



Review:

Prostate cancer: the need for biomarkers and new therapeutic targets*

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Abstract: Prostate cancer (PCa) incidence and mortality have decreased in recent years. Nonetheless, it remains one of the most prevalent cancers in men, being a disquieting cause of men's death worldwide. Changes in many cell signaling pathways have a predominant role in the onset, development, and progression of the disease. These include prominent pathways involved in the growth, apoptosis, and angiogenesis of the normal prostate gland, such as androgen and estrogen signaling, and other growth factor signaling pathways. Understanding the foundations of PCa is leading to the discovery of key molecules that could be used to improve patient management. The ideal scenario would be to have a panel of molecules, preferably detectable in body fluids, that are specific and sensitive biomarkers for PCa. In the early stages, androgen deprivation is the gold standard therapy. However, as the cancer progresses, it eventually becomes independent of androgens, and hormonal therapy fails. For this reason, androgen-independent PCa is still a major therapeutic challenge. By disrupting specific protein interactions or manipulating the expression of some key molecules, it might be possible to regulate tumor growth and metastasis formation, avoiding the systemic side effects of current therapies. Clinical trials are already underway to assess the efficacy of molecules specially designed to target key proteins or protein interactions. In this review, we address that recent progress made towards understanding PCa development and the molecular pathways underlying this pathology. We also discuss relevant molecular markers for the management of PCa and new therapeutic challenges.

Key words: Prostate cancer (PCa), Biomarker, Androgen, Estrogen, Cell signaling pathway, Therapeutical target
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1 Introduction

Prostate cancer (PCa) is the second most common noncutaneous cancer in men (exceeded only by lung cancer), accounting for 14% of all new cancer cases in men worldwide. In 2008, 900 000 cases were identified, and 258 000 of them resulted in death (Jemal *et al.*, 2011). These dark figures resulted in the identification of PCa as the sixth leading cause of death from all cancers among men worldwide (Ferlay *et al.*, 2010).

Old age, black ethnicity, and a family history of the disease are the risk factors most commonly associated with PCa. The average age at the time of diagnosis is 67 years and about two-thirds of cases are diagnosed in men aged 65 years and over-diagnosis before age 40 years is rare. Environmental risk factors such as eating habits, early sexual initiation, and sexually transmitted infections, both viral (herpes simplex virus 2, human papillomaviruses 18 and 16, and human cytomegalovirus) and bacterial (*Neisseria gonorrhoea*, *Treponema pallidum*, and *Chlamydia trachomatis*), are also associated with the disease (Nelson *et al.*, 2003).

In terms of geographic variation, developed countries account for about 72% of all diagnosed PCa cases and 53% of all deaths related to the condition. PCa incidence is high in Australia and New Zealand,

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whereas South-Central Asia has the lowest incidence rate. Variation in PCa incidence is partly attributed to differences in access to diagnostic and treatment procedures (Ferlay *et al.*, 2010; Jemal *et al.*, 2011).

Interestingly, as disease incidence has been increasing, mortality has been decreasing markedly. This is due, in part, to the early detection of the disease as a result of the widespread implementation of prostate-specific antigen (PSA) screening, and significant advances in PCa therapeutics (Miller *et al.*, 2003). Nonetheless, the use of PSA screening is controversial because the early detection of PCa based on this measurement can lead to the diagnosis and treatment of PCa cases that would not otherwise cause symptoms or threaten life (Schröder *et al.*, 2006; Lumen *et al.*, 2012). Recently published data suggest that PSA-based screening results in the detection of more cases of PCa, but the effects on PCa mortality are minimal or non-existent (Djulfbegovic *et al.*, 2010; Ilic *et al.*, 2011). These findings are aggravated when considering that PCa treatment can have major side effects, including urinary incontinence and sexual impotence.

On the other hand, advances in PCa treatment are recognized as a crucial factor in reducing PCa mortality. For instance, sipuleucel T, a cancer treatment vaccine approved by the US Food and Drug Administration (FDA) in 2010, improves the survival of men with advanced PCa (Kantoff *et al.*, 2010; Thara *et al.*, 2011). However, advanced hormone resistant or metastatic disease continues to challenge medical management, which can offer only palliative care in most of these cases. Therefore, effective treatment and management of advanced PCa is still an important preoccupation in clinical practice (Turner *et al.*, 2011; Drudge-Coates and Turner, 2012).

The identification of new molecular markers not only allows a more reliable prediction of the pathological stage of the disease, but can also be useful in the process of selecting therapeutic targets: the ideal scenario would be a panel of molecular biomarkers.

In this review, we briefly describe the pathophysiological features of PCa, focusing on the main molecular processes involved. We also analyze the conventional approaches to PCa diagnosis and treatment. Lastly, we explore recent advances in the establishment of new molecular markers and therapeutic targets for PCa management.

2 Biology of prostate cancer

2.1 Prostate cancer precursors

Despite controversies concerning precancerous prostate lesions, proliferative inflammatory atrophy (PIA), prostatic intraepithelial neoplasia (PIN), and atypical small acinar proliferation (ASAP) have been accepted as the main precursors of PCa (Bostwick *et al.*, 1996; de Marzo *et al.*, 1999; Putzi and de Marzo, 2000; Samaratunga *et al.*, 2006; Davidsson *et al.*, 2011). Several mechanisms have been identified as triggers for each stage of progression (Fig. 1).

PIA consists of a chronic inflammation found mainly in the peripheral zone of the prostate. The hallmarks of PIA are focal glandular atrophy, a low frequency of apoptosis, and a high proliferation of epithelial cells. Apparently, atrophic cells in PIA undergo malignant transformation and originate PCa either directly or indirectly via development of high-grade PIN (HGPIN). This mechanism is corroborated by the common presence of PIA near foci of HGPIN or PCa (Putzi and de Marzo, 2000; Wang *et al.*, 2009).

Like PIA, PIN is found primarily in the peripheral zone (Bostwick and Brawer, 1987). PIN presents many of the genotypic alterations, architectural and cytological features, and markers of differentiation of early invasive carcinoma (Bostwick and Brawer, 1987; Bostwick *et al.*, 1996; Abate-Shen and Shen, 2000). However, unlike PIA, the basement membrane remains intact in PIN (avoiding stromal invasion) and cells express the same proteins and integrin receptors as in normal or hyperplastic stages (Bonkhoff, 1998). Also, these lesions do not produce high levels of PSA (Abate-Shen and Shen, 2000). Microscopically, changes observed in PIN are a continuum between low-grade (almost normal) and high-grade (abnormal) forms thought to be the immediate precursors of early invasive carcinoma (Abate-Shen and Shen, 2000). Alternatively, PIN cells might undergo a senescence period in which their viability is preserved but no proliferation is observed. This phenotype was experimentally demonstrated in cells with complete inactivation of the phosphatase and tensin homolog (PTEN). It was enhanced in the presence of S-phase kinase-associated protein 2 (Skp2) E3-ubiquitin ligase inactivation, and reversed with the inactivation of p53 (Chen *et al.*, 2005; Lin *et al.*, 2010).

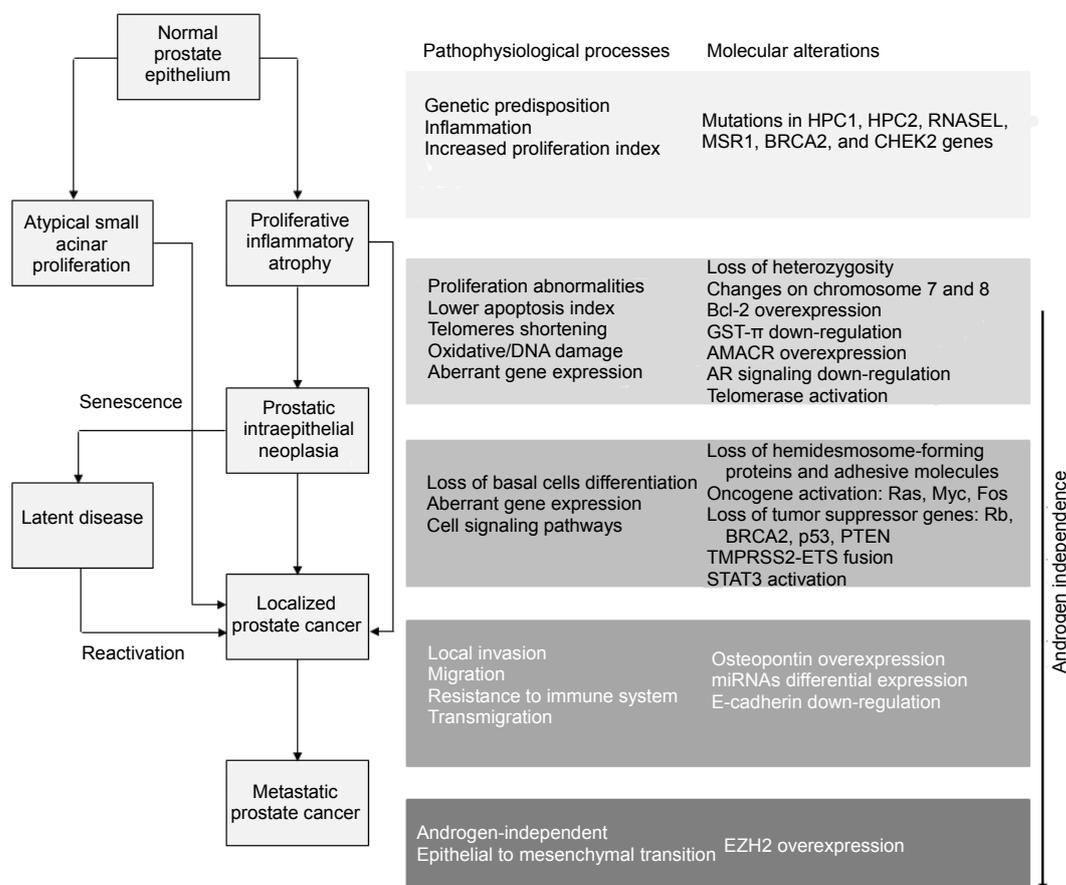


Fig. 1 Stages and processes underlying human prostate cancer (PCa) initiation and development

Genetic predisposition, inflammation, and increased cell proliferation are determinant factors for PCa initiation. The occurrence of these processes in the normal prostate epithelium gives rise to proliferative inflammatory atrophy (PIA) or atypical small acinar proliferation (ASAP). These lesions can directly progress to primary PCa. PIA might also evolve to an intermediate stage, prostatic intraepithelial neoplasia (PIN), in which a loss of proliferation capability in basal cell layers coincides with an increase in the proliferation activity of luminal secretory cells. Loss of tumor suppressor genes, oncogene activation, and altered cell signaling promote the progression to localized PCa. For instance, the loss of PTEN in addition to TMPRSS2-ETS fusion (mainly with the *v-ets* erythroblastosis virus E26 oncogene homolog (avian) member of the ETS family, ERG) promotes progression to PCa through downstream pathways. Alternatively, cells in PIN may undergo a senescence process in which the disease enters a latent stage which, nonetheless, can be reactivated. The establishment of metastases encompasses alterations in cell-cell and cell-matrix interactions that culminate in local invasion, migration, survival to the immune system, and transmigration to secondary sites. While PCa progresses, the dependence on androgens decreases until a completely androgen-independent cancer is formed. The scheme on the left represents the stages of PCa progression up until a metastatic and androgen-independent stage. On the right, each grey rectangle relates to the phase of progression shown immediately to the left. AMACR, α -methylacyl-CoA racemase; AR, androgen receptor; BRCA2, breast cancer 2, early onset; CHEK2, checkpoint kinase 2; ETS, E-twenty six; GST- π , glutathione-S-transferase π ; HPC1, hereditary prostate cancer 1; HPC2, hereditary prostate cancer 2; miRNAs, microRNAs; MSR1, macrophage scavenger receptor 1 gene; p53, protein 53; PTEN, phosphatase and tensin homologue; Rb, retinoblastoma tumor suppressor gene; RNASEL, ribonuclease L (2',5'-oligoadenylate synthetase-dependent); STAT3, signal transducer and activator of transcription 3; TMPRSS2, transmembrane protease serine 2

ASAP is the precancerous lesion most related to PCa (Moore *et al.*, 2005; Amin *et al.*, 2007; Lopez, 2007). By definition, ASAP consists of a focus of small acinar structures formed by atypical epithelial

cells and is considered a strong risk factor for PCa. Consequently, a follow-up of patients with ASAP is mandatory (Mancuso *et al.*, 2007; Koca *et al.*, 2011).

2.2 Underlying prostate cancer development and metastasis formation

PCa is a relatively heterogeneous and multifocal disorder. Around 70% of PCa cases originate in the peripheral zone, 15%–20% in the central zone, and only 10%–15% in the transition zone of the prostate gland (Crawford, 2009). PCa can assume diverse histological patterns, but most cases correspond to acinar adenocarcinomas that arise from prostatic epithelial cells which express androgen receptor (AR) (Bostwick, 1989). Ductal adenocarcinomas, mucinous carcinomas, and signet ring adenocarcinomas are extremely rare (Grignon, 2004).

The cellular origin of PCa has been attributed either to the acquisition of dedifferentiation and mortality by differentiated luminal cells or to the malignant transformation of prostate stem cells that reside among the basal cells (Knudsen and Vasioukhin, 2010). It has also been observed that PCa can arise from basal cells, although the aggressive potential of luminal and basal cell populations differs (Lu *et al.*, 2013).

The severe differentiation and proliferation abnormalities that underlie PCa development might involve multiple genetic changes, such as loss of heterozygosity, activation of oncogenes, and loss of tumor suppressor genes (Foster *et al.*, 2000). Several other factors have been associated with PCa development and progression (Fig. 1). Most are a consequence of the natural aging process—age is the most significant risk factor for PCa development. In addition to the genetic causes already mentioned, factors that contribute to the carcinogenesis of PCa include inflammation (Gueron *et al.*, 2012; Kazma *et al.*, 2012; Sfanos and de Marzo, 2012), oxidative stress and DNA damage (Miyake *et al.*, 2004; Lockett *et al.*, 2006; Battisti *et al.*, 2011; Gupta-Elera *et al.*, 2012), telomere shortening and telomerase activity (Kageyama *et al.*, 1997; Donaldson *et al.*, 1999; Fordyce *et al.*, 2005; Treat *et al.*, 2010; Xu *et al.*, 2011), genomic alterations (Boyd *et al.*, 2012; Nyquist and Dehm, 2013), and epigenetic modifications (Okino *et al.*, 2007; Goering *et al.*, 2012). Efforts have been made to achieve a deep understanding of these factors and consequently improve diagnosis and management of patients. However, the study of the trigger events for PCa initiation and development

is compromised by the small number of PCa cell lines available for *in vitro* studies, the majority of which are derived from metastatic and advanced cancers (Peehl, 2005).

Most tumors are androgen-dependent initially. However, as they develop they eventually become androgen-independent and progress to a hormone-refractory disease. This progression step is followed by metastasis formation (Heinlein and Chang, 2004).

PCa has an incredible propensity to metastasize, a consequence of several molecular mechanisms. Overall, these processes lead to local invasion, migration and site-specific establishment of metastases at secondary sites, usually in the bone, lung, or liver (Bubendorf *et al.*, 2000). Early steps of cancer progression include a down-regulation of cell-cell and cell-matrix characteristics. Malignant cells become motile and acquire the ability to destroy the extracellular matrix through degradative enzymes. In primary PCa, the expression of epithelial cadherin (E-cadherin), a transmembrane glycoprotein that is a key regulator of cell-cell binding and which is critical for epithelial to mesenchymal transition (EMT), was shown to be reduced. The down-regulation of E-cadherin enables EMT and consequently compromises cell-cell binding, so PCa can progress and metastasize. There is also evidence that abnormal signaling of β -catenin, a protein usually found complexed with cadherins, influences PCa progression due to dysfunction of the cadherin-catenin complex, an important early step in metastasis formation. Next, Ras and other GTP-binding proteins promote malignant cell motility and migration. As a solid tumor grows, malignant cells enter the circulation and rapidly bind to endothelial surfaces at secondary sites where they undergo transendothelial migration. In this process, several adhesive interactions occur involving selectin, integrin, platelet-endothelial cell adhesion molecule 1 (PECAM-1), vascular cell adhesion molecule 1 (VCAM-1), and other molecules. Once in a secondary site, tumor cells perturb the normal microenvironment and establish metastases (Clarke *et al.*, 2009).

2.3 Cell signaling pathways in prostate cancer

Cell signaling is essential for the normal function of the prostate gland. For instance, cell growth is controlled by insulin-like growth factor-1 (IGF-1) and

fibroblast growth factor (FGF). Transforming growth factor- β (TGF- β) commands apoptosis, and vascular endothelial growth factor (VEGF) is particularly important for angiogenesis (Reynolds and Kyprianou, 2006). Several cell signaling pathways and crosstalks between them have also been implicated in PCa pathophysiology (Fig. 2). In this section, we briefly describe those that are most prominent.

2.3.1 Androgens

The androgen receptor (AR) signaling pathway is essential for the physiological development and maintenance of the prostate gland. In normal prostate cells, testosterone (T) or dihydrotestosterone (DHT), the more potent androgen, binds to AR, a nuclear receptor that in the absence of ligand is located in the cytoplasm. While no ligand binds to the AR, it remains in the cytoplasm complexed with heat shock proteins, cytoskeletal proteins, and chaperones that negatively regulate its nuclear translocation, transcriptional activity, and androgen dependence. Upon androgen binding, AR is translocated to the nucleus where it binds to androgen response elements in the promoter regions of target genes, regulating their expression (Fig. 2). Overall, it promotes cell proliferation, differentiation, apoptosis, and secretion (Nieto *et al.*, 2007; Lonergan and Tindall, 2011). AR activity is modulated by the action of co-receptors and by phosphorylation of both AR and AR co-receptors (Heinlein and Chang, 2004). Co-receptors can be either co-activators or co-repressors of AR activity. They include: proteins involved in the splicing process, RNA metabolism, DNA repair, endocytosis, and apoptosis; components of the chromatin remodeling complex, ubiquitin/proteasome pathway, and sumoylation pathway; histone modifier enzymes; chaperones and co-chaperones; proteins of the cytoskeleton; signal integrators and transducers; scaffolds and adaptors; cell cycle regulators; viral oncoproteins; nuclear receptor co-regulators; and kinases and phosphatases (Heemers and Tindall, 2007).

Most PCas remain responsive to androgen stimulation for their initial growth and so the blockade of the AR pathway might be sufficient to induce tumor regression (Yang *et al.*, 2005). In the early steps of malignant transformation, AR signaling changes from paracrine to autocrine and patients normally exhibit low levels of serum T and high expression

levels of AR (Gao *et al.*, 2001; Vander Griend *et al.*, 2010). This could be caused by amplification or gain of function mutations in the AR genes, AR proteolytic processing to a constitutively active form, deregulation of AR co-factors, intratumoral androgen production, or crosslinks with other cell signals, such as growth factors, receptor tyrosine kinases, or protein kinase B (Akt) (Craft *et al.*, 1999; Buchanan *et al.*, 2001; Feldman and Feldman, 2001; Kang *et al.*, 2001; Meyer *et al.*, 2004). In contrast, a number of tumor cells present loss of AR expression. This phenomenon has been attributed to silencing by methylation, loss of the X chromosome, or a decrease in AR protein stability. Nevertheless, the real cause is still unclear (Heinlein and Chang, 2004). Notwithstanding, while PCa progresses, it eventually becomes refractory to androgens via diverse cell signaling pathway crosstalks that culminate in abnormal AR signaling (Nieto *et al.*, 2007).

The mechanisms underlying the development of androgen-independence have been extensively investigated. Splice variants of AR were identified and shown to be important for the development of androgen-independence. The characterizations of AR₃ and AR₈ in hormone insensitive PCa cells have been of particular interest (Guo *et al.*, 2009; Yang *et al.*, 2011). AR₃ is constitutively active and is an androgen-independent transcription factor. It was shown to be expressed at higher levels in androgen-independent cells compared to androgen-sensitive cells, resulting in the promotion of cancer cell proliferation (Guo *et al.*, 2009). AR₈ is a less abundant AR splice variant that localizes in the plasma membrane and acts mainly through non-genomic mechanisms, by associating with epidermal growth factor receptor (EGFR). It also enhances Scr activation, AR tyrosine phosphorylation, and the association between AR and EGFR. Overall, AR₈ potentiates AR-mediated proliferative and survival responses to hormones and growth factors (Yang *et al.*, 2011). Moreover, it was recently shown that truncated forms of AR with intact NH₂-terminal and DNA binding domains are constitutively active and able to maintain androgen-independent transcriptional activation of endogenous AR target genes, thus supporting androgen-independent growth of PCa cells (Chan *et al.*, 2012). The expression of these forms is associated with a poor prognosis and they have been recognized

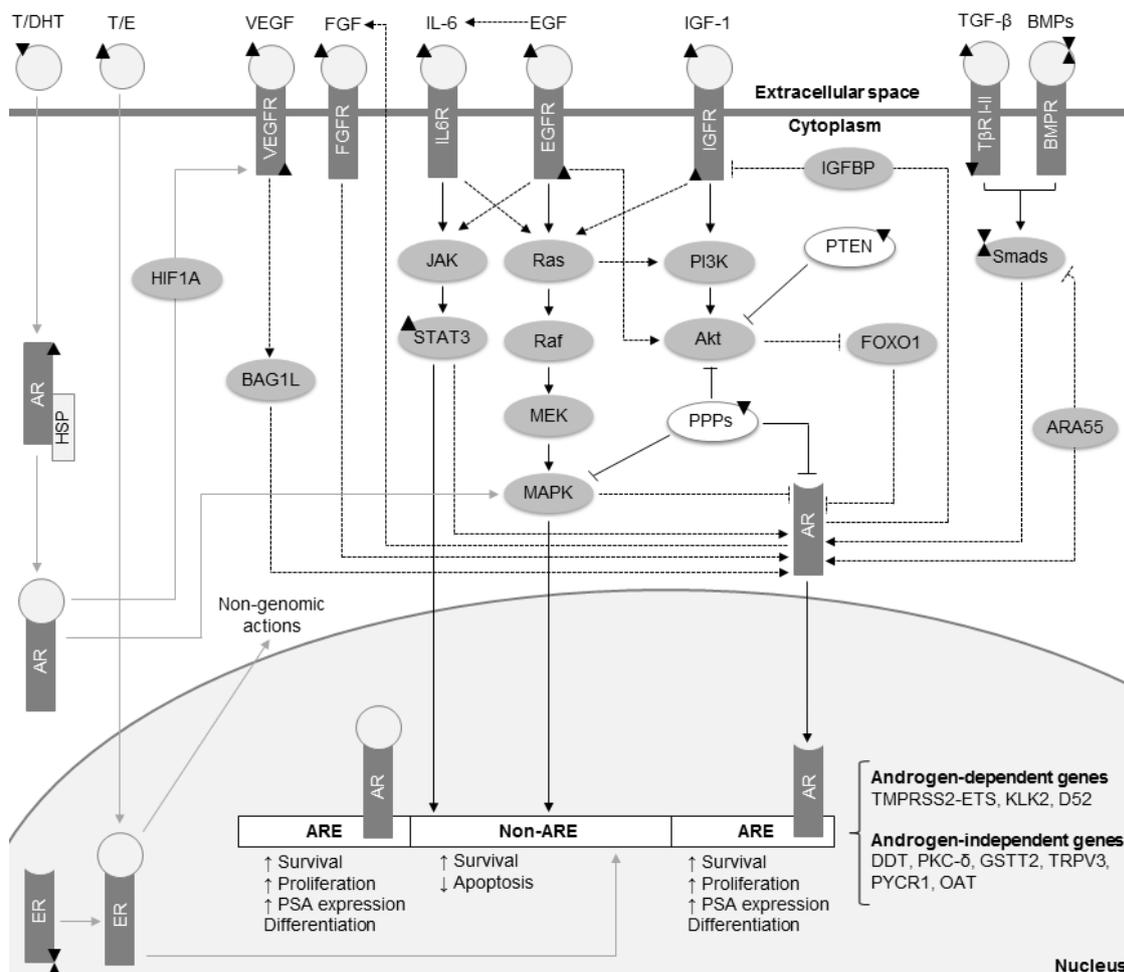


Fig. 2 Essential cell signaling pathways for prostate cancer (PCa) development and crosstalks between them

The interaction of circulating androgens (T/DHT) with AR is critical for PCa growth. Also, estrogens (E) and their corresponding receptors (ER) have a prominent role in prostate carcinogenesis, either by genomic or non-genomic actions. In advanced PCa, when the amount of available androgens is limited, other cytokines and growth factors promote synergic activities of AR. These molecules include TGF- β /BMPs, IL-6, EGF, IGF-1, VEGF, and FGF. Thus, androgen-responsive genes continue to be produced, although target genes differ between androgen-dependent and androgen-independent cells. In addition to their effects on the androgen pathway, these cell signals retain the ability to promote PCa overall survival, growth, and dissemination through alternative molecular targets. Grey arrows represent the androgen signaling in androgen-dependent PCa. Black arrows represent the prominent activated pathways in androgen-independent PCa. Dashed black arrows denote the crosslinks between cell signaling pathways. Black triangles symbolize up/down-regulation of molecules in PCa. Akt, protein kinase B; AR, androgen receptor; ARA55, androgen receptor associated protein 55; ARE, androgen response elements; BAG1L, Bcl-2-associated athanogene 1 long isoform; BMPs, bone morphogenetic proteins; BMPR, bone morphogenetic protein receptor; DDT, D-dopachrome tautomerase; DHT, dihydrotestosterone; D52, tumor protein D52; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FOXO1, forkhead box protein O1; GSTT2, glutathione S-transferase θ 2; HIF1A, hypoxia-inducible factor 1- α ; HSP, heat shock proteins; IGF-1, insulin-like growth factor 1; IGFBP, IGF binding proteins; IGFR, insulin-like growth factor receptor; IL-6, interleukin 6; IL6R, interleukin 6 receptor; JAK, Janus kinase; KLK2, kallikrein-related peptidase 2; MAPK, mitogen-activated protein kinase; MEK, upstream kinases of mitogen-activated protein kinases; OAT, ornithine aminotransferase; PKC- δ , protein kinase C δ ; P13K, phosphatidylinositol 3 kinase; PPPs, phosphoprotein phosphatases; PSA, prostate-specific antigen; PYCR1, pyrroline-5-carboxylate reductase 1; STAT3, signal transducer and activator of transcription 3; T, testosterone; TGF- β , transforming growth factor- β ; T β R, transforming growth factor- β receptors; TMPS2, transmembrane protease serine 2; TRPV3, transient receptor potential cation channel subfamily V member 3; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

as potential therapeutical targets to treat androgen-independent PCa (Hörnberg *et al.*, 2011).

Modern techniques, such as chromatin immunoprecipitation display and microarrays, have been applied to unveil the AR target genes involved in transcriptional regulation in PCa. Genes are expressed differently in normal prostate and PCa tissues. Transmembrane protease serine 2 (TMPRSS2) and its fusions with E-twenty six (ETS) transcription factor family members, mainly v-ets erythroblastosis virus E26 oncogene homolog (ERG) or its variant 1 (ETV1), kallikrein-related peptidase 2 (KLK2), and tumor protein D52, are among the genes expressed by androgen-sensitive cells when stimulated by androgens (Lonergan and Tindall, 2011; Marques *et al.*, 2011). Genes identified in androgen-independent PCa cells include D-dopachrome tautomerase, protein kinase C δ , glutathione S-transferase θ 2, transient receptor potential cation channel subfamily V member 3, pyrroline-5-carboxylate reductase 1, and ornithine aminotransferase (Jariwala *et al.*, 2007). Despite the advances in this field, much remains to be clarified.

2.3.2 Estrogens

Circulating levels of estrogens in males tend to increase during aging. Besides, testosterone can also be continuously converted to 17 β -estradiol by aromatase within the prostate stroma. With aging, there are decomposition of androgens and a predominance of estrogens, which can regulate prostate growth either directly or indirectly via regulation of other hormones, such as prolactin and luteinizing hormone (Huggins and Hodges, 1972; Lee *et al.*, 1981; Marino *et al.*, 2006; Carruba, 2007). Thus, the estrogen receptor (ER) signaling pathway has been receiving increasing attention as an intervenient in PCa carcinogenesis (Carruba, 2007).

ERs belong to a family of nuclear transcription factors which are ubiquitously expressed. Different genes encode two ER subtypes, ER α and ER β , which exhibit several isoforms (Kuiper *et al.*, 1996; Fixemer *et al.*, 2003). In the absence of ligand, ER is found in the nucleus complexed with inhibitory proteins. Upon ligand binding, ER is activated and binds to estrogen response elements in the promoter region of target genes (Fig. 2). In addition to this “genomic action”, ER also exhibits a “non-genomic action” via activation

of other cell signaling pathways: phospholipase C/protein kinase C, Ras/Raf/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K)/Akt, and cyclic-adenosine monophosphate (cAMP)/protein kinase A (PKA) (Marino *et al.*, 2006; Carruba, 2007). There is also evidence that ER activation can occur in a ligand-independent manner via MAPK and cAMP/PKA (Coleman and Smith, 2001).

Estrogens and associated signaling pathways appear to play a critical role in PCa development and progression. Metabolic activation of estrogens to genotoxic metabolites, such as 2- and 4-hydroxyl catechol estrogens, induces DNA damage, oxidative stress, and the formation of high levels of reactive oxygen species (Yager, 2000; Cavalieri and Rogan, 2006; Singh *et al.*, 2008). Concerning the ERs, ER β is progressively lost during human prostate carcinogenesis. ER β isoforms influence the human prostate in different ways depending on the spliced variants expressed and the use of alternative promoters (Horvath *et al.*, 2001; Fixemer *et al.*, 2003; Leung *et al.*, 2010b). For instance, ER β 1 inhibits the proliferation of prostate epithelial cells and EMT, whereas ER β 2/5 promotes metastasis formation (Leung *et al.*, 2010a; Mak *et al.*, 2010; McPherson *et al.*, 2010). In contrast, there is a gradual increase in the expression of ER α from PIN to metastatic lesions. Also, evidence suggests that during PIN, ER α is expressed in both stromal and luminal cells (Ricke *et al.*, 2008). In combination with AR, this receptor stimulates proliferation and differentiation of PCa cells (Bonkhoff *et al.*, 1999). Also, it was found that the enzyme that catalyzes estradiol production from testosterone, aromatase (CYP19), is altered in PCa tissues (Ellem *et al.*, 2004).

2.3.3 TGF- β

TGF- β belongs to the TGF- β superfamily of cytokines that also includes activins and bone morphogenetic proteins (BMPs). Members of this protein family are involved in the regulation of several physiological processes, such as cell proliferation, differentiation, apoptosis, adhesion, chemotaxis, organogenesis, and angiogenesis (Korrodi-Gregorio *et al.*, 2012).

In this signaling pathway, ligands bind to the TGF- β receptor II (T β RII), a transmembrane receptor, which in turns recruits the TGF- β receptor I (T β RI) to

form a heteromeric complex. T β RI is also referred to as activin receptor-like kinase (ALK) and they show different affinities for ligands (Derynck and Feng, 1997). T β RII phosphorylates and activates T β RI and the intracellular signaling proceeds due to phosphorylation of Smads. Smads are classified into three groups: (1) receptor-associated Smads (R-Smads)—Smad-1, -2, -3, -5, -8; (2) common Smad (Co-Smad)—Smad-4; and (3) inhibitory Smads (I-Smad)—Smad-6, -7. T β RI phosphorylates and activates R-Smads which bind with high affinity to Co-Smad. The heteromeric complex formed translocates into the nucleus where it binds to transcription promoters or co-factors, enabling DNA transcription. I-Smads provide a mechanism for negative feedback by preventing the interaction between R-Smads and T β RI or Co-Smad (Bello-DeOcampo and Tindall, 2003; Korrodi-Gregorio *et al.*, 2012).

Components of the TGF- β signaling pathway have been implicated in PCa regulation, with either tumor suppressor or tumor promoter activities being attributed to it. More specifically, TGF- β signaling exhibits growth inhibitory effects in the early stages of PCa and promotes malignancy in later stages. Disruption of TGF- β signaling is referred to as a metastasis promoter (Tu *et al.*, 2003). The loss or reduction of sensitivity to the TGF- β inhibitory effect and the acquired ability to express higher levels of TGF- β are in part explained by the ability of cells to overwhelm the down-regulatory effects of androgens. These phenomena appear to be associated with the transformation of a prostate tumor from benign to malignant (Saez *et al.*, 1998; Lee *et al.*, 1999).

The adult prostate gland expresses mostly TGF- β 1 when compared to TGF- β 2 and TGF- β 3 isoforms (Bello-DeOcampo and Tindall, 2003). TGF- β 1 acts as a tumor suppressor in normal prostate epithelial cells. During progression to metastatic PCa, TGF- β 1 overexpression, due to androgen withdrawal, enhances oncogenesis (Merz *et al.*, 1994; Zhu and Kyprianou, 2008). In contrast, AR expression directly down-regulates or inhibits the transcriptional activity of TGF- β 1 signaling and reduces its growth effects (when DHT is absent) (Zhu and Kyprianou, 2008). PCa cells are able to produce the bioactive form of TGF- β 1 by themselves. Instead of the growth inhibitory effect that TGF- β 1 exhibits in the normal prostate gland, in PCa cells it enhances prostate tumor

growth, angiogenesis, immunosuppression, remodeling of the extracellular matrix, and metastasis formation (Barrack, 1997; Festuccia *et al.*, 1999). However, recent data also show that TGF- β 1 decreases cell viability and induces apoptosis in invasive tumor cells via activation of p38 MAPK and c-Jun N-terminal kinases (JNK), which in turn promote the expressions of the cleaved caspases-8, -9, and -3 (Al-Azayzih *et al.*, 2012). The roles of TGF- β 2 and - β 3 remain unclear.

Regarding the TGF- β receptors, there is a decrease in both T β RI and T β RII receptors with PCa progression (Guo *et al.*, 1997; Kim *et al.*, 1998). This decrease implicates a reduction in the TGF- β growth inhibitory response in tumor cells. Endoglin, an auxiliary receptor of the TGF- β signaling whose expression is lost during PCa progression, was shown to suppress PCa cell motility through activation of the ALK2/Smad-1 pathway (Craft *et al.*, 2007).

BMPs are bone inductive factors of the TGF- β superfamily that have been implicated in bone metastasis formation (Ye *et al.*, 2007a). BMP-6 expression is absent in patients with benign prostatic hyperplasia (BPH), but its expression has been reported in malignant prostatic epithelial cells, mainly in primary PCa with established secondary skeletal metastases (Hamdy *et al.*, 1997). High levels of BMP-6 signaling, a consequence of its increased expression and the decreased expression of its inhibitors, might promote the development of PCa metastases (Yuen *et al.*, 2008). This growth factor may have a direct inductive role in PCa-associated bone metastases (Dai *et al.*, 2005). The increased metastatic potential promoted by BMP-6 is at least in part because of the increase in metalloproteinases and Id-1 (Darby *et al.*, 2008), a downstream BMP target that was previously shown to be indicative of a worse prognosis in early-stage cervical cancer and epithelial ovarian tumors (Schindl *et al.*, 2001; 2003). In contrast, BMP-9 and BMP-10 may function as tumor suppressors and apoptosis regulators in PCa. Their expression is decreased or even absent in PCa. It was demonstrated that their overexpression in PCa cells prevented *in vitro* growth, cell-matrix adhesion, invasion, and migration of cells (Ye *et al.*, 2008; 2009). BMP-9 up-regulates and activates prostate apoptosis response-4 through a Smad-dependent pathway, thus promoting apoptosis of PCa cells (Ye *et al.*, 2008). BMP-10

acts, at least in part, through the Smad-independent pathway X-chromosome-linked inhibitor of apoptosis protein/transforming growth factor- β -activated kinase-1/extracellular regulated kinase (XIAP-TAK1-ERK) (Ye *et al.*, 2009). Another BMP predominantly expressed in normal prostate tissue is BMP-7, whose expression is androgen-dependent (Masuda *et al.*, 2004). It is important for controlling noggin and follistatin expression, antagonists of the BMP signaling pathway, and therefore the regulation of the invasion, motility, and adhesion of PCa cells (Ye *et al.*, 2007b). The loss of endogenous BMP-7 during PCa progression is associated with increased invasiveness and motility (Ye *et al.*, 2007b). The expression of BMP-2 also decreases with the progression to malignancy and the decrease correlates with an increasing Gleason score (Horvath *et al.*, 2004). Its function is dependent on androgens: in the presence of androgens it inhibits the growth of PCa cells, while in the absence of androgens it stimulates cell growth (Langenfeld and Langenfeld, 2004).

Nodal, another member of the TGF- β superfamily, is also expressed in some PCa cell lines. It inhibits proliferation and induces migration depending on the stage in cancer progression and the cell microenvironment (Vo and Khan, 2011).

Several studies also revealed an up-regulation in the expressions of Smad-2, -3, -4, -6, and -7 after androgen deprivation, while the expressions of Smad-1, -5, and -8 are down-regulated in advanced PCa (Romero *et al.*, 2010; Lakshman *et al.*, 2011). The activity of Smad intracellular signaling in PCa has been explored at length. Smad-7 is an effector in TGF- β 1-induced apoptosis and its overexpression in PCa cells induces apoptosis (Brodin *et al.*, 1999; Landstrom *et al.*, 2000). More specifically, Smad-7 acts as a scaffolding protein and facilitates the activation of the p38 MAPK pathway mediated by TGF- β -activated kinase 1 and MAPK-3 (Edlund *et al.*, 2003). Then, p38 modulates the increased adhesion of metastatic PCa cells through phosphorylation and activation of Smad-3 (Hayes *et al.*, 2003). It was also shown that Smad-7 interacts with growth arrest and DNA damage protein (GADD34), a regulatory/targeting subunit of phospho-protein phosphatase 1 (PPP1). The catalytic subunit of PPP1 is then recruited to the T β RI-Smad7-GADD34 complex and dephosphorylates T β RI, establishing a negative

feedback of the TGF- β pathway (Shi *et al.*, 2004). Overexpression of Smad-7 may also be the reason for the decreased levels of phosphorylated Smad-2 observed in PCa. However, this decrease implicates a reduction in the nuclear Smad-4. Smad-4 (either dependent on or independent of Smad-3) interacts with the DNA-binding and the ligand-binding domains of AR, and apparently modulates DHT-induced AR transactivation (Zhu and Kyprianou, 2008). Loss of nuclear Smad-8 during PCa progression has also been reported (Horvath *et al.*, 2004; Perttu *et al.*, 2006).

2.3.4 IGF-1 and PI3K/Akt

IGF-1 is produced by prostatic stromal cells in response to androgen stimulation. This constitutes a paracrine signaling, stimulating the surrounding prostatic epithelial cells that increase cell proliferation. In normal cells, this pathway is repressed by IGF binding proteins (IGFBPs), which bind with high affinity to IGF-1 and prevent its interaction with the receptor. Receptor substrates include PI3K and Ras-MAPK pathways (Pollak, 2008).

The sustained activation of the IGF-1 signaling pathway is responsible for the proliferation of PCa cells. Moreover, a direct correlation was observed between high plasma IGF-1 levels and PCa progression (Stattin *et al.*, 2004). The IGF-1 receptor is overexpressed in primary PCa, in part because of the stimulation by early growth response-1 (EGR-1) (Ma *et al.*, 2012). In general, the effects of this pathway in PCa are due to amplification of the Akt gene or its upstream kinases, such as PI3K, or deletion/mutation of negative regulators, such as PTEN, PPP1, and phospho-protein phosphatase 2 (PPP2) (Li *et al.*, 2005).

Upon phosphorylation, PI3K becomes active and promotes cell proliferation and survival by regulating downstream targets. One most prominent target is Akt that belongs to the serine/threonine protein kinase B family. PI3K mediates both G1 cell cycle progression and cyclin expression by activating the Akt/mammalian target of the rapamycin (mTOR)/p70 (S6K) signaling pathway in PCa cells (Gao *et al.*, 2003). When phosphorylated, the activated Akt (pAkt) inhibits the glycogen synthase kinase 3 β (GSK3 β) which normally increases the degradation of cyclin D1, and consequently prevents the up-regulation of cell proliferation. pAkt also induces the production of

desmoplakin, vimetin, and metalloproteinases and thus regulates EMT and cell invasion (Bellacosa and Larue, 2010). A recent study revealed that the expression of E-cadherin is controlled by the human metastasis-associated gene 1 (MTA1) through a pAkt-dependent mechanism (Wang *et al.*, 2012). Another target of PI3K is the proapoptotic protein Bad, which is inhibited upon phosphorylation, thereby blocking apoptosis of PCa cells. The Ras/MAPK pathway has a similar effect on the bad protein, leading to cell survival and proliferation (Moschos and Mantzoros, 2002).

2.3.5 Other cell signalings

Epidermal growth factor (EGF), its receptor (EGFR), and the subsequent intervenients of this pathway promote cell growth, proliferation, and differentiation. Frequently, EGF and EGFR are up-regulated in advanced PCa. Activation of this signaling in PCa cells induces activation of the MAPK pathway that in turn down-regulates AR. Also, AR activates MAPK and EGFR. Moreover, EGF is able to induce up-regulation of interleukin-6 (IL-6) (Morgan *et al.*, 2009).

The FGF protein family is involved in growth, proliferation, development, and angiogenesis. Altered expression in FGFs and respective receptors correlates with PCa progression towards an androgen-independent state. FGF-2 (or basic FGF, bFGF), FGF-7, and FGF-8 are the growth factors with a more established role in PCa. All three were shown to be overexpressed in hyperplastic tissues.

FGF-2 is a mitogenic agent for prostatic stromal cells, acting mainly in an autocrine manner. It contributes to cell differentiation, migration, and angiogenesis (Reynolds and Kyprianou, 2006). FGF-2 expression and release were shown to up-regulate T β RII/Smad3, enabling the angiogenic and tumor promoting effect observed in reactive stroma (Yang *et al.*, 2008). In contrast, FGF-7 acts as a mitogen for prostatic epithelial cells, exerting its effect in a paracrine manner (Reynolds and Kyprianou, 2006). The function of FGF-8 remains to be elucidated. However, its expression in PCa is at least in part regulated by AR (Gnanapragasam *et al.*, 2002). Recent studies have implicated other members of the FGF superfamily in PCa pathogenesis. A case-control study reported that the Gly-388Arg polymorphism of the

FGF receptor-4 (FGFR4) was a contributing factor to PCa susceptibility (Liwei *et al.*, 2011). Combined with the expression of proteins that stabilize FGFR4, this polymorphism endorses a more stable signal, thus promoting PCa progression (Wang *et al.*, 2008). FGF-9 stimulates proliferation and invasion in PCa cells (Teishima *et al.*, 2012). FGF-19 is expressed in primary and metastatic PCa tissues where it acts as an autocrine growth factor (Feng *et al.*, 2013).

VEGF is a cytokine involved in cellular proliferation, migration, and angiogenesis. VEGF levels are higher in PCa compared to normal or benign hyperplasia (Ferrer *et al.*, 1997) and represent the strongest stimulus for angiogenesis in endothelial PCa cells (Trojan *et al.*, 2004). Moreover, the expressions of VEGF ligands and receptors (VEGFRs) are higher in bone metastasis sites than in the primary tumor. Through VEGF receptor-2 (VEGFR-2), VEGF regulates the migratory responses of PCa cells to fibronectin and bone sialoprotein, proteins of the ECM (Chen *et al.*, 2004). The type of VEGFR expressed in PCa cells influences cell growth, invasion, and metastasis formation. For instance, Qi *et al.* (2003) found that cell lines expressing NP-1 receptor had lower levels of migration, with and without VEGF, compared to those that did not express NP-1. On the other hand, the increased expression levels of VEGF isoform C and its receptor VEGFR-3 are associated with lymph node metastasis formation. This isoform also increases the expression of Bcl-2-associated athanogene 1 long isoform (BAG-1L), an AR co-activator that facilitates AR transactivation (Jennbacken *et al.*, 2005; Chetram *et al.*, 2011).

Interleukins, especially IL-6, have been shown to play an important role in PCa development and progression. Levels of IL-6 are increased in PCa and correlate with worse progression scenarios (Azevedo *et al.*, 2011). IL-6 binds to its receptor and activates Janus kinase (JAK) cell signaling, but also MAPK and PI3K pathways, depending on the cell type. Subsequent to JAK activation, the signal transducer and activator of transcription 3 (STAT3) is phosphorylated and translocated into the nucleus. STAT3 phosphorylates the AR at serine-772, a process essential for its transcriptional activity (Aaronson *et al.*, 2007). Increasing evidence suggests a role for STAT3 in the metastatic behavior of PCa cells (Abdulghani *et al.*, 2008; Gu *et al.*, 2010).

2.3.6 Phosphatases: essential regulators of cell signaling

Many cellular processes are controlled by phosphorylation/dephosphorylation of structural or regulatory proteins. The reversible phosphorylation system encompasses kinases that add a negatively charged phosphate group mainly to serine, threonine or tyrosine residues, and phosphatases that remove those phosphate groups (Fardilha *et al.*, 2011). In cancer, imbalances in protein phosphorylation systems appear to be an important pathophysiologic mechanism. Since the activation of several kinases results in stimulation of cell signaling pathways that potentiate cell growth and proliferation, it is not surprising that tumor suppressive functions have been attributed to phosphatases (Fardilha *et al.*, 2010).

In PCa the tumor suppressor gene that encodes PTEN is often lost. PTEN is a lipid phosphatase that negatively regulates PI3K/Akt signaling by dephosphorylation of phosphatidylinositol 3,4,5-phosphate (PIP3). Thus, mutations of PTEN are frequently associated with cancer development (Salmena *et al.*, 2008). Another element that directly suppresses PTEN expression in PCa cells is the microRNA miR-153 (Wu *et al.*, 2012). Abnormalities in PTEN are observed not only in PCa but also in several cases of PIN, suggesting that PTEN and Akt might be critical for early PCa development (Squire, 2009). PTEN loss enhances the activation of several signaling pathways which are normally deregulated during PCa, including PI3K/Akt and MAPK pathways (Goc *et al.*, 2011). The PTEN/PI3K/Akt pathway was shown to be essential for the maintenance of PCa stem-like cells, being important to their survival and proliferation (Dubrovskaja *et al.*, 2009). In the absence of PTEN, cells also present high levels of mTOR activity which results in uncontrolled growth (Majumder and Sellers, 2005). Moreover, loss of PTEN seems to mediate bone tropism in PCa metastases, at least through Rac1, a small GTPase that is a PTEN effector (Wu *et al.*, 2007). Overall, patients with PCa that exhibit a PTEN mutation have a higher Gleason score, a poorer prognosis, and a greater rate of metastases (Pourmand *et al.*, 2007). Together with VEGF receptor-2, PTEN regulates PCa proliferation and cell adhesion to fibronectin (Chen *et al.*, 2004).

In addition to PTEN, PPP1 and PPP2 modulate PCa progression by interacting with key proteins. In

PCa, both phosphatases seem to act as tumor suppressors. PPP1 and PPP2 activities were previously shown to be needed to maintain endothelial cells in a resting state (Gabel *et al.*, 1999). Inhibition of both phosphatases by caveolin-1 led to increased activities of PDK1, Akt, and ERK1/2. This, in turn, promoted tumor cell survival (Li *et al.*, 2003). Recently, the activation of phospho-protein phosphatase 5 (PPP5) by caveolin-1 was also confirmed (Taira and Higashimoto, 2013). The complex formed between PPP1 and its nuclear inhibitor was recently described as a regulator of PCa direct cell migration via up-regulation of Cdc42 (Martin-Granados *et al.*, 2012). PPP1 also interacts with AR, regulating its stability and nuclear localization through dephosphorylation at Ser650. PPP1 inhibition enhances proteasome-mediated AR degradation, while PPP1 overexpression increases AR expression and markedly enhances AR transcriptional activity in PCa cells (Chen *et al.*, 2009). The activation of PPP1 in malignant cells is potentiated by the down-regulation of Fer, a tyrosine kinase. PPP1 also affects TGF- β signaling in PCa cells through the complex formed with GADD34 and Smad-7, as mentioned above. PPP1 dephosphorylates and activates phosphorylated retinoblastoma protein (Rb), leading to its growth suppressive state and cell-cycle arrest in malignant cells (Pasder *et al.*, 2006). The expression of the catalytic subunit of PPP2 and its activity were shown to be down-regulated in androgen-independent PCa cells compared to androgen-dependent cells. This is particularly interesting as PPP2 is able to sustain the growth of androgen-dependent cells under androgen-deprivation by relieving the androgen deprivation-induced cell-cycle arrest and preventing apoptosis (Bhardwaj *et al.*, 2011).

3 Conventional diagnosis and monitoring of prostate cancer

Currently, the screening for PCa essentially involves digital rectal examination (DRE), determination of serum concentrations of PSA, and transrectal ultrasonography (TRUS). Table 1 describes the main advantages and disadvantages of these techniques.

Definitive diagnosis of PCa always requires the presence of adenocarcinoma in prostate biopsies or

Table 1 Presentation of the three principal methods used for the current screening and diagnosis of prostate cancer: digital rectal examination (DRE), prostate-specific antigen (PSA) measurements, and transrectal ultrasonography (TRUS)

Method	Advantage	Disadvantage	Reference
DRE (physical exam)	Safe exam; May detect PCa in men with normal PSA levels and small tumors; Relatively inexpensive	Most palpable cancers are not early cancers; Cancers can be located in distant regions of the gland, and so are not palpable; Conclusion from the exam is highly dependent on the interpretation of the examiner; Its sensitivity and specificity are low and dependent on tumor stage	Okotie <i>et al.</i> , 2007
PSA measurement (biochemical test)	Safe exam; Used for screening, early detection, and prognosis; Good sensitivity; Relatively inexpensive	Low specificity, thus leading to high number of false positives; Detection rate of PCa when using PSA levels combined with DRE depends on both the PSA threshold used on biopsy and the patient's age; Correlation between grade and progression has been referred to as feeble in some ranges of detection	Stamey <i>et al.</i> , 2002; Bickers and Aukim-Hastie, 2009; Shteynshlyuger and Andriole, 2010
TRUS (imaging exam)	Enables early diagnosis; Useful to guide biopsies; High sensitivity	Invasive procedure; Poor specificity; Time-consuming technique	Shteynshlyuger and Andriole, 2010

operative specimens. Prostate biopsy is an essential procedure for PCa diagnosis, presenting 100% specificity and virtually no false positives. Although major complications have not been associated with this procedure, it is an invasive technique and so its clinical application in daily screening is conditioned (Raaijmakers *et al.*, 2002). Moreover, the number of false negative cases is still higher than desirable (Haas *et al.*, 2007). Histopathological examination of prostate specimen enables both grading and determination of tumor extent. The Gleason score is the standard system used by clinicians to rate PCa. This system classifies the biopsy specimen from 1 to 5, with 5 being the most aggressive with no glands being recognized. The final Gleason score is a sum of the most common primary and secondary grades. Sometimes a tertiary grade can also be given (Borley and Feneley, 2008).

4 Emerging biomarkers: new tools to improve prostate cancer diagnosis and management

A biomarker, as defined by the National Cancer Institute, is “a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease” (<http://www.cancer.gov/dictionary?cdrid=45618>). These molecules are useful tools in supporting several

steps of a patient's management: prevention, screening, diagnosis, prognosis, prediction of treatment effectiveness, and monitoring responses to treatment. The establishment of a specific panel of biomarkers, in either tissue or body fluids, might complement the routinely applied diagnostic techniques to achieve earlier and more accurate diagnoses. This is a major challenge as PCa is a silencing disease in the early stages, hence not presenting symptoms until it becomes locally advanced or metastasizes (Smith *et al.*, 2003). In addition, to enable the evaluation of the course of the disease, prognostic biomarkers might support treatment decisions. Predictive biomarkers are helpful in choosing the treatment type. Biomarkers can also aid drug development, and the advantage of their use in clinical trials has been widely explored (Rolan, 1997; Alaoui-Jamali and Xu, 2006; Committee on Developing Biomarker-Based Tools for Cancer Screening, Diagnosis, and Treatment, 2007).

Nowadays, only two biomarkers are approved by the FDA for use in PCa management: total PSA (tPSA) and free PSA (fPSA) (Rhea and Molinaro, 2011). Increased levels of serum tPSA are associated with PCa. tPSA has been widely used for screening, diagnosis, and monitoring of the disease, especially for the detection of recurrent disease after treatment (Makarov *et al.*, 2009). However, as previously noted,

it lacks specificity as conditions such as prostate infection, irritation, benign prostatic hypertrophy, recent ejaculation, or medical interventions can also cause an upsurge in tPSA levels (Makarov *et al.*, 2009; Rhea and Molinaro, 2011). fPSA corresponds to the portion of secreted PSA that does not form complexes with serum antiproteases. Patients with PCa present a lower proportion of fPSA to tPSA (%fPSA), and so this percentage can be used to reduce unnecessary biopsies and to follow the progression of the disease. The %fPSA is, nevertheless, modulated by prostate volume, patient age, tPSA levels, and prostatic manipulation (Makarov *et al.*, 2009).

Prior to PSA, prostatic acid phosphatase (PAP) was extensively used as a serum prognostic biomarker for PCa. Higher levels of expression and activity of PAP are associated with PCa with established metastases on bone. Also, post-treatment PAP levels are predictive of the clinical outcome.

Efforts have been made to discover new tissue biomarkers and molecules measurable in body fluids with high sensitivity and specificity. Table 2 summarizes some of the most relevant molecular markers that can be used in the context of PCa.

5 Current options and challenges in prostate cancer treatment

PCa treatment must be adjusted according to: (1) the tumor stage and grade, (2) PSA levels, and (3) the estimated baseline for patient life expectancy (Heidenreich *et al.*, 2012). In terms of response to treatment, four stages can be identified while the tumor progresses: hormone-sensitive, androgen-independent, symptomatic metastatic, and advanced metastatic or anaplastic. Nowadays, treatment options are available for each stage. However, the ideal therapy has not yet been found and treatment effectiveness varies with cancer progression (Oudard, 2013).

Localized tumors can be efficiently treated by radical surgery or radiotherapy. However, this type of treatment is associated with urinary, sexual, and bowel dysfunction. The severity and frequency of these adverse effects differ between treatments (Wilt *et al.*, 2008). When tumors disseminate, androgen deprivation therapy is adopted, since cells will not grow and survive without androgens. The standard

procedure of androgen deprivation (AD) by surgical or chemical castration usually results in incomplete tumor regression and the tumor eventually becomes castration-independent. This can be explained, at least in part, because AD induces a senescent-like phenotype in a subset of androgen-sensitive cancer cells (Ewald *et al.*, 2013).

Recent advances in PCa treatment culminated in the approval of Firmagon (degarelix), Jevtana (cabazitaxel), Provenge (sipuleucel-T), and Zytiga (abiraterone acetate) (US Food and Drug Administration, 2010; European Medicines Agency, 2009; 2011a; 2011b). Degarelix is a gonadotrophin-releasing hormone antagonist, which reduces the amount of T, and it is indicated for the hormone-sensitive stage of PCa (European Medicines Agency, 2009; Oudard, 2013). Sipuleucel-T has been a remarkable development as it was the first FDA-approved vaccine for PCa treatment, and it is currently used to treat androgen-independent (non-metastatic or minimally metastatic) PCa (Oudard, 2013). Despite the improved survival in patients with metastatic castration-resistant PCa, sipuleucel-T lacks accurate biomarkers to assess the response to immunotherapy (Kim *et al.*, 2012). In more advanced symptomatic metastatic stages, cabazitaxel or abiraterone acetate is commonly applied (Oudard, 2013). The optimism of patients is increasing with these new products for PCa treatment. However, overall survival remains relatively short (Antonarakis and Eisenberger, 2011).

Simultaneous with progress in the comprehension of PCa genetics and the molecular pathways involved, new therapeutic targets have been investigated, especially for advanced and metastatic cancers. Manipulation of cell signaling pathways, targeting of specific protein interaction genes, antisense therapy, anti-angiogenic and pro-apoptotic therapies are now challenges for biomedical research.

Several attempts have been made to eradicate advanced metastatic and hormone-refractory PCa mainly through targeting of AR signaling (Leibowitz-Amit and Joshua, 2012). Apparently, AR targeting via ASC-J9, an AR degradation inducer, leads to tumor suppression through the induction of autophagy (Jiang *et al.*, 2012). Other studies have been focused on the PI3K/Akt/mTOR pathway, testing inhibitors for each kinase. Inhibitors of mTOR are most promising, and phases I and II clinical trials are already

Table 2 Emerging biomarkers for diagnosis, prognosis, and prediction of prostate cancer (PCa): a brief characterization of each biomarker and observations that justify its use in the management of patients with PCa

Biomarker	Characterization	Observation in PCa	Detection	Use	Reference
α methylacyl coenzyme A racemase (AMACR)	Protein involved in the β -oxidation of branched fatty acids	Overexpressed in PCa specimens, with levels greater than PSA. mRNA levels of AMACR and survivin together aid the identification and risk stratification of patients with PCa	Tissue	Diagnosis, predictive	Rubin <i>et al.</i> , 2002; Liu <i>et al.</i> , 2007; Al-Maghrebi <i>et al.</i> , 2012; Yap <i>et al.</i> , 2012
Adenomatous polyposis coli (APC)	Tumor suppressor gene	Loss of heterozygosity, lower expression, and methylation of APC are described in PCa	Tissue, serum, urine	Diagnosis	Gao <i>et al.</i> , 1995; Liu <i>et al.</i> , 2011; Chen Y. <i>et al.</i> , 2013
Bone turnover markers (BTMs)	Markers of bone formation, such as osteocalcin, total alkaline phosphatase activity, and serum bone-specific alkaline phosphatase, as well as, bone resorption, including C-telopeptides of type I collagen	Higher levels of BTM are associated with negative clinical outcomes in patients presenting bone metastases. A reduction in their levels after treatment is related to patient survival, both in androgen-dependent or -independent PCa	Serum	Diagnosis, prognosis, predictive	Gamero <i>et al.</i> , 2000; Gamero, 2001; Hegele <i>et al.</i> , 2007; Som <i>et al.</i> , 2012
Circulating tumor cells (CTCs)	Tumor cells that enter the bloodstream as a consequence of tumor growth. Responsible for establishing metastases	Higher amounts of CTCs are related to worse prognoses. They also provide information about other molecular markers, including TMPRSS2-ERG and EMT-related genes. Commercial kits are already available to count CTCs in metastatic PCa	Blood	Prognosis	Danila <i>et al.</i> , 2011; Chen C.L. <i>et al.</i> , 2013; Thalgott <i>et al.</i> , 2013
Endoglin (CD105)	Transmembrane glycoprotein that constitutes a co-receptor for TGF- β signaling	Primarily detected in newly formed vessels, being related to the angiogenic process. Preoperative serum levels of endoglin correlate with pelvic lymph node metastasis after radical prostatectomy	Tissue, serum	Prognosis, predictive	Wikstrom <i>et al.</i> , 2002; Karam <i>et al.</i> , 2008
Early prostate cancer antigen (EPCA)	PCa-specific nuclear matrix protease	PCa patients have higher serum levels of EPCA. The highest preoperative serum levels correspond to metastatic PCa cases	Serum	Diagnosis, prognosis	Zhao <i>et al.</i> , 2010; 2012
Golgi membrane protein 1 (GOLM1)	Type II Golgi transmembrane protein that collaborates in protein synthesis and transport of protein cargo	Up-regulation of GOLM1 expression in PCa compared with normal prostate and BPH tissues	Tissue, urine	Diagnosis	Varambally <i>et al.</i> , 2008; Li <i>et al.</i> , 2012

To be continued

Table 2

Biomarker	Characterization	Observation in PCa	Detection	Use	Reference
Glutathione S-transferase-P1 gene (GSTP1)	Encodes a detoxifying enzyme that protects DNA from free radical damage	Serum methylation profiles differ between healthy subjects, BPH, and PCa patients. PIN and PCa tissues present loss of GSTP1 expression due mainly to promoter hypermethylation. Together with APC levels it might improve the distinction between PCa and BPH. Methylation status of GSTP1 gene and Gleason score are strictly related	Tissue, serum	Diagnosis, prognosis	Bernardini et al., 2004; Liu et al., 2007; Bryzgunova et al., 2008; Karam et al., 2008; Woodson et al., 2008; Yoon et al., 2012
Hepsin	Type II transmembrane serine protease	Overexpressed in PCa when compared to healthy or BHP tissues	Tissue	Diagnosis	Klezovitch et al., 2004; Kelly et al., 2008; Goel et al., 2011; Pace et al., 2012
Human kallikrein 2 (KLK2)	Serine protease that belongs to the human kallikrein family, as well as PSA. It is predominantly expressed in the prostate under androgen control	Overexpressed in HGPIN and PCa when compared to healthy tissue. Increased serum levels are associated with worse outcomes	Tissue, serum	Diagnosis, prognosis	Darson et al., 1997; Raaijmakers et al., 2007; He et al., 2009
Ki-67	Mitotic index that regulates the cell-cycle	Increased Ki-67 staining index correlates with higher Gleason score. Together with other molecular markers, it helps to determine the risk of PCa progression	Tissue	Prognosis	Steuber et al., 2007; Madani et al., 2011; Josefsson et al., 2012; Fisher et al., 2013
Lactate dehydrogenase (LDH)	Metabolic enzyme that participates in glycolysis and gluconeogenesis pathway	Increased levels of LDH are associated with poor clinical outcomes in PCa patients and aggressive phenotypes in patients with bone metastases. Expression of the gene encoding LDH subunit is lost through promoter methylation	Blood	Prognosis	Leiblich et al., 2006; Naruse et al., 2007
MicroRNAs	Noncoding regulatory RNAs, such as miR-21, miR-75, miR-141, and miR-221 crucial in development and post-transcriptional processes	Increased levels in patients with metastatic PCa. Higher levels of miR-75 inhibit Sec23A protein, promoting cell proliferation	Tissue, serum	Diagnosis, prognosis	Mattie et al., 2006; Mitchell et al., 2008; Szczyrba et al., 2011; Yaman Agaoglu et al., 2011
p63	Gene that belongs to the p53 gene family. It inhibits PCa metastases through regulation of miR-205 expression	The majority of PCa tissues do not express p63, unlike normal and preneoplastic tissue. Aberrant expression of p63 is associated with oncogenic progression and survival	Tissue	Diagnosis	Parsons et al., 2001

To be continued

Table 2

Biomarker	Characterization	Observation in PCa	Detection	Use	Reference
Prostate cancer antigen 3 (PCA3)	PCa-specific gene that modulates the transcriptional activity of androgen receptor target genes. Involved in PCa survival	Highly overexpressed in PCa tissues, but not in normal or BPH tissues. In contrast to PSA, it is a marker independent of prostate size. As observed in PCa tissues, PCA3 is up-regulated in patients' urine. Combining the PCA3 level with the PSA level may decrease the number of unnecessary prostate biopsies	Tissue, urine	Diagnosis	Bussemakers et al., 1999; Crawford et al., 2012; Ferreira et al., 2012; Yutkin et al., 2012
Phospho-choline-containing phospholipid (PcCP)	Lipids are involved in several functions, including cellular energy storage, structure, and signaling	Patients with PCa have increased plasma lipid concentrations of almost all classes of lipids. Levels of lipids, such as myristic acid, α -linolenic acid, eicosapentaenoic acids, and cholesterol modulate the risk of PCa	Serum	Diagnosis	Zhou et al., 2012
Prostate-specific antigen (PSA)-derived markers	Include PSA density, PSA velocity, pro-PSA, and PSA doubling time	Patients with PCa present increased PSA density, higher PSA velocity, lower PSA doubling time, and higher levels of pro-PSA	Serum	Predictive, prognosis	Makarov et al., 2009
Phosphatase and tensin homolog (PTEN)	Tumor suppressor gene that encodes the phosphatase responsible for the conversion of PIP3 to inactive phosphatidylinositol (4,5) biphosphate	PTEN deletion is common in PCa and is associated with decreased expression of PTEN and AR, which represents a poorer outcome	Tissue	Prognosis	Lotan et al., 2011; Antonarakis et al., 2012; Choucair et al., 2012
Transforming growth factor- β 1 (TGF- β 1)	Regulator of cellular proliferation, differentiation, and angiogenesis	Decreased expression levels in PCa. Polymorphisms also aid the determination of PCa progression (single nucleotide polymorphisms at C-509T correlate with lower risk of aggressive PCa)	Tissue	Predictive	Soulitzis et al., 2006; Faria et al., 2007; Brand et al., 2008
Trans-membrane protease serine 2-v-ets erythroblastosis virus E26 oncogene homolog (avian) (TMPRSS2-ETS gene fusion)	Fusion of TMPRSS2 gene, encoding a serine protease, with an ETS family member oncogene, such as ERG, culminates in ETS oncogene being overexpressed under androgen control	TMPRSS2-ERG fusion is the most frequent. TMPRSS2-ETS gene fusion is common in PCa tissues and precancerous lesions closed to PCa. TMPRSS2-ETS gene fusion together with PCA3 levels supports risk stratification	Tissue, urine	Predictive, diagnosis, prognosis	Tomlins et al., 2005
Urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR)	Cell signaling involved in the degradation of the extracellular matrix and basement membrane	Increased expression of uPA is associated with bone metastases. High serum levels of the uPAR forms correlate with poorer outcomes	Tissue, serum	Prognosis, predictive	Ohta et al., 2003; Kumano et al., 2009; Almási et al., 2011

underway (Morgan *et al.*, 2009). The verification that loss of PTEN expression results in a down-regulation of CXCR4-mediated events and in the subsequent activation of PI3K/AKT and ERK1/2, also drew attention to the promising antagonistic effect of this receptor (Chetram *et al.*, 2011).

Despite the attention given to kinases as therapeutic targets, efforts are now being made by pharmaceutical companies to investigate the role of phosphatases. Nevertheless, the only FDA-approved drugs targeting a protein phosphatase are cyclosporine A and FK506, immunosuppressors that inhibit phospho-protein phosphatase 3 (PPP3) (or calcineurin). Because of the numerous functions of PPP1 and PPP2 catalytic subunits, the long-term usage of these enzyme inhibitors is associated with nephrotoxicity and hepatotoxicity. For this reason, a more satisfactory option seems to be to target PPP1 interacting proteins (PIPs) instead of protein phosphatases directly, as they are more event, tissue, and subcellular compartment specific (Fardilha *et al.*, 2010). Two targeted PPP1-PIP complexes have already been described: (1) the PPP1-GADD34 complex, which is diminished in cells treated with salubrinal—a small molecule that protects the cell from ER-stress-induced apoptosis; and (2) PPP1 and histone deacetylases (HDACs), an example of which is trichostatin A, which disrupt the PPP1-HDAC6 complex in glioblastoma and PCa cells (McConnell and Wadzinski, 2009).

Other potential therapeutic agents are miRNAs. Either by anti-sense oligonucleotide inactivation of oncogenic miRNAs or by restoration of down-regulated mRNAs, it is possible to inhibit tumor growth, decrease lung metastases, and extend survival in mice (Vandenboom II *et al.*, 2008; Gordanpour *et al.*, 2012). A recent study proved that miR-185 could function as a tumor suppressor gene by targeting AR signaling (Qu *et al.*, 2013).

6 Future directions

Despite the recent positive progress observed in PCa incidence and mortality rates, efforts have to be made to achieve a better understanding of PCa precancerous lesions and of the factors triggering PCa development. This comprehension would enable a

more proactive action against PCa progression. Emerging biomarkers need validation to improve PCa management and to reduce population differences in access to accurate diagnostic and prognostic procedures. The exploration of molecular targets and interactions for PCa treatment has been surprisingly rewarding and promising, with several benefits achieved in blocking progression and causing regression of metastases.

Compliance with ethics guidelines

Juliana FELGUEIRAS, Joana Vieira SILVA, and Margarida FARDILHA declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要:

本文题目: 前列腺癌: 亟待肿瘤标志物和治疗新靶点

Prostate cancer: the need for biomarkers and new therapeutic targets

研究目的: 这篇综述系统地阐述了前列腺癌的发生、发展、相关分子信号通路以及分子标志物用于前列腺癌的临床诊断和前列腺癌诊治所面临的挑战。

重要结论: 许多分子信号通路通过影响细胞生长、凋亡、血管生成等病理生理过程而参与了前列腺癌的起始、发生和发展。这些研究基础有助于寻找前列腺癌的肿瘤标志物和改进前列腺癌的治疗。虽然去势治疗是早期前列腺癌治疗的金标准,但是晚期的激素抵抗型前列腺癌(CRPC)的治疗仍面临着巨大的挑战。所幸的是,目前研究发现可以通过切断特异的蛋白-蛋白相互作用或者通过调节某些影响肿瘤生长和转移的关键分子来治疗前列腺癌,能减少传统治疗所带来的副作用;相关临床研究已开始实施。这些研究进展给前列腺癌的诊治带来了新的曙光。

关键词组: 前列腺癌; 肿瘤标志物; 分子信号通路; 治疗新靶点