. The prostatic secretion, which accounts for approximately 20% of

the seminal fluid, confers a characteristic odor of this flowing, and participates in the activation of spermatozoa [8]. The ducts of the prostatic glands open into a sulcus located on each side of the urethral ridge, called the prostatic sinus. The prostate is traversed throughout the prostatic portion of the urethra, from the base to the apex, with a slightly curved course in the anterior-posterior direction, and closer to its anterior face [5–8].

The prostate is anatomically described as an inverted pyramid whose apex is the lowest portion, and which is located about 1.5 cm behind the lower border of the pubic symphysis and is directly related to the upper face of the urogenital diaphragm. The base of the prostate gland is in a horizontal plane that passes through the middle part of the pubic symphysis, and it is directly related to the cervix of the bladder and the inner ostium of the urethra. Inferior-lateral surfaces are convex and are separated from the superior fascia of the pelvic diaphragm by a venous plexus, and are related to the pubococcygeal muscles [6–9]. The posterior surface is flattened and triangular, and it is related to the bladder of the rectum. The anterior surface is narrow and separated from the pubic symphysis by retropubic fat tissue. The upper part is related to the seminal glands and to the lower extremities of the vas deferens, and near its base presents small depressions for the entrance of the ejaculatory ducts [6].

Despite not being clearly distinguished anatomically, the following prostate lobes are traditionally defined: right, left and a middle lobe [5–8]. In pathology, the prostate is described in different zones (peripheral zone, central zone, transition zone and anterior fibro-muscular zone) [9]. The right and left lobes are not isolated from each other, being connected, prior to the urethra, by the isthmus of the prostate, constituted by fibromuscular tissue. Their muscular fibers represent the superior continuation of the external sphincter muscle of the urethra to the cervix of the bladder, and it is devoid of glandular tissue. The middle lobe, of variable size, is the part of the prostate that protrudes internally from the upper part of the posterior face of the organ, between the ejaculatory duct and the urethra [5–8]. However, structurally, the middle lobe is indeed inseparable from the right and left lobes. In each prostate lobe we can identify four lobules: (I) Posterior-Inferior, located posterior to the urethra, and inferior to the ejaculatory ducts. It constitutes the face of the prostate, palpable to digital rectal examination; (II) Lateral-Inferior, directly lateral to the urethra, forming the major part of the right or left lobe; (III) Superomedial, deeply to the inferoposterior lobe, surrounding the ipsilateral ejaculatory duct; (IV) Anteromedial, deeply to the inferolateral lobe, directly lateral to the proximal portion of the prostatic urethra.

The superior fascia of the pelvic diaphragm reflects in the superior direction from the visceral fascia of the pelvis to envelop the prostate, and then continues superiorly over the bladder. The portion covering the prostate is dense and fibrous, being called the fascia of the prostate. It is located externally to the prostate capsule and is separated from it, laterally and anteriorly, by the loose connective tissue harboring the prostatic venous plexus. The fascia of the prostate fuses anteriorly with the tendinous arch of the pelvic fascia, which at the level of the pube is called the medial puboprostatic ligament [5–7]. Smooth muscle fibers fulfill this ligament, and it is called the puboprostatic muscle. The lateral puboprostatic ligament extends from the fascia of the prostate to the tendon arch of the pelvic fascia. Inferior to the puboprostatic ligaments, the prostate associates with the medial borders of the pubococcygeus muscle, and

from this point the muscle fibers extend in the superior direction to fuse with the fascia of the prostate, forming the prostate lifting muscle. Later, the fascia of the prostate is separated from the tunica of the rectum by the rectovesical septum [6, 10].

The prostatic arteries are usually direct branches of the inferior bladder artery from one of the branches of the internal iliac artery. In some cases, it may be a branch of the internal pudendal artery, or the medial rectal artery [5–7, 10]. The veins draining the prostate girdle, to form the prostatic venous plexus, located in the fascia of the prostate. The prostatic venous plexus continuous superiorly with the bladder venous plexus, and communicates posteriorly with the internal vertebral venous plexus. The lymphatic vessels of the prostate drain into the internal iliac lymph nodes [5–7, 10]. Finally, the prostate is innervated by sympathetic fibers from the lower hypogastric plexus. These fibers innervate smooth muscle fibers and blood vessels [5–7, 10].

## 2. Prostate gland hormonal regulation

There are considerable variations related to the prostate anatomy, biochemistry and pathology of several mammal species. In humans, the sexual accessory tissues (or glands) produce high concentration of several biologically active substances, such as fructose, citric acid, spermine, prostaglandins, zinc, proteins including immunoglobulins, and specific enzymes (i.e. esterases and phosphatases) [11].

The growth, differentiation and maintenance of the activity of the prostate gland [12] are mainly controlled by androgens, which is the basis of the anti-androgenic therapies for the treatment of primary prostate cancer. The development and physiology of the prostate is also directly modulated by somatotrophic hormones (such as insulin, prolactin and growth hormone), retinoic acid and estrogen [13, 14], as well as a biomolecular scenario of complex interactions between the epithelium and stroma [15], which sum up to a complicated and poorly understood regulatory mechanism.

Receptors type androgen receptor (AR) and estrogen receptor (ER) are responsible for mediating the physiological effects of androgens and estrogens, respectively [16, 17]. Briefly, the receptor located in the cytoplasm binds to testosterone or dihydrotestosterone, dissociates a heat shock protein (HSP), dimerizes, and it is translocated to the nucleus, where, together with a variety of co-activators and co-repressors, activates or inactivates different sets of genes [18]. The classic AR has 110 kDa and several features in common with members of the nuclear receptor family, such as estrogen receptors, progesterone, thyroid hormones, and peroxisome proliferator-activated receptors (PPARs) [19].

Testosterone and dihydrotestosterone (DHT) act through AR. The AR primarily functions as a transcription factor. It is an extremely important molecule, responsible for the primary male sex differentiation (formation of gonads and external genitalia), and for the pubertal acquisition of the male secondary characteristics (events associated with puberty and adolescence) [20]. It is also liable for most cases of complete androgen insensitivity (resulting in infertile XY

karyotype female) [21], and it is deeply associated with the origin of prostate tumors and, particularly, with the recurrence of androgen independent cancer [22–24].

The most striking androgen dependence of the prostate gland is observed by hormonal or surgical castration. In a rat model, removal of the testes results in prostate involution to approximately 10% of its original size after 21 days. Epithelial cells death and stroma reorganization are responsible by such event [25]. Similar to AR, estrogen receptors (ERs) belong to the family of nuclear receptors. The two subtypes, ER $\alpha$  and ER $\beta$ , have different physiological roles. They share homology with each other, but are the products of different genes [26]. Both ER $\alpha$  and ER $\beta$  are expressed in the prostate. In adults, ER $\alpha$  and ER $\beta$  are preferentially found in the stroma and in the epithelium, respectively [27]. Similarly to the AR, ER expression might be suppressed by methylation of its promoters, and this epigenetic alteration was suggested to be involved in both benign prostatic hyperplasia and prostate cancer development [28, 33].

The action of estrogens on prostatic ductal morphogenesis and cell differentiation is complex [14]. However, a brief exposure of rodents to estrogens during neonatal development causes irreversible and dose-dependent effects on morphology, cellular organization and function of the gland [29, 30]. Reduced prostate size at adulthood was associated with decreased responsiveness at puberty due to reduced AR content [31]. The reduced AR levels were justified by increased proteasomal degradation of AR protein at postnatal day 10 [32].

Estrogen exposure to occasional doses during the gestation period causes increased concentrations of androgen receptor in mice, ductal budding and prostate weight later in the adulthood [34]; whereas the neonatal exposure to high doses compromises the growth epithelial differentiation, and accounts for changes in the secretory function, as well as for incidence of prostatic intraepithelial neoplasia (PIN) and prostatitis [14, 29]. The effect of high doses of estrogens on the neonatal prostate is due not only to the changes in the androgen concentrations, via permanent actions on the hypothalamic-gonadal pituitary gland, but also due to direct effects on the prostate gland, since the administration of testosterone is not able to reverse those effects [35]; this phenomena is known as *estrogenic imprinting*.

High doses of estrogen administered in adult animals function as castration, resulting in the inhibition of the hypothalamic-pituitary-gonadal axis, by suppression of the gonadotrophin releasing hormone, and consequent blockage of the hormone testosterone by the testes [36, 37]. Nonetheless, such effects can be reversed (contrary to those observed in neonates), by replacing testosterone or dihydrotestosterone hormones.

It is well established that some of the circulating androgens are converted into estrogens in various peripheral tissues by the enzyme aromatase [38]. The aromatase was also identified in the human prostate, suggesting that this gland is able to perform the aromatization reaction and it is a feasible local source of estrogen production [39]. Estrogens acts in target cells all over the body and in addition to sexual organs they influence growth, health and cell activity. Despite early work of estrogens used as therapy for androgen-resistant prostate cancer, it can be critical in predisposing prostate cancer.

Estrogens also participate in several pathological changes in the prostate; among the very well described pathologies is the induction of chronic inflammation [40, 41], squamous metaplasia reported in several species of mammals [42–44], and human prostate cancer [45].

There are several pathological complications, including benign and malign proliferative alterations, often aging escalate-associated, that affect prostate gland. So, studies focusing on the growth regulation and physiology of the prostate are very precious to understand the origin and progression of these pathologies.

### **3. Benign prostate hyperplasia (BPH)**

Benign prostate hyperplasia (BPH) is a common urological issue that causes prostate enlargement in men after 40-years-old. It is a noncancerous augmentation of the prostate gland size, with stromal and glandular epithelial hyperplasia in the transition zone. It is estimated that 50% of 50 year old, and 75% of 80 year old men could have some lower urinary tract symptom (LUTS). In such condition the urethra can be partially or totally blocked, resulting in urinary retention, weak urination stream, incomplete bladder emptying and hesitancy; and so carrying secondary problems as urinary tract infections, bladder stones and chronic kidney disease, culminating in kidney failure. The LUTS is reflection of the hormonal changes rising with age, and resulting in abnormal stromal and epithelial cell proliferation (hyperplasia) in the transition zone of the prostate. The molecular etiology of these events remains unclear, but few studies attempt to correlate it to sex steroids hormones [46], also known as gonadocorticoids and gonadal steroids, that interact with vertebrate androgen and estrogen receptors. It is important to mention that the BPH is generally not a precursor lesion to a prostate cancer (PCa) condition.

Some animal models studies, including dogs and chimpanzees, have been performed in order to understand the prostate conditions. Chimpanzees sporadically suffer from age-associated BPH, and are the closest match to human prostate gland. Throughout the time, dogs are like human counterpart because they develop BPH containing distinct nodules of hyperplasia with diffuse areas of compression of the rectum producing constipation, a symptom opposed to the urinary retention in men [47, 48]. In order to supply these deficiencies, some transgenic animal models using other normal mammal species were developed. Prostate-specific 15-LOX-2 transgenic mouse and PPAR $\delta$  knockdown mice naturally develop increased prostate size with age, in addition to epithelial-hyperplasia, and prostatic intraepithelial neoplasia progression [49, 50].

### **4. Prostate cancer**

Nearly 14 million new cases of cancer occurred worldwide during 2012 [51], generating around 8.2 million deaths. More than a half of cancer deaths arose in countries of medium or low human development index (HDI). The four most common types, in this order were lung, female breast, bowel and PCa. Among malignant neoplasms that affect men, PCa is the most common, after non-melanoma skin tumors, especially in the male population from the sixth decade of life. This is a recognized public health problem, since according to data from the Mortality Information System (MIS), 13,773 deaths were caused by PCa in Brazil in 2013 [52].

Considering the statistics worldwide, PCa prevalence is only beaten by lung cancer in men. Unlike some types of tumors, the incidence of PCa has increased over the years. There are two

main factors for this association: the improvement of diagnostic methods and the extended life expectancy of men over the years; since PCa has slow growth and its incidence is age-associated, it is very comprehensible the increased detection of this malignant neoplasia lately in the years. The origin of PCa and the several processes giving direction to PCa carcinogenesis are still unclear, but often are assumed that several components may influence it, among which stands out: diet, genetic, hormonal, and environmental factors; all currently being widely investigated in the literature.

The treatment of PCa can be very controversial because there are many variables, such as the patient's age, prostatic specific antigen (PSA) concentrations and the stage of the tumor. Patients in inoperable conditions, due to age, are treated with hormone therapy or radiation. The most common hormone therapy for PCa is the androgen deprivation, since the prostate gland is a highly androgen dependent gland, and because the majority of prostate tumors originate from androgen-dependent glandular epithelial cells of the prostate [53]. The therapies in use for PCa will be best addressed later in this chapter.

## 5. Clinical diagnosis and biomarkers for PCa

The diagnosis and follow-up of PCa patients are often difficult because of the absence of specific markers that could change accordingly to the status of disease, the best therapy, and the existence of future complications caused by the chosen treatment.

For several decades many researchers joined efforts to study biomarkers of prognosis and treatment for PCa. Almost 50-years, PSA measurement represented the best marker for PCa. The primary idea was to substitute the digital rectal examination by PSA screening; nevertheless this was not possible despite the low specificity and false positive rate, as it is also observed in BPH [54]. No significant progress in the use of PSA as a precise biomarker of PCa was achieved during the past years.

Beyond this scenario, advances in genetic testing for PCa risk and new molecular diagnostic assays have been designed to improve diagnostic accuracy and treatment decision beyond prostate-specific antigen (PSA) testing. PSA is a protein of the kallikrein family synthesized in the prostatic epithelium and secreted in the seminal fluid. From its discovery in 1970 to the present day, it is a diagnostic tool used as a tumor marker for early diagnosis, treatment and monitoring of patients with neoplasia in conjunction with the rectal examination. However, many studies have questioned the use of this biomarker for a diagnosis, due to the exponential increase in the diagnosis of PCa and, consequently, the increase of unnecessary hormonal, radiotherapeutic, chemotherapeutic and surgical treatments such as radical prostatectomy [55, 56]. PSA evaluation is performed by its measurement in serum using immunoassay (34 kDa). Normal values vary according to the method used. In most tests, values of up to 2.5 ng/mL are allowed as normal. If this value is higher, it is indicated to request the dosage of fractionated PSA, which relates total PSA to free PSA (fPSA). The result is expected to be equal to or greater than 20%; if it is lower, there is a probability that it is a PCa [57]. However, this test does not have 100% of specificity or sensitivity, insofar as there is PCa whose PSA is not altered, and there are other transient factors that can raise serum PSA levels, such as prostatitis

[58], benign prostatic hyperplasia [59], prostatic biopsies [60] and trauma, due to prostatic cell lysis releasing PSA into the bloodstream [61].

Despite results enhancing detection at earlier stage and decreasing the number of metastatic patients, the use of prostate-specific antigen (PSA) to detect PCa has low specificity, unnecessary biopsies and frequently mistaken diagnoses. Also, PCa has various features so prognosis following diagnosis is greatly variable. Hence, there is a requirement for new prognostic biomarkers, particularly to differentiate between inactive and aggressive forms of the disease, to improve clinical management of PCa patients. Research continues into finding additional markers that may allow this goal to be attained.

In order to improve the specificity of PSA as a tumor biomarker, tests called PHI (Prostatic Health Index), that predicts the risk of having PCa and 4 K score<sup>TM</sup> (predicts the risk of having high-risk of PCa) were launched on the American and European markets [62]. 4 K score<sup>TM</sup> blood test combines 4 prostatic biomarkers (total PSA, fPSA, intact PSA, and human kallekrein 2(hK2)) with the age of the patient, the digital rectal exam (DRE) findings (presence of a nodule or not), and the result of previous biopsies [63]. The higher the score, the greater the probability of finding tumor cells in a biopsy (Gleason  $\geq 7$ ). This test combination is interesting because it does not allow unnecessary biopsies to be performed, whereas post-operative, as well as any surgery, has risks and can lead to future complications for the patient, affecting his quality of life.

Another non-invasive test available is the ExoDx<sup>TM</sup> Prostate (IntelliScore) Test18, which, through urinalysis, assesses the risk of developing invasive PCa, and thereby target the best treatment by molecular analysis of three specific genes in exosome and microvesic RNAs released by tumor cells, called extracellular vesicles (further discussed in this chapter) [64]. These related genes (*ERG*, *PCA3* and *SPDEF*) are most commonly related to tumor progression and, consequently, its aggressiveness and invasion [65].

It is important to note that these tests are not accessible to the entire population, either because of the high cost of the technology, or because some countries have still not approved it. Thus, the main diagnostic method used nowadays for the screening and detection of the PCa remains PSA testing and rectal examination (DRE). If the results of these exams are altered, a biopsy is necessary to confirm the diagnosis, and determine the aggressiveness and prognosis of the cancer. This is done by histological analysis of the biopsied tissue, following classification according to the Gleason Scale. This system consists of the sum of 2 values that represent the degree of the tumor, and that determine the dominant cellular pattern and the most frequent cellular pattern, respectively. Tumor grades range from 1 to 5, the former representing more differentiated and prostate restricted tumors, while the latter represents totally undifferentiated tumors that have normally infiltrated the glandular stroma. The score, therefore, ranges from 2 (1 + 1) to 10 (5 + 5), and values below 4 on the Gleason Scale represent a well differentiated PCa; between 5 and 7, an intermediate PCa; and between 8 and 10, advanced PCa [66]. The determination of the degree and stage of cancer allows classification into high, intermediate and low risk categories.

The clinical picture of castrated-resistant prostate cancer (CRPC) is quite heterogeneous, ranging from the asymptomatic increase in the PSA indices to the distant metastasis (commonly bone metastasis), with an important impairment of the patient's quality of life [66]. This is a

reflection of the complexity and diversity of biomolecular alterations already found in biopsies. Tumor progression is related to a number of genetic changes that can affect AR, signaling cascades, apoptosis mechanisms and cell regulation, or, as in many cases, a combination of all of them [67].

Biomolecular techniques, such as fluorescent *in situ* hybridization (FISH) and Microarray, for example, have identified a variety of key factors genes, oncogenes and tumor suppressor genes, related to the development and progression of PCa [68, 69]. The use of molecular techniques also allowed the identification of some genes related to the suppressive function of metastasis, opening a new perspective for researching the phenomenon of tumor invasion to other tissues and, with that, to identify and elucidate new indicators of prognosis, or even PCa target therapies. As example, some studies have focused attention on the CDH1 gene and its protein expression, located on chromosome 16q22, which encodes the E-cadherin, a glycoprotein responsible for cell-cell adhesion, an important cellular function that prevents EMT in tumor progression [70].

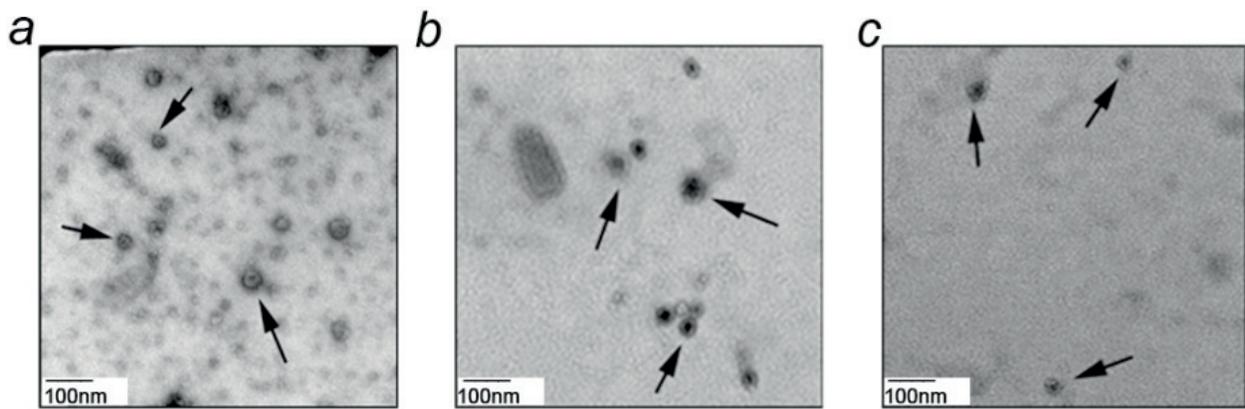
The Metastatic prostate adenocarcinoma (metPA) is diagnosed by immunohistochemistry. Nowadays very promising biomarkers have been used to determine prostatic origin of metPA, such as prostate specific membrane antigen (PSMA) and NKX3.1 [71]. PSMA is a type II membrane protein not secreted and is expressed in all forms of prostate tissue, but it is expressed at high levels on malignant prostate cells with limited extraprostatic expression [72]. Many approaches to target PSMA include DNA-based vaccines, as well as passive administration of monoclonal antibodies (PSMA-mAb), including 7E11.C5.3, that has already been approved by USA FDA (Food and Drug Administration); the medication is commercially available as ProstaScint<sup>®</sup> [72, 73].

Compared to PSA, PSMA is upregulated with androgen deprivation, and its expression was correlated with cancer aggressiveness and poor prognosis, while PSA decreases with androgen deprivation [72]. PSMA was also evaluated in PCa using PET molecular imaging system. After all, PSMA is not specific only to prostate gland; it is expressed in other normal tissues (such as salivary glands, duodenal mucosa, renal tubular cells, and neuroendocrine cells in the colon), and in malignant cells (renal cell carcinomas, colon carcinomas, and endothelial cells that surround or are into the tumors) [74].

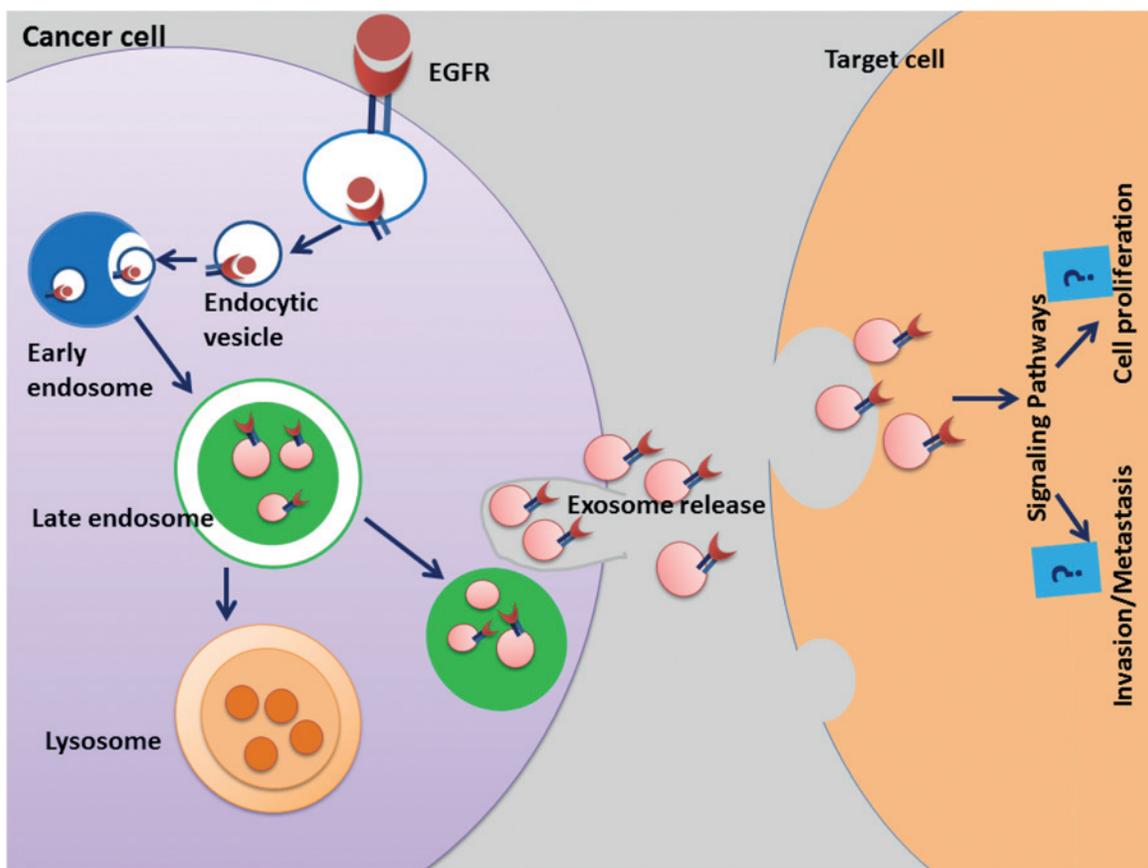
Although multiple independent studies sought to demonstrate evidence that genetic variations may be independent predictors of PCa risk in addition to family history and serum PSA levels, the challenge in the years to come will be to introduce these new gene-based diagnostic and prognostic tests in algorithms integrating the other known risk factors including age, ethnicity, family history and PSA level to better tailor diagnostic and therapeutic strategies for PCa.

### 5.1. The extracellular vesicles (exosomes) and PCa: beyond classical biomarkers

Several studies have related to novel PCa biomarkers that can precisely detect, and treat, types of aggressive cancer by headlining circulating tumor cells (CTCs) and circulating extracellular vesicles (EVs) (**Figure 1**). Notably, EVs are released by almost all the cells, and brings lots of molecular information. The study based on EVs provides lots of information about its content,



**Figure 1.** Representative TEM images of exosomes derived from (a) C42 PCa cell line, (b) LNCaP xenograft serum and (c) patient plasma by ultracentrifugation method. Exosomes were negatively stained with 2% uracyl acetate after removal of moisture. Arrows indicate cup-shaped structures which are identified as exosomes (30–100 nm in diameter). From: Kharmate et al. [86]. Online available at: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0154967>.



**Figure 2.** Schematic representation of possible role of EGFR-exosomes in cancer progression. Ligand binding induces rapid activation and internalization of EGFR and endocytosis. Whether EGFR escapes lysosomal degradation and is released extracellularly via exosomes is unknown. The transfer of EGFR via exosomes may significantly alter the tumor microenvironment and could be relevant to progression of an aggressive PCa. From: Kharmate et al. [86]. Online available at: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0154967>.

such as: lipids, proteins, nucleic acids and metabolites [75, 76]. All of each can be isolated in small volumes from body fluids, just by using some steps of ultracentrifugation, as a non-invasive method to monitor disease progression, and are proposed to function as tumor-specific molecular signatures. They are small structures (50–150 nm) that carry genetic and/or nongenetic materials from tumor cells. Recent study analyzed the presence of CD9 and CD63 (a housekeeping exosome marker) positive EVs, demonstrating that patients with metastatic cancer and detectable CTCs have higher CD9 detectable in plasma [77]. The CD9 positive EVs were found higher in plasma of PCa patients compared to HPB patients, and were related to paracrine signaling that contributes to PCa progression [77]. *In silico* reanalysis of genes involved in vesicular trafficking demonstrated that the expression of required well-known endosomal sorting complexes, such as *RAB27A*, *RAB27B* and *VPS36*, are downregulated in patients with advanced PCa [78].

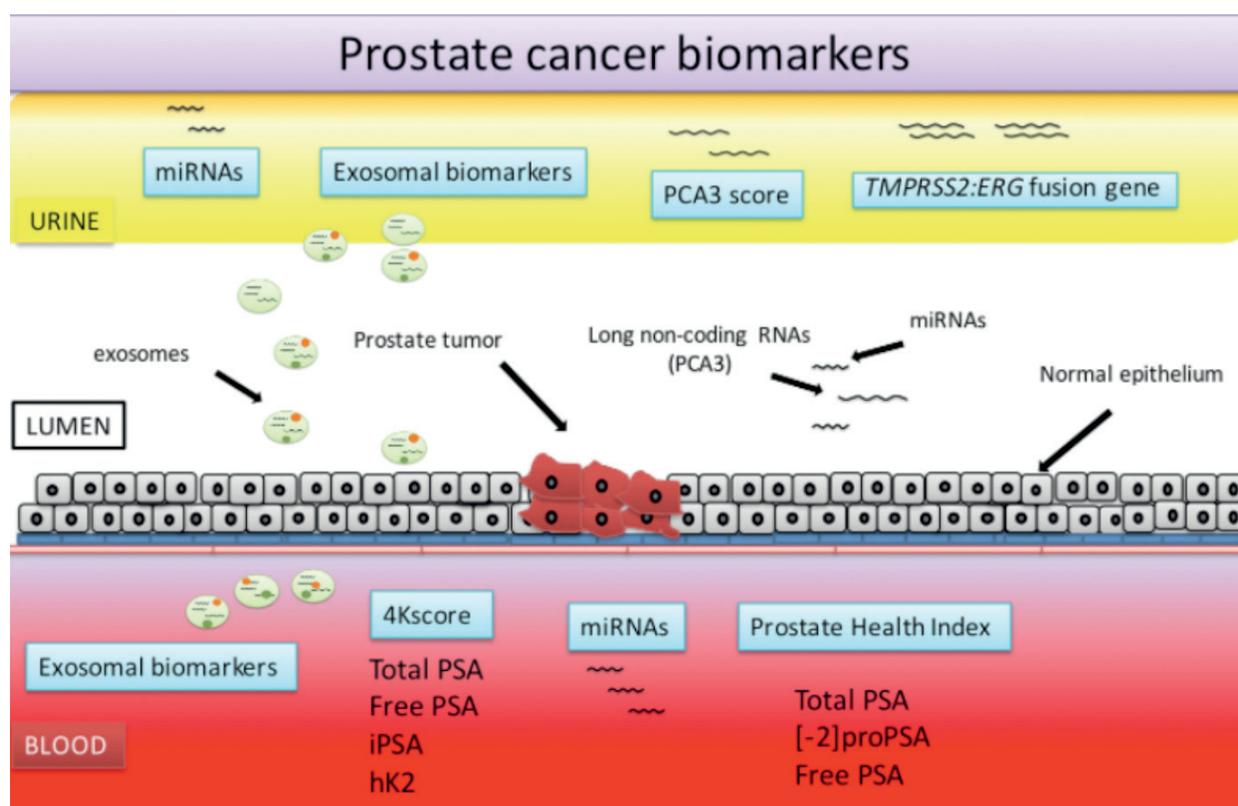
Other studies suggest possible micro-RNAs roles in PCa [79] due to their recruitment to EVs present in various human body fluids; they are miR-2909 and miR-615-3p, which was detected in urinary-exosomal of PCa patients [80, 81]. Also EVs was useful to monitor the response to radiation therapy, in the search for a personalized treatment according to different profiling levels [82].

Biomarkers	Biomarkers Measurement	Sample	Recommendation
Prostate Health Index (PHI)	PSA, fPSA, [-2]proPSA	serum	Approved by the Food and Drug Administration (FDA) * Related to PCa aggressiveness
4Kscore	PSA, fPSA, iPSA, hK2	serum	The test provides information about the probability of having a high-risk PCa * Related to PCa aggressiveness
PCA3 score	<i>mRNA PCA3</i> in relation to <i>mRNA PSA</i>	urine obtained after prostate massage	Approved by the Food and Drug Administration (FDA) * Inconclusive results about its relationship with PCa aggressiveness
<i>miRNAs</i> and other exosomal biomarkers	No standardized methodology	blood and urine	Directly related to development and progression of cancer No standardized methodology Preliminary results

**Table 1.** Biomarkers in PCa detection and prognosis.

Additionally to androgens (as described in Section 3), prostate physiology is, in part, regulated by the epidermal growth factor (EGF), whose action is mediated by its receptor (EGFR). EGFR is one of the mediators of cell proliferation, and its overexpression has been associated with aggressiveness and invasion of PCa. It has been described and identified as an important anti-PCa target, and some inhibitors of EGFR were tested with limited effectiveness in prostate cancer patients; they are Gefitinib, Lapatinib, and Erlotinib [83–85]. Recently, EGFR was also observed in EVs (Figure 2) of PCa patients [86].

Previous studies have demonstrated that PSA can be detectable in plasma and urine derived EV's [87]. Logozzi et al. [88] demonstrated that an acid microenvironment (such as the tumor microenvironment), functions as a key factor for the exosomal releasing, and determines the quality and quantity of released vesicles, including the ones containing PSA, an enzyme that needs an acidic microenvironment for full activation, in PCa.



**Figure 3.** The prostate specific antigen (PSA) remains the most used biomarker in the management of early prostate cancer (PCa), in spite of the problems related to false positive results and overdiagnosis. New biomarkers have been proposed in recent years with the aim of increasing specificity and distinguishing aggressive from non-aggressive PCa. The emerging role of the prostate health index and the 4Kscore: both are blood-based tests related to the aggressiveness of the tumor, which provide the risk of suffering PCa and avoiding negative biopsies. Furthermore, the use of urine has emerged as a non-invasive way to identify new biomarkers in recent years, including the *PCA3* and *TMPRSS2: ERG* fusion gene. Available results showed *PCA3* score usefulness to decide the repetition of biopsy in patients with a previous negative result, although its relationship with the aggressiveness of the tumor is controversial. More recently, aberrant the microRNA expression in PCa has been reported by different authors. The utility of circulating and urinary microRNAs in the detection and prognosis of PCa has also been explored. Although several of these new biomarkers have been recommended by different guidelines, large prospective and comparative studies are necessary to establish their value in PCa detection and prognosis. From: Filella and Foj [69]. Online available at: <http://www.mdpi.com/1422-0067/17/11/1784>.

To summarize, in recent years, many new promising PCa biomarkers have been identified (**Table 1**) (**Figure 3**), and found to be associated with tumor aggressiveness. Multiplied studies showed the utility of the PHI, the 4Kscore™ and the PCA3 score to reduce the number of unnecessary prostate biopsies in PSA tested men. Actually, these biomarkers have been recommended for different guidelines. Still, large prospective studies, avoiding bias due to selection of patients according to PSA serum levels, are necessary to compare the value of these biomarkers. Also, new efforts are necessary to standardize the methodology for the measurement of exosomal and non-exosomal miRNAs, in order to analyze accurately their usefulness in the management of patients with early PCa. Finally, the combined role of these biomarkers together with magnetic resonance imaging data should be elucidated [89].

Adapted from: Filella and Foj [69]. (\*Recommended by the National Comprehensive Cancer Network).

## 6. Treatment modalities for PCa

PCa treatment is variable, and it is chosen according to the staging of the cancer and, mainly, according to the patient's own preference. Since this type of cancer has slow growth, the presence of low-risk groups, where tumor is diagnosed still *in situ*, is indicative of an active surveillance treatment in which the patient only accompanies the tumor through regular PSA testing, and digital touch every 3–6 months [90]. During this follow-up period, if the existence of a tumor progression is observed, radiotherapy or surgery is indicated by total removal of the prostate gland (radical prostatectomy).

Radical prostatectomy may be the first choice of the patient who opts for complete removal of the gland, by caution of future metastasis. It is an effective procedure, however, just like any surgical procedure, there may be complications and compromise the patient's quality of life. For this procedure, the most common complications are the urinary incontinence, erectile dysfunction, and inguinal hernia; anyhow, the prognosis tends to be positive and long-lasting [91]. Nonetheless, some tumors may recur over time even after radical prostatectomy. In such cases, it is important to evaluate whether the recurrence was local or occurred at a distance (lymph nodes or other organs, such as liver, bone, or lung).

Hormone therapy is usually used in patients with lymph node involvement or distant metastasis. It consists of reducing androgen concentrations to the level of castration. This can be done by surgical method through bilateral orchiectomy, or through drugs that act on the androgen receptor (AR) pathways; the latter being more commonly used nowadays. At first, hormone deprivation therapy has great effects on the control of advanced PCa. However, it is known that part of the cases evolves to the state of CRPC. The mechanisms responsible for progression of tumor growth, despite hormonal blockade, have not been fully elucidated yet. Current studies have shown that molecular changes in the androgen receptor (AR) are related to such progression. Among these changes, it is relevant to mention the overexpression of AR, mutations in the

AR gene that allow its activation by other endogenous steroids, increased production of growth factors activating AR even in the absence of androgen, changes in co-regulatory proteins and upregulation of enzymes related to androgen synthesis [92].

There are two drug lines for hormone therapy; the first line accounts for the central blockers that constitute the agonists of gonadotrophin-releasing hormone (GnRH agonists), and the peripheral androgen receptor blockers. Usually they are used in a comminuted way, since the central blockers, for example, Leuprolide (Lupron) and Gosserelin Acetate (Zoladex), acts through the interruption of the pituitary feedback mechanism, inhibiting LH realizing by the pituitary gland, and leading to a decreased testosterone production [63]. However, because these drugs initially boosted testosterone production, the combination with peripheral androgen receptor blockers, such as Bicalutamide (Casodex), Flutamide (Flutamide) and Androcur (Androcur), shall be indicated due to their binding capacity to the ARs in a way that inhibits androgenic stimulation, deactivating their genetic expression [93].

The second line of therapy is most commonly used when PCa is resistant to the first-line hormonal therapies stage. Abiraterone Acetate (Zytiga) is a drug that primarily acts on the adrenal gland through the inhibition of the  $17\alpha$ -hydroxylase/C17, 20-lyase (CYP17) enzyme, essential for androgen biosynthesis in tissues [94, 95]. Enzalutamide (Xtandi) is another drug of this therapeutic line that works by inhibiting androgen receptors, their signaling pathways, and is able to act on anti-androgen-resistant tumor cells.

It is important to emphasize that hormonal therapy is a palliative treatment, in that it acts to contain the progression of advanced PCa, and not its elimination. In this context, given the scarcity of effective treatments for these types of tumors, it is promising to still search for new biomarkers capable of not only diagnosing PCa early, but also being able to evaluate its aggressiveness and prognosis.

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