Cancer and Metastasis Reviews (2019) 38:333–346
https://doi.org/10.1007/s10555-019-09815-3

Beyond the biomarker role: prostate-specific antigen (PSA) in the prostate cancer microenvironment

Afshin Moradi[1,2] · Srilakshmi Srinivasan[1,2] · Judith Clements[1,2] · Jyotsna Batra[1,2]

Published online: 28 October 2019

Abstract

The prostate-specific antigen (PSA) blood test is the accepted biomarker of tumor recurrence. PSA levels in serum correlate with disease progression, though its diagnostic accuracy is questionable. As a result, significant progress has been made in developing modified PSA tests such as PSA velocity, PSA density, 4Kscore, PSA glycoprofiling, Prostate Health Index, and the STHLM3 test. PSA, a serine protease, is secreted from the epithelial cells of the prostate. PSA has been suggested as a molecular target for prostate cancer therapy due to the fact that it is not only active in prostate tissue but also has a pivotal role on prostate cancer signaling pathways including proliferation, invasion, metastasis, angiogenesis, apoptosis, immune response, and tumor microenvironment regulation. Here, we summarize the current standing of PSA in prostate cancer progression as well as its utility in prostate cancer therapeutic approaches with an emphasis on the role of PSA in the tumor microenvironment.

Keywords Prostate-specific antigen · Prostate cancer · Kallikrein-related peptidase · Angiogenesis · Immune response

1 Introduction

Prostate-specific antigen (PSA), encoded by the *KLK3* gene, is a member of the tissue kallikrein-related peptidase family (KLKs). The KLK family is comprised of a large family of 15 conserved trypsin- or chymotrypsin-like serine proteases encoded by a contiguous cluster of protease genes (*KLK1-KLK15*) at chromosome 19q13.3-13.4 [1–3]. PSA, a chymotryptic serine protease, is a 261-amino acid long preproprotein with a 17-amino acid signal peptide (pre-) and a 7-amino acid pro-peptide [4]. The signal sequence contributes to transporting the protein to the secretory pathway and is normally eliminated before it is released into the prostatic ducts. Enzymes including trypsin and the related KLK2, KLK4, and KLK5 proteases activate this proenzyme [5]. Active PSA (237 amino acids) contains five disulfide bridges, a surface loop with a length of 11 residues that partially embraces the catalytic cleft and is thought to influence the enzymatic specificity [6, 7].

✉ Jyotsna Batra
jyotsna.batra@qut.edu.au

[1] School of Biomedical Sciences, Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

[2] Translational Research Institute, Queensland University of Technology, Brisbane, Australia

In comparison with other organs, PSA is expressed almost exclusively in the prostate [8]. Under normal physiological conditions, PSA is secreted into the seminal fluid rather than into the circulatory system. PSA activity is modulated by Zn^{2+} ions together with inhibitors such as α1-antichymotrypsin (ACT), inter-α-trypsin inhibitor, α1-protease inhibitor (API), α-2-macroglobulin (α2M), and protein C inhibitor (PCI). During ejaculation, the semenogelins (SG) sequester Zn^{2+} liberating free active PSA and start a proteolytic activation cascade to hydrolyse fibronectin (FN) and SG1 and SG2 thus leading to seminal clot liquefaction and release of motile spermatozoa [9, 10]. Serum PSA levels are reported to be increased in conditions such as inflammation or neoplasia owing to the leakage of PSA into the circulation when the architecture of the prostate gland is disrupted [11]. Increased levels of PSA in serum and prostate make it a sensitive marker for prostate cancer [12]. Serum total PSA (tPSA) comprises both the complexed and free PSA (fPSA) forms. PSA-ACT complex is a major form of PSA and it is increased with increasing PSA concentrations. A complex with API was also observed in serum of patients with prostate cancer and very high levels of PSA [13, 14]. Prostate cancer patients have a lower f/t PSA ratio (%fPSA) than those with benign prostatic hyperplasia (BPH). In addition, measuring %fPSA was shown to have the potential to predict prostate cancer morbidity in men with levels of PSA in the range of 4 to 10 ng/mL [15].

As described above, proteases such as KLK2 process pro-PSA to generate a mature and active form of PSA by

removing the first 7 amino acids from the pro-form. It is noteworthy that, under-processing and over-processing of pro-PSA leads to an another form of PSA. These types of PSA which can be found in the prostate tissue and serum of cancer patients are termed the (-5), (-4) and (-2) pro-forms [16]. The (-2) pro-PSA form is used as a biomarker for the detection of prostate cancer clinically, where the combination of pro-PSA isoforms and fPSA or tPSA assists in the diagnosis of aggressive cancers [17]. The Prostate Health Index (PHI) combines tPSA, fPSA, and (-2) proPSA, according to the mathematical formula [(-2) proPSA/fPSA] × √ tPSA. PHI is considered to have a better accuracy than tPSA and %fPSA to predict the outcome of biopsy [18]. This model reduced the number of biopsies by 15% of the 700 men tested (PSA levels 2–10 ng/mL) but missed 1% of high-grade cancers [19–21]. Several PSA transcript variants have been identified to date [22]. For example, *PSA-RP2* is a transcript variant of the PSA gene which is documented to be upregulated in prostate cancer compared with BPH. Similarly, the PSA protein sequence is affected by some genetic variants [23]. For example, the rs61752561 single-nucleotide polymorphism (SNP) has a potential role in the pathogenesis of prostate cancer by changing the glycosylation as well as PSA activity and protein stability, all of which can affect the f/t PSA ratio measured in clinical settings. It is asserted that the accuracy of the current PSA test would be enhanced if the effect of the genetic variants is considered while measuring tPSA and f/tPSA ratio [24]. PSA glycoprofiling in addition to SNP-based change in glycosylation has been suggested as a prognostic biomarker for patients with aggressive prostate cancer. For example, measuring increased sialylation of PSA glycans could distinguish prostate cancer from BPH patients [25].

The rise in level of PSA is considered to indicate the progression of prostate cancer. During cancer progression, changes in PSA level over time is measured by doubling time or PSA velocity (PSAv). Some studies have shown that there is significant improvement using PSAv compared to the ratio of the fPSA to tPSA screening [26, 27]. However, monitoring PSAv can have variability due to the high PSA levels in BPH patients and variation in intervals between PSA measurements [28]. Similarly, the PSA density (PSAD) can be achieved by the ratio of tPSA to the prostate volume [29]. Prostate volume measurement requires use of either digital rectal exam (DRE) or transrectal ultrasonography (TRUS) techniques which are both invasive procedures. Improving the efficacy of prostate cancer diagnosis is achievable by combination of the PSA test with other diagnostic factors. For example, the 4Kscore model including tPSA, fPSA, intact PSA (iPSA), and KLK2 was shown to improve prostate cancer detection. The 4Kscore outperforms tPSA in predicting the outcome of a prostate biopsy [18]. The STHLM3 test is a clinical diagnostic test for prostate cancer detection. This test combines plasma protein biomarkers [PSA, iPSA, KLK2, microseminoprotein-beta, and macrophage inhibitory cytokine 1 (MIC-1)], 232 SNPs, and clinical variables such as age, family history, prostate examination, and previous prostate biopsy [30–32]. The STHLM3 trial improved specificity in early diagnosis of prostate cancer by detecting men at increased risk of harboring significant prostate cancer. This test also improved discrimination of high-grade prostate cancer in comparison with conventional PSA testing [31].

In addition to its well established biomarker role, PSA has been shown to have a profound impact on the signaling pathways of proliferation, angiogenesis, and metastasis; for example, a number of IGF binding proteins (IGFBPs) are cleaved by PSA which indirectly supports tumor growth by the release of IGFs [33]. Also, PSA may play its antiangiogenic activity through its action with cell surface receptors. It is known that PSA stimulates expression of tumor-suppressor genes, for example *IFN-γ* and suppresses oncogene promoters, such as *VEGF*, *urokinase-type plasminogen activator (uPA)*, and *Pim-1* which can prevent tumor growth in the PC-3M prostate cancer cell line [34]. Interestingly, PSA has also been shown to increase prostate cancer cell proliferation by stimulating androgen receptor associated protein 70 (ARA70)-induced AR transactivation by modulating the p53 pathway [35]. As a result, besides being a diagnostic and prognostic biomarker for prostate cancer progression, PSA has been suggested to have an important role in prostate cancer genesis and progression and proposed as a suitable target, albeit controversially, for prostate cancer treatment. In this review, we summarize these diverse roles of PSA focusing on recent literature on its role in the tumor microenvironment.

2 Functional role of PSA in prostate cancer genesis

Prostate cancers are almost entirely adenocarcinomas (95%) which are described as tumors originating from malignant growth in epithelial tissues. The remainder (5%) of less prevalent prostate cancers may comprise transitional cell carcinomas, small cell carcinomas, intraductal carcinomas, and squamous cell sarcomas [36, 37]. Within the initial stage of prostate cancer, the tumor is restricted to the prostatic capsule and may be indolent throughout the lifespan of the patient. Even so, some tumors predisposed to aggressiveness will later develop by invading from the prostate capsule to adjacent tissues in the pelvis, urinary bladder, urethra, and seminal vesicle. Such metastatic spread happens principally through lymph nodes in the pelvis and subsequently to the bones; in some cases, prostate cancer also spreads to the lungs and other organs such as the kidney and liver [38–40]. In contrast, head and neck metastases from prostate cancer arise because of hematogenous spreading through the vertebral venous system [41, 42].

2.1 PSA has a role in sustaining cancer cell growth and apoptosis

Cancer cells essentially require proliferation and the ability to evade growth inhibitory signals. As mentioned before, PSA catalyzes IGFBPs, in particular IGFBP-3, to release insulin growth factor-1 (IGF-1) which is a proliferative factor involved in multiple cancers such as prostate cancer [43]. *In vitro* growth of LNCaP cells was reduced when endogenous PSA expression was ablated using gene-specific shRNA, and reduced tumor weight compared to controls in a mice model. PSA is, therefore, likely to be pro-tumorigenic in prostate tumors, as demonstrated by both *in vitro* and *in vivo* models [44]. One of the barriers in the progression of cancer is programmed cell death. To be resistant to cell death during cancer progression, cancer cells use alternatives such as overexpression of anti-apoptotic regulators (e.g., Bcl-2) or signals related to survival (e.g., Interleukin-6) and decreased expression of pro-apoptotic regulators (such as Bax, Bak) [45–47]. Interestingly, knockdown of PSA in LNCaP and CWR22rv1 cells induced cell apoptosis [35]. By modulating the p53 pathway, increased PSA levels led to increased ARA70-induced AR transactivation that led to a decline in apoptosis and increased cell proliferation in prostate cancer cells [47]. On the contrary, there are several reports suggesting that PSA may act as an inducer of apoptosis, for example, treatment of PC3 cells with fPSA can downregulate antiapoptotic genes such as *Bcl-xl* and upregulate apoptotic genes such as *Fas-activated serine kinase* [48]. PSA can cleave galectin-3; knockdown of galectin-3 decreased migration, invasion, and proliferation of tumor cells in immunosuppressed mice [43, 49, 50]. Another study showed that PSA overexpression in the PC3 cell line led to increased proliferation and migration [24].

2.2 PSA mediates epithelial to mesenchymal transition (EMT), invasion, and metastasis

Primary tumor cells have a tendency to move from their stromal niche and invade neighboring tissues, intravasate local and circulating blood vessels, and finally settle in distant tissues, through the EMT process. They then, with the purpose of penetrating into the interstitial stroma, break down the basement membrane, connective tissue, and the surrounding extracellular matrix (ECM) of nearby normal epithelial cells [51]. In this context, PSA plays a key role as a protease cleaving proteins including collagen type IV [52], fibronectin, and laminin (Fig. 1) [53, 54] or as an activator of other ECM enzymes including

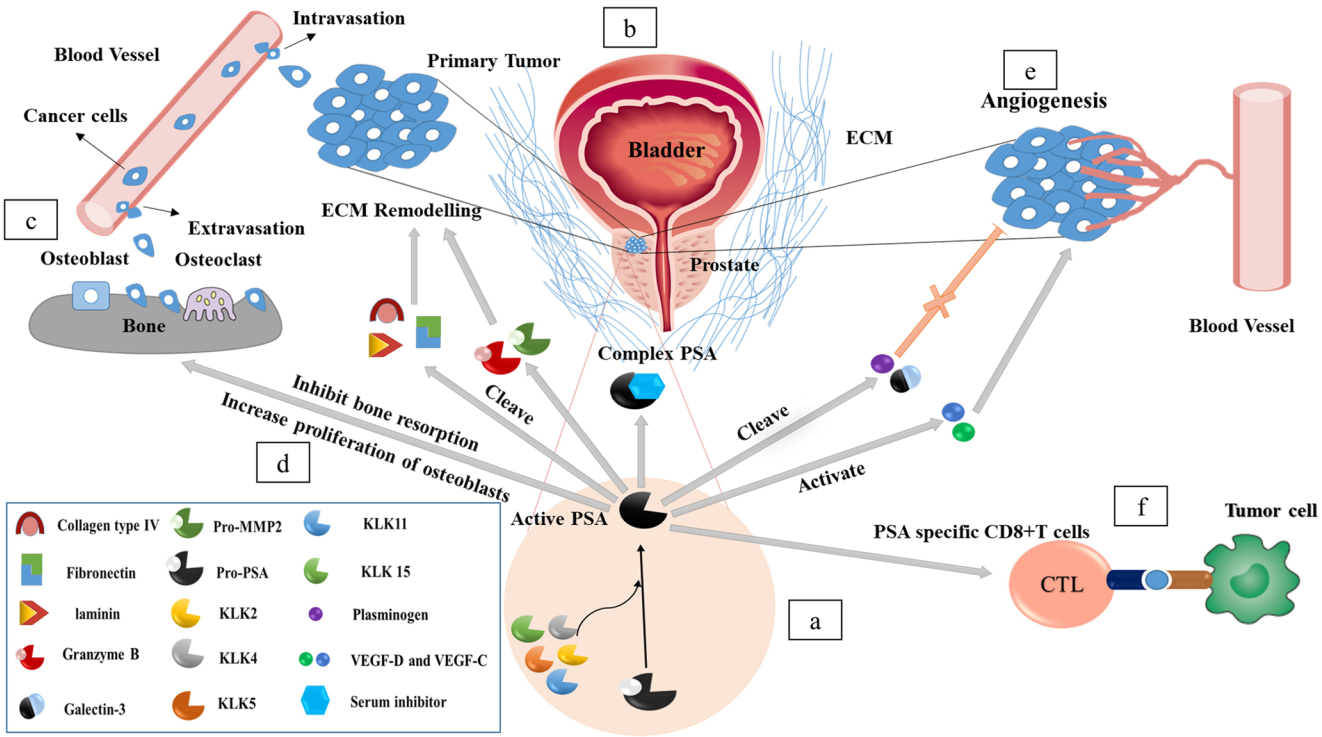

Fig. 1 PSA has a multi-faceted role in prostate cancer progression. a) Other KLKs such as KLK2, KLK4, KLK5, KLK11, and KLK15 can cleave pro-PSA and release active PSA. b) PSA cleaves proteins including collagen type IV, fibronectin, and laminin and is able to activate Granzyme B and pro-MMP2 leading to ECM remodeling. c) PSA has been shown to be involved in the EMT process leading to metastasis of the primary tumor. d) PSA also has a potential to inhibit bone resorption leading to osteogenesis. Exposure of osteoblasts to PSA *in vitro* led to cell proliferation. e) PSA cleaves plasminogen leading to inhibition of new endothelial tube formation and thus angiogenesis inhibition. PSA cleaves galectin-3, a mediator of VEGF and basic bFGF-mediated angiogenic response. In contrast, PSA activates tumor-derived VEGF-C and VEGF-D which may lead to the production of angiogenic and lymphangiogenic tumors. f) PSA-specific epitopes can activate CTLs leading to the killing of the cancer cells, thus has a role to play in the immune response

pro-MMP2 [55] (Fig. 1). PSA overexpressing clones have increased migration towards a membrane barrier and are inclined to grow as single cells with a more fusiform morphology and changed focal adhesions. These cells were also shown to have lower E-cadherin and elevated vimentin expression, all changes typically representative of EMT [56, 57]. Cumming et al, in another study, over-expressed different forms of PSA, and determined a role of PSA and pro-PSA, in invasion and bone metastasis. Pro-PSA-secreting subclones increased invasion and migration. Conversely, the PSA-secreting subclones significantly reduced both invasion and migration. The opposing effects show that different forms of PSA may have different functions in tumorigenesis [58]. Furthermore, PSA stimulates the transitory receptor potential melastatin 8 (TRPM8), which in turn functionalizes the bradykinin 2 receptor signal pathway leading to a decline in migration of PC3 cells [59]. In addition, PSA levels in serum from mice-bearing tumors derived from cells expressing mutant p53 were increased in comparison with PSA levels in mice-bearing tumors derived from control cells. The tumors derived from cells with mutant p53 had increased vascularization and induced lymph node metastases, supporting the idea that p53 mutations lead to increased PSA levels [60]. Suppression of the enzymatic function of endogenous PSA through the use of a PSA-specific antibody in LNCaP cells lowered the invasive behavior of these cancer cells, implying that PSA may significantly contribute to the invasion of LNCaP cells [61]. A recent study presented evidence that the human protease Granzyme B, like other extracellular proteases, is able to cleave an array of ECM proteins, hence, disturbing cell growth, integrity, signaling, and motility (Fig. 1). PSA strongly activated trypsin and Granzyme B which caused ECM impairment leading to *in vitro* death of prostate cancer cells [62].

In prostate cancer, osteoblastic bone metastasis is more prevalent, with reduced function of osteoclasts and enhanced proliferation of osteoblasts [63, 64]. PSA overexpression in the SaoS-2 cell line led to induction of osteoblastic differentiation by upregulating osteoblastic markers such as collagen type I, osteocalcin, alkaline phosphatase, and bone sialoprotein [65]. PSA can hydrolyze parathyroid hormone-related protein (PTHrP) aiding in the conversion from an osteoclastic to an osteoblastic phenotype that has an essential role in osteoblastic bone metastasis [58, 66]. Exposure of osteoblasts to PSA *in vitro* led to cell proliferation and significant upregulation of TGF-β mRNA expression [67]. PSA also has the potential of inhibiting bone resorption leading to osteogenesis [68], suggesting that PSA may lead to the osteoblastic phenotype of bone metastasis which is prevalent in prostate cancer [58].

2.3 The role of PSA in angiogenesis

An extremely important process in tumor metastasis is angiogenesis, during which new blood vessels are created from the existing vasculature [69]. It has been shown that PSA is able to inhibit angiogenesis *in vitro*. PSA can cleave plasminogen into angiostatin-like fragments that inhibit new endothelial tube formation and thus angiogenesis [70]. PSA can cleave galectin-3, that has been reported as a mediator of vascular endothelial growth factor (VEGF)- and basic FGF-mediated angiogenic response [43]. There are reports indicating that suppression of PSA function by Zn^{+2} hindered new blood vessel formation and also migration and invasion of endothelial cells confirming an anti-angiogenic activity of PSA, independent of enzymatic activity [71]. VEGF-C is a tumor spreading factor with an established role in lymphangiogenesis. Tumor-derived VEGF-C and VEGF-D are also activated by PSA, which leads to the production of angiogenic and lymph-angiogenic tumor activity suggesting the function of PSA could depend on the tumor context and bio-availability of these substrates (Fig. 1) [72].

2.4 The effects of PSA on the immune response

Tumor cells evade immune response by the process of the activation of soluble immune suppressive factors. TGF-β and other immunosuppressive agents such as cytokines (i.e., IL-10) are generated by tumor cells which prevent normal cell activities. Interestingly, PSA can contribute to the stimulation of TGFβ, so this suggests an indirect role in tumorigenesis regulation [73, 74]. CD4+ T cells improve the antigen-presenting capacity of dendritic cells (DCs) and support the stimulation of tumor-reactive cytotoxic T lymphocytes (CTLs). More importantly, a number of PSA-specific epitopes have been recognized that can activate CTLs and sequentially lead to the killing of tumor cells targeted by peptide-specific CTLs (Fig. 1) [75].

3 The function of other KLKs in prostate cancer

KLK2, KLK4, KLK5, KLK11, and KLK15 are among the several other KLKs which can activate PSA (Fig. 1) [73, 76, 77]. Similarly, PSA can activate KLK4 and KLK11 [78]. These KLK family members have also been shown to play a role in cell proliferation, extracellular matrix degradation, tumor cell invasion, metastasis, and prostate tumor microenvironment regulation. For example, KLK2 is highly expressed in prostate cancer compared with BPH. KLK2 activates pro-PSA, pro-KLK2, and the zymogen form of uPA. Increased KLK2 expression in prostate cancer tissues leads to activation of uPA and also inactivation of its primary inhibitor, PAI-1 [79]. KLK2 cleaves high molecular weight kininogen to release bradykinin which induces smooth muscle cell contraction, facilitating vasodilation and cancer cell intravasation [80]. KLK4 is highly expressed in the prostate and primarily localized to the nucleus. Furthermore, KLK4 protein is significantly overexpressed in malignant prostate in comparison to normal prostate. Adenovirus-mediated expression of KLK4

intensely induces proliferation of prostate cancer cells, and knockdown of endogenous KLK4 in LNCaP prostate cancer cells leads to cell growth inhibition [81]. Some studies suggest a positive association between *KLK4* mRNA levels on prostate tissue biopsy and higher Gleason Score and disease stage [82]. In the prostate microenvironment, upregulation of KLK4 significantly enhances the activity of androgen receptor (AR) and mammalian target of rapamycin (mTOR) signaling [83]. Transfection of PC3 cells with KLK4 led to increased migration towards Saos2 conditioned medium and better attachment to collagens I and IV [84, 85]. Furthermore, KLK4 and PSA overexpression lead to the loss of E-cadherin and an EMT-like effect in prostate cancer cells [56]. The efficacy of KLK2 and KLK4 activity adjacent to the prostate is explained by formation of complex between serpins and active forms of these KLKs in seminal fluid, as serpins only inhibit active proteases [80, 86].

KLK5 is highly expressed in the normal prostate in comparison to prostate cancer tissue with reports of a negative correlation between the levels of KLK5 and pathologic stage and grade of tumor [87, 88]. No other studies have been reported to date on the biological action of KLK5 in prostate cancer. Similarly, the expression levels of KLK7 are lower in prostate cancer, compared to BPH and normal prostate tissue, with its decreased expression closely associated with higher Gleason Score and higher levels of PSA [89]. On the other hand, another study suggested that by inducing the EMT of prostate cancer cells, KLK7 can promote invasion and metastasis [90]. *KLK10* mRNA was also found to be downregulated in prostate cancer cell lines [91]. KLK10 overexpression in PC3 cells downregulates tumor proliferation, combined with increased apoptosis and decreased glucose metabolism. Notably, a negative feedback was recognized between KLK10 and Bcl-2/HK-2 expression; therefore, KLK10 may act as a tumor suppressor in PC3 cells [91]. *KLK10* DNA methylation is increased in prostate cancer tissue vs. normal which can serve as cancer biomarker [92]. Increased expression of *KLK11* mRNA is associated with less advanced stage and lower Gleason Score in prostate cancer and downregulated in advanced tumors. Thus, *KLK11* has been suggested as a useful negative prognostic biomarker to distinguish aggressive prostate cancer [93] and indolent prostate cancer. KLK12 is overexpressed in prostate in cancer tissues over normal prostate tissue [94]. Also, a shift in subcellular localization of KLK12 was observed indicating a potential role of this enzyme during prostate progression [95]. Increased levels of *KLK14* mRNA and protein have been associated with aggressive tumors [96]. Similarly, *KLK15* and its transcript variants are overexpressed in aggressive prostate cancers which can help to discriminate prostate cancer and BPH [97, 98]. Moreover, rs2659053 and rs35711205, two *KLK15* SNPs, present in the putative promoter region of *KLK15* gene are associated with the risk of prostate cancer [99]. Generally, unveiling the role of the KLK family in prostate cancer progression may pave the way for targeting this protease family in prostate cancer treatment.

4 PSA as imaging tools

Imaging techniques have been developed for selective detection for the presence of malignant regions within the prostate as well as metastases. For example, targeted imaging agents for prostate-specific membrane antigen (PSMA) have been developed [100]. Another approach is targeting PSA, for example, a peptide boronic acid PSA inhibitor with the sequence of Cbz-Ser-Ser-Gln-Nle-(boro)-Leu was identified with an affinity (K_i) for PSA of 25 nM. A bulky metal chelating group, that did not affect the inhibitory ability of the engineered compound, was added to the amino terminal end of the boronic acid-type PSA inhibitor for use in imaging techniques [101]. Similarily, A ^{125}I-labeled monoclonal antibody against fPSA has been developed which was able to selectively target fPSA in LNCaP tumor-bearing mice using digital autoradiography [102]. The rationale in considering unbound PSA for imaging is that abundant amounts of fPSA are present in close proximity to its local site of production, while complex forms of PSA are generally found in the circulation [102]. Likewise, the ^{89}Zr-labeled 5A10 anti-PSA mAb is localized to multiple AR- and PSA-positive prostate cancer cells and can be used for measuring fPSA synthesis in a xenograft model of castration-resistant prostate cancer. It has been shown that ^{89}Zr-5A10 can detect osseous prostate cancer lesions, where bone scans fail to discriminate malignant and non-malignant signals [103]. Aside from KLK3, successful targeting of KLK2 has also been reported for imaging prostate cancer *in vivo*. Specifically, the 111In-labeled 11B6 radiotracer demonstrated specific targeting of KLK2 in both subcutaneous and intra-tibial bone xenografts [104].

5 PSA-based therapeutic approaches

The increased understanding of the role of PSA in the progression of prostate cancer has attracted much attention to targeting PSA in the tumor microenvironment and led to the development of potential PSA activators and anti-metastatic inhibitors. Because PSA displays antiangiogenic activity and, as a result, is able to suppress the expansion of prostatic tumors, there has been an interest in molecules that induce PSA activity and suppress prostate cancer growth. Stimulators are limited in number and include small peptides and molecules that increase the anti-angiogenic properties of PSA and its enzymatic activity [105]. In prostate cancer, PSA is

overexpressed. Thus, downregulation of PSA activity by inhibitor compounds can be an attractive therapeutic approach. PSA activity is regulated by several types of inhibitors such as metallic ions, small molecules, and proteins.

5.1 PSA inhibitors

There is a high concentration of Zn^{2+} in the prostatic fluid that inhibits KLK activity, including that of PSA, both reversibly and allosterically [106]. In prostate cancer, there is a significant reduction in Zn^{2+} concentration caused by the diminished levels of Zn^{2+} transporters. A recent method for consideration in the treatment of prostate malignancy is to restore Zn^{2+} levels in prostate tumors. In murine models, intratumoral injection of Zn^{2+} decelerated tumor growth and improved survival time with no severe toxicity consequences in other organs [107]. Given the function of PSA is tightly regulated by controls such as inhibitors in healthy tissues, deregulation of these controls may lead to their aberrant function in conditions such as prostate cancer.

With respect to small molecule inhibitors, synthesis of a monocyclic β-lactam 2-azetidinone and two triazole family compounds has been utilized to inhibit PSA. But their limitation is the lack of specificity for PSA and binding to other similar proteases [84, 107]. Koistinen et al. screened a collection of 50,000 chemical compounds and recognized benzoxazinone derivatives and triazole compounds that suppress the anti-angiogenic function of PSA [108]. Fukugetin is also a suppressor obtained from a Brazilian plant and is able to suppress PSA with an IC_{50} of 13 ± 0.8 μM [109]. A study on synthetic peptide substrates led to identification of first, peptide aldehyde and then, boronic acid suppressors of PSA, of which the sequence Cbz-Ser-Ser-Lys-Leu-(boro)Leu, had a K_i of 65 nM for PSA [110]. Another example is AhxFSQn(boro)Bpg, a selective peptidyl boronic acid-based PSA inhibitor that contains a bromo propylglycine group. This compound was proven to significantly alter PSA levels in the serum of animal models; nonetheless, it only exerted a small effect on tumor growth [111]. Inhibition of PSA proteolytic activity by small compounds as well as by peptide inhibitors maybe a promising therapeutic strategy (Table 1) [111]. Azapeptides suppress cysteine and serine proteases, and it is thought that azapeptides may suppress expressed human PSA in *Escherichia coli* in salt-rich environments [113]. Another PSA inhibitor is glutamine aldehyde which is not the best inhibitor of PSA, but it is incapable of inhibiting the related chymotrypsin ($K_i > 1000$ μM), hence has some specificity [114]. Serpins are a superfamily of 33–46 kDa macromolecular suppressors of serine and cysteine proteases. Their action leads to irreversible suppression of protease activity, of which the mode of action is based on a change in the conformation inside the proteases, whereby the functional site is deformed for the formation of a stable covalent adduct [115, 116].

Table 1 PSA inhibitors and miRNAs

Type	Inhibitor/activator	Description	Ref
Metallic ions	Zn^{2+}	Intratumoral injection of Zn^{2+} decelerated tumor growth and improved survival time	[106]
Small-molecule inhibitors	Triazole and azetidinone	Identified by the screening a library of 50,000 chemical compounds	[108, 112]
Heterocyclic derivatives	Fukugetin	Extracted from a Brazilian plant	[109]
Peptides inhibitors	With the sequence Cbz-Ser-Ser-Lys-Leu-(boro)Le	Peptidyl boronic acid inhibitor/restricting the progression of subcutaneous prostate cancer xenografts	[110]
	Ahx-FSQn(boro)Bpg	Reduced PSA levels in serum of animal models and reduced tumor growth	[111]
	Azapeptides	These compounds represent the first described inhibitors designed to utilize the substrate binding subsites of PSA	[113]
	Peptides contained a glutamine residue	Inhibit PSA but in capable to inhibit chymotrypsin	[114]
Serpins	α1-antitrypsin, ACT, antithrombin III (ATIII), PCI, Monocyte/neutrophil elastase inhibitor (MNEI)	Serpins are a superfamily of macromolecular inhibitors of serine and cysteine proteases	[115, 116]
miRNA	miR-3162-5p	PSA protein expression was regulated by miR-3162-5p consistent with lower PSA proteolytic activity observed in LNCaP-conditioned media	[117]
	miR-183	Increased mRNA and protein levels of PSA by binding to the 3′ UTR of PSA	[118]
	miR-99 family	The suppression of PSA expression and prostate cancer cell proliferation	[119]

5.2 MicroRNA (miRNA)-mediated targeting of PSA

It is proposed that miRNAs may play a role in the modulation of the expression of the *KLK3* gene. miR-3162-5p regulates expression of the PSA protein in LNCaP cells which is consistent with the lower proteolytic activity of PSA observed in LNCaP-conditioned media. Interestingly, the expression of miR-3162-5p is increased in prostate tumor tissues with higher Gleason grade [117]. The synthesis of PSA and its serum levels are positively affected by miR-183 which may be a factor to be considered during clinical decision making [118]. Besides, PSA is regulated post-transcriptionally by miR-99 family members; transfection of prostate cancer cell lines with miR-99 decreased PSA expression levels and inhibited the growth of cells (Table 1) [119]. Although miRNAs are emerging as therapeutic targets for many diseases especially cancers, further studies are needed before miRNAs can be used in therapeutic strategies to regulate PSA expression and ultimately for prostate cancer treatment.

5.3 PSA-activated prodrugs and gene therapy

Protease-stimulated prodrugs present promise for targeted supply of drugs into a specific tissue. The inactive pro-drug comprises a peptide-conjugated toxic drug molecule. The cleavage of the peptide activates the prodrug in the target tissue via a specific protease releasing the active drug molecule. PSA is mostly active in the prostate cancer microenvironment, but PSA is deactivated in the serum by complex formation with protein inhibitors. Therefore, PSA is becoming an ideal molecular target for prodrug development due to the fact that it is present in its active from in the prostate tissue microenvironment [120]. PSA has unique substrate specificity among serine proteases to hydrolyze amide bonds on peptides containing a glutamine residue. The most effective cleavage occurs between Gln349 and Ser350 in SG I. This characteristic was used to produce pro-drugs for selective therapies in the prostate preneoplastic or tumor microenvironment [121]. A peptide with the amino acid sequence His-Ser-Ser-Lys-Leu-Gln (HSSKLQ) was detected to possess a high level of specificity for PSA. HSSKLQ-Leu- doxorubicin was observed to be potent in killing LNCaP cells. The proteolytic activity of PSA resulted in site-specific release of Leu-Dox at elevated concentrations in the microenvironment encompassing prostate cancer cells and exhibited less cardiac toxicity than Dox in animal models [122].

The peptide prodrug of L12ADT, a modified form of a thapsigargin analog, showed selective toxicity to PSA-generating prostate cancer cells. The development of PSA-producing prostate cancer xenograft tumors was completely inhibited by continual hypodermic prodrug medication in mice while it does not affect PSA non-producing renal carcinoma xenograft tumors (Table 2) [123]. Another example is L-377202, a peptide conjugated to doxorubicin, that could

Table 2 PSA pro-drugs and PSA-based immunotherapy

Drug	Role of the drug	Type	Ref
HSSKLQ-Leu- doxorubicin	Killing LNCaP cells with less cardiac toxicity than Dox in animal models	Pro-drug	[122]
PSA-activated L12ADT	Selective toxicity to PSA-generating prostate cancer cells	Pro-drug	[123]
PSA-activated L-377202	Reduction of tumor growth in xenografts	Pro-drug	[124]
PSA-activated TGX-D1-based	High cellular uptake in LNCaP cells	Pro-drug	[125]
PSA-activated LY294002-based	Reduction of PI3-kinase in C4-2 cells	Pro-drug	[126]
PSA-activated BSD352-based	Increase of pro-apoptotic molecules	Pro-drug	[127]
Emetine prodrug	Emetine prodrug is activated by PSA with an IC50 of 75 nM and 59 nM in LNCaP and CWR22Rv1 respectively	Pro-drug	[128]
PRX302	Selective antitumor impact on PSA-producing murine tumor xenografts	PSA-activated protoxin	[129]
5-fluoro-2′-deoxyuridine anti PSA IgG immunoconjugate	Induced cell death in tumors that express PSA	Immunoconjugate	[130]
PROSTVAC	CD4+ and CD8+ cell activation	Vaccines	[131, 132]
Ad5-PSA/PSCA	The vaccine inhibited the growth of tumors	Vaccines	[133]
pVAX/PSA	DNA-based vaccine can induce PSA-specific immune response in hormone-refractory prostate cancer patients	Vaccines	[134]
Engineered bispecific antibody	Induced anti-tumor response	Antibody	[135]
AR47.47	Anti-PSA IgE antibody causes immune activation both *in vitro* and *in vivo*	Antibody	[136]

selectively kill LNCaP cells as PSA-producing prostate cancer cells and is 15 times as effective as conventional doxorubicin in growth inhibition of human prostate tumor in nude mice [84, 124, 137]. TGX-221 is also a prodrug that inhibits phosphoinositide 3-kinase β (PI3Kβ) with a high potency. Peptide-drug conjugate comprising TGX-D1 (TGX-221 derivatives with additional hydroxyl groups) was found to undergo gradual cleavage by PSA for releasing TGX-D1. A significant elevated cellular uptake was reported for the peptide-drug conjugate in prostate cancer cells in comparison to the original drug [125]. Moreover, a modified form of the quercetin analogue, LY294002 (HO-CH(2)-LY294002), was chemically coupled to the peptide Mu-LEHSSKLQL to generate a prostate cancer-specific PI3K suppressor, wherein the internal sequence HSSKLQ is a substrate for the PSA. This yielded a water-soluble prodrug with PI3K inhibition latency. Upon activation, the L-O-CH(2)-LY294002 is capable of specific inhibition of PI3K in PSA-releasing prostate cancer cells and induction of apoptosis with a strength equivalent to that of the parent LY294002 compound [126].

Another prodrug, BSD352 contains three domains, a protein transduction domain from HIV transactivating regulatory protein (TAT) and then the BH3 domain of the p53 upregulated modulator of apoptosis (TAT-BH3), an anti-vascular endothelial growth factor peptide (SP5.2), and an anti-basic fibroblast growth factor peptide (DG2). The above-described domains in BSD352 were connected to one another by a linking sequence equivalent to a PSA substrate peptide. The BSD352 fusion peptide was subjected to selective cleavage by PSA in PSA-generating LNCaP prostate cancer cells. Additionally, the BH3 domain was detected to stimulate tumor cell apoptosis by raising the expression of Bax, cytochrome C release, and caspase-9 cleavage [127]. As a small molecule protein biosynthesis suppressor, emetine shows toxicity to the entire cell types making it appropriate for thorough eradication of the various cancer cell types. Emetine is a small protein synthesis inhibitor. Its pro-drug activity was shown to be cytotoxic against PSA-generating LNCaP and CWR22Rv1 cells [128]. EMC-Arg-Ser-Ser-Tyr-Tyr-Ser-Arg-DOXO (EMC = ε-maleimidocaproic acid) is a recently developed PSA pro-drug, that could bind to circulating albumin; albumin-bound drug was cleaved rapidly releasing the pro-drug and showed superiority relative to doxorubicin against LNCaP cells in terms of antitumor effectiveness and tolerability [138]. PRX302, modified proaerolysin, is an engineered protoxin. This compound displayed selective antitumor impact on PSA-producing murine tumor xenografts and also damaged PSA-producing prostate cells in monkeys extensively, and exhibited no toxicity in other neighboring tissues [129]. Research has shown that the antibody immunoglobulin G (IgG) against PSA (anti-PSA-IgG) was able to act as a carrier protein for coupled chemotherapeutic drugs (e.g., 5-fluoro-2′-deoxyuridine, and doxorubicin) and that the immunoconjugate was capable of selective delivery to PSA-generating neoplastic prostate [130].

A number of PSA-targeted pro-drugs carrying 5-fluorodeoxyuridine [139], vinblastine [140], and paclitaxel [141] were synthesized following the same method. Macromolecular carriers, including albumin [138] or polymers [N-(2-hydroxypropyl) methacrylamide copolymer] [142], have been utilized with success as pro-drug carriers with the aim of increasing prodrug half-life time and delivery to the target site. Owing to cytotoxicity of drugs on non-targeted tissues, there is a growing attention in improving the tumor selectivity of PSA-activated pro-drugs. Although, there are still challenges that need to be addressed, these targeted pro-drug approaches are very promising in contributing new treatment approaches to prostate cancer.

Since PSA expression is known to be tissue-specific and that PSA is expressed at all stages of prostate cancer, the regulatory site of the *KLK3* gene is a possible contender for a specific gene therapy approach for prostate cancer [143]. Different elements of the *KLK3* gene can be utilized for gene therapy. Such as a proximal promoter that contains a TATA box and two practically critical binding sites for the transcription factor AR, named androgen responsive elements (AREs), and upstream enhancer regions, which impact on the augmentation of prostate-specific gene expression [144]. The *KLK3* promoter in a lentiviral vector maintains its tissue specificity and induces gene expression in prostate cells with acceptable efficiency and specificity. It is worth mentioning that deletion of redundant sequences and engineering of multiple copies of functional regions further induced *KLK3* promoter activity [145].

5.4 PSA-based immunotargeted therapy

Immunotherapeutic strategies are a promising alternative for prostate cancer treatment. Considering the significant role of PSA in immune response, this protease might be considered as a significant candidate for prostate cancer immunotherapy. An example of successful application is PROSTVAC. The mechanism of action of PROSTVAC is supplying antigen-presenting cells (APCs) with PSA epitopes which lead to activation of cytotoxic T cells (CD8+) and T helper (CD4+) that organizes targeting of PSA-expressing prostate cancer cells. Consequently, not only prostate cancer cell proliferation but also tumor growth declined significantly [131, 132, 146]. A Phase III study showed that although PROSTVAC was safe and well tolerated, it had no effect on overall survival so there is no clinical benefit from this treatment [147]. Another example is that of anti-PSA IgG1 and IgE antibodies stimulating T cell activation that targets prostate tumor cells and stimulated a patient's antitumor immunity with cancer vaccines. Adenoviral (Ad) vector-based vaccines using the replication-deficient Ad serotype 5 (Ad5) having the full-length PSA gene inserted have been used for the induction of anti-PSA immune responses. In vaccinated

mice, injection of Ad5-PSA stimulated the cytotoxic CD8+ T cell-intervened suppression of PSA-generating tumors' growth rate [133, 148, 149]. Also, a DNA-based vaccine, the pVAX/PSA, comprising a plasmid encoding PSA, has been assessed for prostate cancer therapy. This vaccine can induce PSA-specific cellular immune responses in patients with hormone-refractory prostate cancer. A phase I clinical trial demonstrated that the vaccine was able to activate immune reactions in patients with hormone-refractory prostate cancer. Improving DNA vaccination in cancer therapy may cause more potent and durable immune responses [134]. PSA-based vaccines are summarized in Table 2.

Bispecific monoclonal antibodies can help in the effective achievement of crosslinks between tumor antigens and the T cell-linked CD3 antigen resulting in a rise of antigen-specific cytotoxicity in T cells. Since PSA is highly organ-specific, a bispecific antibody (BiAb) targeted towards this antigen, and CD3 can provide a means for prostate cancer treatment with a high immune specificity [135]. It was reported that the IgG1 monoclonal antibody AR47.47 in mice, specific for human PSA, could improve antigen demonstration by human dendritic cells and activate both CD4 and CD8 T cells once combined with PSA. Notably, the anti-PSA IgE coupled with PSA activates immunity both *in vitro* and *in vivo,* stimulating a patient's antitumor immunity with cancer vaccines [136] suggesting PSA has potential to be a target for prostate cancer immunotherapy.

6 Conclusion

Beyond its biomarker role, PSA has a key role in cancer signaling pathways such as angiogenesis, invasion and metastasis. Moreover, due to its exclusivity within prostate (cancer)-derived tissues, PSA will continue to be a focus for prostate cancer therapy and diagnostic imaging/theranostics. Combined strategies to specifically suppress PSA activity or growth of aggressive/metastatic cells with PSA-targeted therapies will be an ongoing research focus into the future.

Acknowledgments Afshin Moradi, Queensland University of Technology Postgraduate Research Award; Srilakshmi Srinivasan, Advance QLD ECR Research Fellowship, PCFA John Mills Young Investigator Award; Judith Clements, National Health & Medical Research Council of Australia, Cancer Council Queensland, Prostate Cancer Foundation of Australia project grants; Jyotsna Batra, National Health & Medical Research Council (NHMRC) Career Development Fellowship, Cancer Council of Queensland, Prostate Cancer Foundation of Australia project grants and TRI Spore grant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Yousef, G. M., Kopolovic, A. D., Elliott, M. B., & Diamandis, E. P. (2003). Genomic overview of serine proteases. *Biochemical and Biophysical Research Communications, 305*(1), 28–36. https://doi.org/10.1016/s0006-291x(03)00638-7.
2. Lundwall, A., Band, V., Blaber, M., Clements, J. A., Courty, Y., Diamandis, E. P., et al. (2006). A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. *Biological Chemistry, 387*(6), 637–641. https://doi.org/10.1515/BC.2006.082.
3. Clements, J. A., Willemsen, N. M., Myers, S. A., & Dong, Y. (2004). The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. *Critical Reviews in Clinical Laboratory Sciences, 41*(3), 265–312. https://doi.org/10.1080/10408360490471931.
4. Lundwall, A., & Lilja, H. (1987). Molecular cloning of human prostate specific antigen cDNA. *FEBS Letters, 214*(2), 317–322. https://doi.org/10.1016/0014-5793(87)80078-9.
5. Paju, A., Bjartell, A., Zhang, W. M., Nordling, S., Borgstrom, A., Hansson, J., et al. (2000). Expression and characterization of trypsinogen produced in the human male genital tract. *The American Journal of Pathology, 157*(6), 2011–2021. https://doi.org/10.1016/S0002-9440(10)64840-7.
6. Carvalho, A. L., Sanz, L., Barettino, D., Romero, A., Calvete, J. J., & Romao, M. J. (2002). Crystal structure of a prostate kallikrein isolated from stallion seminal plasma: a homologue of human PSA. *Journal of Molecular Biology, 322*(2), 325–337.
7. Skala, W., Utzschneider, D. T., Magdolen, V., Debela, M., Guo, S., Craik, C. S., et al. (2014). Structure-function analyses of human kallikrein-related peptidase 2 establish the 99-loop as master regulator of activity. *The Journal of Biological Chemistry, 289*(49), 34267–34283. https://doi.org/10.1074/jbc.M114.598201.
8. Denmeade, S. R., Sokoll, L. J., Chan, D. W., Khan, S. R., & Isaacs, J. T. (2001). Concentration of enzymatically active prostate-specific antigen (PSA) in the extracellular fluid of primary human prostate cancers and human prostate cancer xenograft models. *Prostate, 48*(1), 1–6. https://doi.org/10.1002/pros.1075.
9. Lilja, H. (1985). A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *The Journal of Clinical Investigation, 76*(5), 1899–1903. https://doi.org/10.1172/JCI112185.
10. Lilja, H., & Laurell, C. B. (1985). The predominant protein in human seminal coagulate. *Scandinavian Journal of Clinical and Laboratory Investigation, 45*(7), 635–641.
11. Stenman, U. H., Leinonen, J., Zhang, W. M., Finne, P., & Wu, P. (1998). The clinical importance of free prostate-specific antigen (PSA). *Current Opinion in Urology, 8*(5), 393–399.
12. Lilja, H., Ulmert, D., & Vickers, A. J. (2008). Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. *Nature Reviews. Cancer, 8*(4), 268–278. https://doi.org/10.1038/nrc2351.
13. Zhu, L., Leinonen, J., Zhang, W. M., Finne, P., & Stenman, U. H. (2003). Dual-label immunoassay for simultaneous measurement of prostate-specific antigen (PSA)-alpha1-antichymotrypsin complex together with free or total PSA. *Clinical Chemistry, 49*(1), 97–103.
14. Stenman, U. H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K., & Alfthan, O. (1991). A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Research, 51*(1), 222–226.
15. Ito, K., Yamamoto, T., Ohi, M., Kurokawa, K., Suzuki, K., & Yamanaka, H. (2003). Free/total PSA ratio is a powerful predictor

of future prostate cancer morbidity in men with initial PSA levels of 4.1 to 10.0 ng/ml. *Urology, 61*(4), 760–764. https://doi.org/10.1016/S0090-4295(02)02427-5.
16. Bangma, C. H., Wildhagen, M. F., Yurdakul, G., Schroder, F. H., & Blijenberg, B. G. (2004). The value of (-7, -5) pro-prostate-specific antigen and human kallikrein-2 as serum markers for grading prostate cancer. *BJU International, 93*(6), 720–724. https://doi.org/10.1111/j.1464-410X.2003.04733.x.
17. Mikolajczyk, S. D., Catalona, W. J., Evans, C. L., Linton, H. J., Millar, L. S., Marker, K. M., et al. (2004). Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. *Clinical Chemistry, 50*(6), 1017–1025. https://doi.org/10.1373/clinchem.2003.026823.
18. Kornberg, Z., Cooperberg, M. R., Spratt, D. E., & Feng, F. Y. (2018). Genomic biomarkers in prostate cancer. *Translational Andrology and Urology, 7*(3), 459–471. https://doi.org/10.21037/tau.2018.06.02.
19. Morgan, T., Palapattu, G., & Wei, J. (2015). Screening for prostate cancer-beyond total PSA, utilization of novel biomarkers. *Current Urology Reports, 16*(9), 63. https://doi.org/10.1007/s11934-015-0537-3.
20. Saini, S. (2016). PSA and beyond: alternative prostate cancer biomarkers. *Cellular Oncology (Dordrecht), 39*(2), 97–106. https://doi.org/10.1007/s13402-016-0268-6.
21. Punnen, S., Pavan, N., & Parekh, D. J. (2015). Finding the wolf in sheep's clothing: the 4K score is a novel blood test that can accurately identify the risk of aggressive prostate cancer. *Revista de Urología, 17*(1), 3–13.
22. Whitbread, A. K., Veveris-Lowe, T. L., Dong, Y., Tan, O. L., Gardiner, R., Samaratunga, H. M., et al. (2010). Expression of PSA-RP2, an alternatively spliced variant from the PSA gene, is increased in prostate cancer tissues but the protein is not secreted from prostate cancer cells. *Biological Chemistry, 391*(4), 461–466. https://doi.org/10.1515/BC.2010.043.
23. Farashi, S., Kryza, T., Clements, J., & Batra, J. (2019). Post-GWAS in prostate cancer: from genetic association to biological contribution. *Nature Reviews. Cancer, 19*(1), 46–59. https://doi.org/10.1038/s41568-018-0087-3.
24. Srinivasan, S., Stephens, C., Wilson, E., Panchadsaram, J., DeVoss, K., Koistinen, H., et al. (2019). Prostate cancer risk-associated single-nucleotide polymorphism affects prostate-specific antigen glycosylation and its function. *Clinical Chemistry, 65*(1), e1–e9. https://doi.org/10.1373/clinchem.2018.295790.
25. Tkac, J., Gajdosova, V., Hroncekova, S., Bertok, T., Hires, M., Jane, E., et al. (2019). Prostate-specific antigen glycoprofiling as diagnostic and prognostic biomarker of prostate cancer. *Interface Focus, 9*(2), 20180077. https://doi.org/10.1098/rsfs.2018.0077.
26. Schroder, F. H., Roobol, M. J., van der Kwast, T. H., Kranse, R., & Bangma, C. H. (2006). Does PSA velocity predict prostate cancer in pre-screened populations? *European Urology, 49*(3), 460–465; discussion 465. https://doi.org/10.1016/j.eururo.2005.12.026.
27. Vickers, A. J., Wolters, T., Savage, C. J., Cronin, A. M., O'Brien, M. F., Pettersson, K., et al. (2009). Prostate-specific antigen velocity for early detection of prostate cancer: result from a large, representative, population-based cohort. *European Urology, 56*(5), 753–760. https://doi.org/10.1016/j.eururo.2009.07.047.
28. Loeb, S., Roehl, K. A., Nadler, R. B., Yu, X., & Catalona, W. J. (2007). Prostate specific antigen velocity in men with total prostate specific antigen less than 4 ng/ml. *The Journal of Urology, 178*(6), 2348–2352; discussion 2352-2343. https://doi.org/10.1016/j.juro.2007.08.016.
29. Freedland, S. J. (2005). Can we preoperatively identify men who are at high risk for aggressive prostate cancer? *Nature Clinical Practice. Urology, 2*(12), 584–585. https://doi.org/10.1038/ncpuro0355.
30. Gronberg, H., Adolfsson, J., Aly, M., Nordstrom, T., Wiklund, P., Brandberg, Y., et al. (2015). Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population-based diagnostic study. *The Lancet Oncology, 16*(16), 1667–1676. https://doi.org/10.1016/S1470-2045(15)00361-7.
31. Sooriakumaran, P. (2011). Smarter screening for prostate cancer: for the few, not the many? A stratified approach based on baseline risk. *Expert Review of Anticancer Therapy, 11*(2), 169–172. https://doi.org/10.1586/era.10.233.
32. Tan, G. H., Nason, G., Ajib, K., Woon, D. T. S., Herrera-Caceres, J., Alhunaidi, O., et al. (2019). Smarter screening for prostate cancer. *World Journal of Urology, 37*(6), 991–999. https://doi.org/10.1007/s00345-019-02719-5.
33. Rehault, S., Monget, P., Mazerbourg, S., Tremblay, R., Gutman, N., Gauthier, F., et al. (2001). Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3. *European Journal of Biochemistry, 268*(10), 2960–2968. https://doi.org/10.1046/j.1432-1327.2001.02185.x.
34. Bindukumar, B., Schwartz, S. A., Nair, M. P., Aalinkeel, R., Kawinski, E., & Chadha, K. C. (2005). Prostate-specific antigen modulates the expression of genes involved in prostate tumor growth. *Neoplasia, 7*(5), 544.
35. Niu, Y., Yeh, S., Miyamoto, H., Li, G., Altuwaijri, S., Yuan, J., et al. (2008). Tissue prostate-specific antigen facilitates refractory prostate tumor progression via enhancing ARA70-regulated androgen receptor transactivation. *Cancer Research, 68*(17), 7110–7119. https://doi.org/10.1158/0008-5472.CAN-07-6507.
36. Humphrey, P. A. (2007). Diagnosis of adenocarcinoma in prostate needle biopsy tissue. *Journal of Clinical Pathology, 60*(1), 35–42. https://doi.org/10.1136/jcp.2005.036442.
37. Li, J., & Wang, Z. (2016). The pathology of unusual subtypes of prostate cancer. *Chinese Journal of Cancer Research, 28*(1), 130–143. https://doi.org/10.3978/j.issn.1000-9604.2016.01.06.
38. Datta, K., Muders, M., Zhang, H., & Tindall, D. J. (2010). Mechanism of lymph node metastasis in prostate cancer. *Future Oncology, 6*(5), 823–836. https://doi.org/10.2217/fon.10.33.
39. Chen, C., He, H., Yu, Z., Qiu, Y., & Wang, X. (2016). Renal and retroperitoneal metastasis from prostate adenocarcinoma: a case report. *World Journal of Surgical Oncology, 14*, 74. https://doi.org/10.1186/s12957-016-0834-4.
40. Sepulveda, L., Gorgal, T., Pires, V., & Rodrigues, F. (2015). Prostate cancer metastatic to the cervical lymph nodes. *Case Reports in Urology, 2015*, 263978. https://doi.org/10.1155/2015/263978.
41. Messiou, C., Cook, G., & deSouza, N. M. (2009). Imaging metastatic bone disease from carcinoma of the prostate. *British Journal of Cancer, 101*(8), 1225–1232. https://doi.org/10.1038/sj.bjc.6605334.
42. Batson, O. V. (1940). The function of the vertebral veins and their role in the spread of metastases. *Annals of Surgery, 112*(1), 138–149. https://doi.org/10.1097/00000658-194007000-00016.
43. Markowska, A. I., Liu, F. T., & Panjwani, N. (2010). Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response. *The Journal of Experimental Medicine, 207*(9), 1981–1993. https://doi.org/10.1084/jem.20090121.
44. Williams, S. A., Jelinek, C. A., Litvinov, I., Cotter, R. J., Isaacs, J. T., & Denmeade, S. R. (2011). Enzymatically active prostate-specific antigen promotes growth of human prostate cancers. *Prostate, 71*(15), 1595–1607. https://doi.org/10.1002/pros.21375.
45. Nabatchian, F., Moradi, A., Aghaei, M., Ghanadian, M., Jafari, S. M., & Tabesh, S. (2017). New 6(17)-epoxylathyrane diterpene: aellinane from Euphorbia aellenii induces apoptosis via mitochondrial pathway in ovarian cancer cell line. *Toxicology Mechanisms and Methods, 27*(8), 622–630. https://doi.org/10.1080/15376516.2017.1347735.

46. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013.
47. Filippou, P. S., Karagiannis, G. S., Musrap, N., & Diamandis, E. P. (2016). Kallikrein-related peptidases (KLKs) and the hallmarks of cancer. *Critical Reviews in Clinical Laboratory Sciences, 53*(4), 277–291. https://doi.org/10.3109/10408363.2016.1154643.
48. Bindukumar, B., Schwartz, S. A., Nair, M. P., Aalinkeel, R., Kawinski, E., & Chadha, K. C. (2005). Prostate-specific antigen modulates the expression of genes involved in prostate tumor growth. *Neoplasia, 7*(3), 241–252. https://doi.org/10.1593/neo.04529.
49. Saraswati, S., Block, A. S., Davidson, M. K., Rank, R. G., Mahadevan, M., & Diekman, A. B. (2011). Galectin-3 is a substrate for prostate specific antigen (PSA) in human seminal plasma. *Prostate, 71*(2), 197–208. https://doi.org/10.1002/pros.21236.
50. Wang, Y., Nangia-Makker, P., Tait, L., Balan, V., Hogan, V., Pienta, K. J., et al. (2009). Regulation of prostate cancer progression by galectin-3. *The American Journal of Pathology, 174*(4), 1515–1523. https://doi.org/10.2353/ajpath.2009.080816.
51. Emami, N., & Diamandis, E. P. (2007). New insights into the functional mechanisms and clinical applications of the kallikrein-related peptidase family. *Molecular Oncology, 1*(3), 269–287. https://doi.org/10.1016/j.molonc.2007.09.003.
52. Pezzato, E., Sartor, L., Dell'Aica, I., Dittadi, R., Gion, M., Belluco, C., et al. (2004). Prostate carcinoma and green tea: PSA-triggered basement membrane degradation and MMP-2 activation are inhibited by (-)epigallocatechin-3-gallate. *International Journal of Cancer, 112*(5), 787–792. https://doi.org/10.1002/ijc.20460.
53. Webber, M. M., Waghray, A., & Bello, D. (1995). Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. *Clinical Cancer Research, 1*(10), 1089–1094.
54. Lilja, H., Oldbring, J., Rannevik, G., & Laurell, C. B. (1987). Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen. *The Journal of Clinical Investigation, 80*(2), 281–285. https://doi.org/10.1172/JCI113070.
55. Lawrence, M. G., Lai, J., & Clements, J. A. (2010). Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. *Endocrine Reviews, 31*(4), 407–446. https://doi.org/10.1210/er.2009-0034.
56. Veveris-Lowe, T. L., Lawrence, M. G., Collard, R. L., Bui, L., Herington, A. C., Nicol, D. L., et al. (2005). Kallikrein 4 (hK4) and prostate-specific antigen (PSA) are associated with the loss of E-cadherin and an epithelial-mesenchymal transition (EMT)-like effect in prostate cancer cells. *Endocrine-Related Cancer, 12*(3), 631–643. https://doi.org/10.1677/erc.1.00958.
57. Whitbread, A. K., Veveris-Lowe, T. L., Lawrence, M. G., Nicol, D. L., & Clements, J. A. (2006). The role of kallikrein-related peptidases in prostate cancer: potential involvement in an epithelial to mesenchymal transition. *Biological Chemistry, 387*(6), 707–714. https://doi.org/10.1515/BC.2006.089.
58. Cumming, A. P., Hopmans, S. N., Vukmirovic-Popovic, S., & Duivenvoorden, W. C. (2011). PSA affects prostate cancer cell invasion in vitro and induces an osteoblastic phenotype in bone in vivo. *Prostate Cancer and Prostatic Diseases, 14*(4), 286–294. https://doi.org/10.1038/pcan.2011.34.
59. Gkika, D., Flourakis, M., Lemonnier, L., & Prevarskaya, N. (2010). PSA reduces prostate cancer cell motility by stimulating TRPM8 activity and plasma membrane expression. *Oncogene, 29*(32), 4611–4616. https://doi.org/10.1038/onc.2010.210.
60. Downing, S., Bumak, C., Nixdorf, S., Ow, K., Russell, P., & Jackson, P. (2003). Elevated levels of prostate-specific antigen (PSA) in prostate cancer cells expressing mutant p53 is associated with tumor metastasis. *Molecular Carcinogenesis, 38*(3), 130–140. https://doi.org/10.1002/mc.10154.
61. Ishii, K., Otsuka, T., Iguchi, K., Usui, S., Yamamoto, H., Sugimura, Y., et al. (2004). Evidence that the prostate-specific antigen (PSA)/Zn2+ axis may play a role in human prostate cancer cell invasion. *Cancer Letters, 207*(1), 79–87. https://doi.org/10.1016/j.canlet.2003.09.029.
62. Rogers, O. C., Anthony, L., Rosen, D. M., Brennen, W. N., & Denmeade, S. R. (2018). PSA-selective activation of cytotoxic human serine proteases within the tumor microenvironment as a therapeutic strategy to target prostate cancer. *Oncotarget, 9*(32), 22436–22450. https://doi.org/10.18632/oncotarget.25091.
63. Goya, M., Ishii, G., Miyamoto, S., Hasebe, T., Nagai, K., Yonou, H., et al. (2006). Prostate-specific antigen induces apoptosis of osteoclast precursors: potential role in osteoblastic bone metastases of prostate cancer. *Prostate, 66*(15), 1573–1584. https://doi.org/10.1002/pros.20375.
64. Sohara, Y., Shimada, H., & DeClerck, Y. A. (2005). Mechanisms of bone invasion and metastasis in human neuroblastoma. *Cancer Letters, 228*(1-2), 203–209. https://doi.org/10.1016/j.canlet.2005.01.059.
65. Nadiminty, N., Lou, W., Lee, S. O., Mehraein-Ghomi, F., Kirk, J. S., Conroy, J. M., et al. (2006). Prostate-specific antigen modulates genes involved in bone remodeling and induces osteoblast differentiation of human osteosarcoma cell line SaOS-2. *Clinical Cancer Research, 12*(5), 1420–1430. https://doi.org/10.1158/1078-0432.CCR-05-1849.
66. Cramer, S. D., Chen, Z., & Peehl, D. M. (1996). Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. *The Journal of Urology, 156*(2 Pt 1), 526–531. https://doi.org/10.1097/00005392-199608000-00076.
67. Yonou, H., Aoyagi, Y., Kanomata, N., Kamijo, T., Oda, T., Yokose, T., et al. (2001). Prostate-specific antigen induces osteoplastic changes by an autonomous mechanism. *Biochemical and Biophysical Research Communications, 289*(5), 1082–1087. https://doi.org/10.1006/bbrc.2001.6129.
68. D'Oronzo, S., Coleman, R., Brown, J., & Silvestris, F. (2019). Metastatic bone disease: pathogenesis and therapeutic options: up-date on bone metastasis management. *Journal of Bone Oncology, 15*, 004–004. https://doi.org/10.1016/j.jbo.2018.10.004.
69. Fidler, I. J. (2000). Angiogenesis and cancer metastasis. *Cancer Journal, 6*(*Suppl 2*), S134–S141.
70. Heidtmann, H. H., Nettelbeck, D. M., Mingels, A., Jager, R., Welker, H. G., & Kontermann, R. E. (1999). Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. *British Journal of Cancer, 81*(8), 1269–1273. https://doi.org/10.1038/sj.bjc.6692167.
71. Chadha, K. C., Nair, B. B., Chakravarthi, S., Zhou, R., Godoy, A., Mohler, J. L., et al. (2011). Enzymatic activity of free-prostate-specific antigen (f-PSA) is not required for some of its physiological activities. *Prostate, 71*(15), 1680–1690. https://doi.org/10.1002/pros.21385.
72. Jha, S. K., Rauniyar, K., Chronowska, E., Mattonet, K., Maina, E. W., Koistinen, H., et al. (2019). KLK3/PSA and cathepsin D activate VEGF-C and VEGF-D. *Elife, 8*. https://doi.org/10.7554/eLife.44478.
73. Pampalakis, G., & Sotiropoulou, G. (2007). Tissue kallikrein proteolytic cascade pathways in normal physiology and cancer. *Biochimica et Biophysica Acta, 1776*(1), 22–31. https://doi.org/10.1016/j.bbcan.2007.06.001.
74. Gorelik, L., & Flavell, R. A. (2001). Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nature Medicine, 7*(10), 1118–1122. https://doi.org/10.1038/nm1001-1118.

75. Kiessling, A., Wehner, R., Fussel, S., Bachmann, M., Wirth, M. P., & Schmitz, M. (2012). Tumor-associated antigens for specific immunotherapy of prostate cancer. *Cancers (Basel), 4*(1), 193–217. https://doi.org/10.3390/cancers4010193.
76. Guo, S., Briza, P., Magdolen, V., Brandstetter, H., & Goettig, P. (2018). Activation and activity of glycosylated KLKs 3, 4 and 11. *Biological Chemistry, 399*(9), 1009–1022. https://doi.org/10.1515/hsz-2018-0148.
77. Yoon, H., Blaber, S. I., Evans, D. M., Trim, J., Juliano, M. A., Scarisbrick, I. A., et al. (2008). Activation profiles of human kallikrein-related peptidases by proteases of the thrombostasis axis. *Protein Science, 17*(11), 1998–2007. https://doi.org/10.1110/ps.036715.108.
78. Yoon, H., Laxmikanthan, G., Lee, J., Blaber, S. I., Rodriguez, A., Kogot, J. M., et al. (2007). Activation profiles and regulatory cascades of the human kallikrein-related peptidases. *The Journal of Biological Chemistry, 282*(44), 31852–31864. https://doi.org/10.1074/jbc.M705190200.
79. Mikolajczyk, S. D., Millar, L. S., Kumar, A., & Saedi, M. S. (1999). Prostatic human kallikrein 2 inactivates and complexes with plasminogen activator inhibitor-1. *International Journal of Cancer, 81*(3), 438–442.
80. Charlesworth, M. C., Young, C. Y., Miller, V. M., & Tindall, D. J. (1999). Kininogenase activity of prostate-derived human glandular kallikrein (hK2) purified from seminal fluid. *Journal of Andrology, 20*(2), 220–229.
81. Klokk, T. I., Kilander, A., Xi, Z., Waehre, H., Risberg, B., Danielsen, H. E., et al. (2007). Kallikrein 4 is a proliferative factor that is overexpressed in prostate cancer. *Cancer Research, 67*(11), 5221–5230. https://doi.org/10.1158/0008-5472.CAN-06-4728.
82. Avgeris, M., Mavridis, K., & Scorilas, A. (2010). Kallikrein-related peptidase genes as promising biomarkers for prognosis and monitoring of human malignancies. *Biological Chemistry, 391*(5), 505–511. https://doi.org/10.1515/BC.2010.056.
83. Jin, Y., Qu, S., Tesikova, M., Wang, L., Kristian, A., Maelandsmo, G. M., et al. (2013). Molecular circuit involving KLK4 integrates androgen and mTOR signaling in prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America, 110*(28), E2572–E2581. https://doi.org/10.1073/pnas.1304318110.
84. Avgeris, M., & Scorilas, A. (2016). Kallikrein-related peptidases (KLKs) as emerging therapeutic targets: focus on prostate cancer and skin pathologies. *Expert Opinion on Therapeutic Targets, 20*(7), 801–818. https://doi.org/10.1517/14728222.2016.1147560.
85. Gao, J., Collard, R. L., Bui, L., Herington, A. C., Nicol, D. L., & Clements, J. A. (2007). Kallikrein 4 is a potential mediator of cellular interactions between cancer cells and osteoblasts in metastatic prostate cancer. *Prostate, 67*(4), 348–360. https://doi.org/10.1002/pros.20465.
86. Obiezu, C. V., Shan, S. J., Soosaipillai, A., Luo, L. Y., Grass, L., Sotiropoulou, G., et al. (2005). Human kallikrein 4: quantitative study in tissues and evidence for its secretion into biological fluids. *Clinical Chemistry, 51*(8), 1432–1442. https://doi.org/10.1373/clinchem.2005.049692.
87. Yousef, G. M., Scorilas, A., Chang, A., Rendl, L., Diamandis, M., Jung, K., et al. (2002). Down-regulation of the human kallikrein gene 5 (KLK5) in prostate cancer tissues. *Prostate, 51*(2), 126–132. https://doi.org/10.1002/pros.10067.
88. Kurlender, L., Yousef, G. M., Memari, N., Robb, J. D., Michael, I. P., Borgono, C., et al. (2004). Differential expression of a human kallikrein 5 (KLK5) splice variant in ovarian and prostate cancer. *Tumour Biology, 25*(3), 149–156. https://doi.org/10.1159/000079147.
89. Zhang, C. Y., Zhu, Y., Rui, W. B., Dai, J., & Shen, Z. J. (2015). Expression of kallikrein-related peptidase 7 is decreased in prostate cancer. *Asian Journal of Andrology, 17*(1), 106–110. https://doi.org/10.4103/1008-682X.137613.
90. Mo, L., Zhang, J., Shi, J., Xuan, Q., Yang, X., Qin, M., et al. (2010). Human kallikrein 7 induces epithelial-mesenchymal transition-like changes in prostate carcinoma cells: a role in prostate cancer invasion and progression. *Anticancer Research, 30*(9), 3413–3420.
91. Hu, J., Lei, H., Fei, X., Liang, S., Xu, H., Qin, D., et al. (2015). NES1/KLK10 gene represses proliferation, enhances apoptosis and down-regulates glucose metabolism of PC3 prostate cancer cells. *Scientific Reports, 5*, 17426. https://doi.org/10.1038/srep17426.
92. Olkhov-Mitsel, E., Van der Kwast, T., Kron, K. J., Ozcelik, H., Briollais, L., Massey, C., et al. (2012). Quantitative DNA methylation analysis of genes coding for kallikrein-related peptidases 6 and 10 as biomarkers for prostate cancer. *Epigenetics, 7*(9), 1037–1045. https://doi.org/10.4161/epi.21524.
93. Stavropoulou, P., Gregorakis, A. K., Plebani, M., & Scorilas, A. (2005). Expression analysis and prognostic significance of human kallikrein 11 in prostate cancer. *Clinica Chimica Acta, 357*(2), 190–195. https://doi.org/10.1016/j.cccn.2005.03.026.
94. Lose, F., Batra, J., O'Mara, T., Fahey, P., Marquart, L., Eeles, R. A., et al. (2013). Common variation in Kallikrein genes KLK5, KLK6, KLK12, and KLK13 and risk of prostate cancer and tumor aggressiveness. *Urologic Oncology, 31*(5), 635–643. https://doi.org/10.1016/j.urolonc.2011.05.011.
95. Memari, N., Diamandis, E. P., Earle, T., Campbell, A., Van Dekken, H., & Van der Kwast, T. H. (2007). Human kallikrein-related peptidase 12: antibody generation and immunohistochemical localization in prostatic tissues. *Prostate, 67*(13), 1465–1474. https://doi.org/10.1002/pros.20596.
96. Rabien, A., Fritzsche, F., Jung, M., Diamandis, E. P., Loening, S. A., Dietel, M., et al. (2008). High expression of KLK14 in prostatic adenocarcinoma is associated with elevated risk of prostate-specific antigen relapse. *Tumour Biology, 29*(1), 1–8. https://doi.org/10.1159/000132565.
97. Mavridis, K., Avgeris, M., Koutalellis, G., Stravodimos, K., & Scorilas, A. (2010). Expression analysis and study of the KLK15 mRNA splice variants in prostate cancer and benign prostatic hyperplasia. *Cancer Science, 101*(3), 693–699. https://doi.org/10.1111/j.1349-7006.2009.01450.x.
98. Mavridis, K., Stravodimos, K., & Scorilas, A. (2013). Quantified KLK15 gene expression levels discriminate prostate cancer from benign tumors and constitute a novel independent predictor of disease progression. *Prostate, 73*(11), 1191–1201. https://doi.org/10.1002/pros.22667.
99. Batra, J., Lose, F., O'Mara, T., Marquart, L., Stephens, C., Alexander, K., et al. (2011). Association between Prostinogen (KLK15) genetic variants and prostate cancer risk and aggressiveness in Australia and a meta-analysis of GWAS data. *PLoS One, 6*(11), e26527. https://doi.org/10.1371/journal.pone.0026527.
100. Schmitthenner, H. F., Dobson, D. E., Jones, K. G., Akporji, N., Soika, D. Q. M., Nastiuk, K. L., et al. (2019). Modular synthesis of DOTA-metal based PSMA targeted imaging agents for MRI and PET of prostate cancer. *Chemistry*. https://doi.org/10.1002/chem.201903390.
101. LeBeau, A. M., Banerjee, S. R., Pomper, M. G., Mease, R. C., & Denmeade, S. R. (2009). Optimization of peptide-based inhibitors of prostate-specific antigen (PSA) as targeted imaging agents for prostate cancer. *Bioorganic & Medicinal Chemistry, 17*(14), 4888–4893. https://doi.org/10.1016/j.bmc.2009.06.012.
102. Evans-Axelsson, S., Ulmert, D., Orbom, A., Peterson, P., Nilsson, O., Wennerberg, J., et al. (2012). Targeting free prostate-specific antigen for in vivo imaging of prostate cancer using a monoclonal antibody specific for unique epitopes accessible on free prostate-specific antigen alone. *Cancer Biotherapy &*

Radiopharmaceuticals, 27(4), 243–251. https://doi.org/10.1089/cbr.2011.1088.

103. Ulmert, D., Evans, M. J., Holland, J. P., Rice, S. L., Wongvipat, J., Pettersson, K., et al. (2012). Imaging androgen receptor signaling with a radiotracer targeting free prostate-specific antigen. *Cancer Discovery, 2*(4), 320–327. https://doi.org/10.1158/2159-8290.CD-11-0316.
104. Timmermand, O. V., Ulmert, D., Evans-Axelsson, S., Pettersson, K., Bjartell, A., Lilja, H., et al. (2014). Preclinical imaging of kallikrein-related peptidase 2 (hK2) in prostate cancer with a (111)In-radiolabelled monoclonal antibody, 11B6. *EJNMMI Research, 4*(1), 51. https://doi.org/10.1186/s13550-014-0051-5.
105. Koistinen, H., Wallen, E., Ylikangas, H., Meinander, K., Lahtela-Kakkonen, M., Narvanen, A., et al. (2016). Development of molecules stimulating the activity of KLK3 - an update. *Biological Chemistry, 397*(12), 1229–1235. https://doi.org/10.1515/hsz-2016-0189.
106. Costello, L. C., & Franklin, R. B. (2011). Zinc is decreased in prostate cancer: an established relationship of prostate cancer! *Journal of Biological Inorganic Chemistry, 16*(1), 3–8. https://doi.org/10.1007/s00775-010-0736-9.
107. Mavridis, K., Avgeris, M., & Scorilas, A. (2014). Targeting kallikrein-related peptidases in prostate cancer. *Expert Opinion on Therapeutic Targets, 18*(4), 365–383. https://doi.org/10.1517/14728222.2014.880693.
108. Koistinen, H., Wohlfahrt, G., Mattsson, J. M., Wu, P., Lahdenpera, J., & Stenman, U. H. (2008). Novel small molecule inhibitors for prostate-specific antigen. *Prostate, 68*(11), 1143–1151. https://doi.org/10.1002/pros.20773.
109. Santos, J. A., Kondo, M. Y., Freitas, R. F., dos Santos, M. H., Ramalho, T. C., Assis, D. M., et al. (2016). The natural flavone fukugetin as a mixed-type inhibitor for human tissue kallikreins. *Bioorganic & Medicinal Chemistry Letters, 26*(5), 1485–1489. https://doi.org/10.1016/j.bmcl.2016.01.039.
110. LeBeau, A. M., Singh, P., Isaacs, J. T., & Denmeade, S. R. (2008). Potent and selective peptidyl boronic acid inhibitors of the serine protease prostate-specific antigen. *Chemistry & Biology, 15*(7), 665–674. https://doi.org/10.1016/j.chembiol.2008.05.020.
111. Kostova, M. B., Rosen, D. M., Chen, Y., Mease, R. C., & Denmeade, S. R. (2013). Structural optimization, biological evaluation, and application of peptidomimetic prostate specific antigen inhibitors. *Journal of Medicinal Chemistry, 56*(11), 4224–4235. https://doi.org/10.1021/jm301718c.
112. Adlington, R. M., Baldwin, J. E., Becker, G. W., Chen, B., Cheng, L., Cooper, S. L., et al. (2001). Design, synthesis, and proposed active site binding analysis of monocyclic 2-azetidinone inhibitors of prostate specific antigen. *Journal of Medicinal Chemistry, 44*(10), 1491–1508. https://doi.org/10.1021/jm000145g.
113. Huang, X., Knoell, C. T., Frey, G., Hazegh-Azam, M., Tashjian Jr., A. H., Hedstrom, L., et al. (2001). Modulation of recombinant human prostate-specific antigen: activation by Hofmeister salts and inhibition by azapeptides. Appendix: thermodynamic interpretation of the activation by concentrated salts. *Biochemistry, 40*(39), 11734–11741. https://doi.org/10.1021/bi010364j.
114. LeBeau, A. M., Kostova, M., Craik, C. S., & Denmeade, S. R. (2010). Prostate-specific antigen: an overlooked candidate for the targeted treatment and selective imaging of prostate cancer. *Biological Chemistry, 391*(4), 333–343. https://doi.org/10.1515/BC.2010.044.
115. Goettig, P., Magdolen, V., & Brandstetter, H. (2010). Natural and synthetic inhibitors of kallikrein-related peptidases (KLKs). *Biochimie, 92*(11), 1546–1567. https://doi.org/10.1016/j.biochi.2010.06.022.
116. Law, R. H., Zhang, Q., McGowan, S., Buckle, A. M., Silverman, G. A., Wong, W., et al. (2006). An overview of the serpin superfamily. *Genome Biology, 7*(5), 216. https://doi.org/10.1186/gb-2006-7-5-216.
117. Mating, F., Jeet, V., Srinivasan, S., Cristino, A. S., Panchadsaram, J., Clements, J. A., et al. (2019). MicroRNA-3162-5p-Mediated crosstalk between kallikrein family members including prostate-specific antigen in prostate cancer. *Clinical Chemistry, 65*(6), 771–780. https://doi.org/10.1373/clinchem.2018.295824.
118. Larne, O., Ostling, P., Haflidadottir, B. S., Hagman, Z., Aakula, A., Kohonen, P., et al. (2015). miR-183 in prostate cancer cells positively regulates synthesis and serum levels of prostate-specific antigen. *European Urology, 68*(4), 581–588. https://doi.org/10.1016/j.eururo.2014.12.025.
119. Sun, D., Lee, Y. S., Malhotra, A., Kim, H. K., Matecic, M., Evans, C., et al. (2011). miR-99 family of MicroRNAs suppresses the expression of prostate-specific antigen and prostate cancer cell proliferation. *Cancer Research, 71*(4), 1313–1324. https://doi.org/10.1158/0008-5472.CAN-10-1031.
120. Kostova, M. B., Brennen, W. N., Lopez, D., Anthony, L., Wang, H., Platz, E., et al. (2018). PSA-alpha-2-macroglobulin complex is enzymatically active in the serum of patients with advanced prostate cancer and can degrade circulating peptide hormones. *Prostate, 78*(11), 819–829. https://doi.org/10.1002/pros.23539.
121. Thorek, D. L., Evans, M. J., Carlsson, S. V., Ulmert, D., & Lilja, H. (2013). Prostate-specific kallikrein-related peptidases and their relation to prostate cancer biology and detection. Established relevance and emerging roles. *Thrombosis and Haemostasis, 110*(3), 484–492. https://doi.org/10.1160/TH13-04-0275.
122. Ast, G. (2003). Drug-targeting strategies for prostate cancer. *Current Pharmaceutical Design, 9*(6), 455–466.
123. Denmeade, S. R., Jakobsen, C. M., Janssen, S., Khan, S. R., Garrett, E. S., Lilja, H., et al. (2003). Prostate-specific antigen-activated thapsigargin prodrug as targeted therapy for prostate cancer. *Journal of the National Cancer Institute, 95*(13), 990–1000. https://doi.org/10.1093/jnci/95.13.990.
124. DeFeo-Jones, D., Garsky, V. M., Wong, B. K., Feng, D. M., Bolyar, T., Haskell, K., et al. (2000). A peptide-doxorubicin 'prodrug' activated by prostate-specific antigen selectively kills prostate tumor cells positive for prostate-specific antigen in vivo. *Nature Medicine, 6*(11), 1248–1252. https://doi.org/10.1038/81351.
125. Tai, W., Shukla, R. S., Qin, B., Li, B., & Cheng, K. (2011). Development of a peptide-drug conjugate for prostate cancer therapy. *Molecular Pharmaceutics, 8*(3), 901–912. https://doi.org/10.1021/mp200007b.
126. Baiz, D., Pinder, T. A., Hassan, S., Karpova, Y., Salsbury, F., Welker, M. E., et al. (2012). Synthesis and characterization of a novel prostate cancer-targeted phosphatidylinositol-3-kinase inhibitor prodrug. *Journal of Medicinal Chemistry, 55*(18), 8038–8046. https://doi.org/10.1021/jm300881a.
127. Li, B., Zhang, L. J., Zhang, Z. L., Long, M., Ren, J. H., Lin, F., et al. (2011). Synergistic tumor growth-inhibitory effect of the prostate-specific antigen-activated fusion peptide BSD352 for prostate cancer therapy. *Anti-Cancer Drugs, 22*(3), 213–222. https://doi.org/10.1097/CAD.0b013e3283401f4d.
128. Akinboye, E. S., Rosen, M. D., Bakare, O., & Denmeade, S. R. (2017). Anticancer activities of emetine prodrugs that are proteolytically activated by the prostate specific antigen (PSA) and evaluation of in vivo toxicity of emetine derivatives. *Bioorganic & Medicinal Chemistry, 25*(24), 6707–6717. https://doi.org/10.1016/j.bmc.2017.11.015.
129. Williams, S. A., Merchant, R. F., Garrett-Mayer, E., Isaacs, J. T., Buckley, J. T., & Denmeade, S. R. (2007). A prostate-specific antigen-activated channel-forming toxin as therapy for prostatic disease. *Journal of the National Cancer Institute, 99*(5), 376–385. https://doi.org/10.1093/jnci/djk065.

130. Sinha, A. A., Quast, B. J., Reddy, P. K., Elson, M. K., & Wilson, M. J. (1999). Intravenous injection of an immunoconjugate (anti-PSA-IgG conjugated to 5-fluoro-2′-deoxyuridine) selectively inhibits cell proliferation and induces cell death in human prostate cancer cell tumors grown in nude mice. *Anticancer Research, 19*(2A), 893–902.
131. Madan, R. A., Arlen, P. M., Mohebtash, M., Hodge, J. W., & Gulley, J. L. (2009). Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. *Expert Opinion on Investigational Drugs, 18*(7), 1001–1011. https://doi.org/10.1517/13543780902997928.
132. Sonpavde, G., Agarwal, N., Choueiri, T. K., & Kantoff, P. W. (2011). Recent advances in immunotherapy for the treatment of prostate cancer. *Expert Opinion on Biological Therapy, 11*(8), 997–1009. https://doi.org/10.1517/14712598.2011.575357.
133. Karan, D., Dubey, S., Van Veldhuizen, P., Holzbeierlein, J. M., Tawfik, O., & Thrasher, J. B. (2011). Dual antigen target-based immunotherapy for prostate cancer eliminates the growth of established tumors in mice. *Immunotherapy, 3*(6), 735–746. https://doi.org/10.2217/imt.11.59.
134. Miller, A. M., Ozenci, V., Kiessling, R., & Pisa, P. (2005). Immune monitoring in a phase 1 trial of a PSA DNA vaccine in patients with hormone-refractory prostate cancer. *Journal of Immunotherapy, 28*(4), 389–395.
135. Katzenwadel, A., Schleer, H., Gierschner, D., Wetterauer, U., & Elsasser-Beile, U. (2000). Construction and in vivo evaluation of an anti-PSA x anti-CD3 bispecific antibody for the immunotherapy of prostate cancer. *Anticancer Research, 20*(3A), 1551–1555.
136. Daniels-Wells, T. R., Helguera, G., Leuchter, R. K., Quintero, R., Kozman, M., Rodriguez, J. A., et al. (2013). A novel IgE antibody targeting the prostate-specific antigen as a potential prostate cancer therapy. *BMC Cancer, 13*, 195. https://doi.org/10.1186/1471-2407-13-195.
137. DiPaola, R. S., Rinehart, J., Nemunaitis, J., Ebbinghaus, S., Rubin, E., Capanna, T., et al. (2002). Characterization of a novel prostate-specific antigen-activated peptide-doxorubicin conjugate in patients with prostate cancer. *Journal of Clinical Oncology, 20*(7), 1874–1879. https://doi.org/10.1200/JCO.2002.07.001.
138. Elsadek, B., Graeser, R., Warnecke, A., Unger, C., Saleem, T., El-Melegy, N., et al. (2010). Optimization of an albumin-binding prodrug of doxorubicin that is cleaved by prostate-specific antigen. *ACS Medicinal Chemistry Letters, 1*(5), 234–238. https://doi.org/10.1021/ml100060m.
139. Mhaka, A., Denmeade, S. R., Yao, W., Isaacs, J. T., & Khan, S. R. (2002). A 5-fluorodeoxyuridine prodrug as targeted therapy for prostate cancer. *Bioorganic & Medicinal Chemistry Letters, 12*(17), 2459–2461.
140. DeFeo-Jones, D., Brady, S. F., Feng, D. M., Wong, B. K., Bolyar, T., Haskell, K., et al. (2002). A prostate-specific antigen (PSA)-activated vinblastine prodrug selectively kills PSA-secreting cells in vivo. *Molecular Cancer Therapeutics, 1*(7), 451–459.
141. Elsadek, B., Graeser, R., Esser, N., Schafer-Obodozie, C., Abu Ajaj, K., Unger, C., et al. (2010). Development of a novel prodrug of paclitaxel that is cleaved by prostate-specific antigen: an in vitro and in vivo evaluation study. *European Journal of Cancer, 46*(18), 3434–3444. https://doi.org/10.1016/j.ejca.2010.08.018.
142. Chandran, S. S., Nan, A., Rosen, D. M., Ghandehari, H., & Denmeade, S. R. (2007). A prostate-specific antigen activated N-(2-hydroxypropyl) methacrylamide copolymer prodrug as dual-targeted therapy for prostate cancer. *Molecular Cancer Therapeutics, 6*(11), 2928–2937. https://doi.org/10.1158/1535-7163.MCT-07-0392.
143. Latham, J. P., Searle, P. F., Mautner, V., & James, N. D. (2000). Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. *Cancer Research, 60*(2), 334–341.
144. Figueiredo, M. L., Sato, M., Johnson, M., & Wu, L. (2006). Specific targeting of gene therapy to prostate cancer using a two-step transcriptional amplification system. *Future Oncology, 2*(3), 391–406. https://doi.org/10.2217/14796694.2.3.391.
145. Yu, D., Chen, D., Chiu, C., Razmazma, B., Chow, Y. H., & Pang, S. (2001). Prostate-specific targeting using PSA promoter-based lentiviral vectors. *Cancer Gene Therapy, 8*(9), 628–635. https://doi.org/10.1038/sj.cgt.7700344.
146. Gerritsen, W. R. (2012). The evolving role of immunotherapy in prostate cancer. *Annals of Oncology, 23*(*Suppl 8*), viii22–viii27. https://doi.org/10.1093/annonc/mds259.
147. Gulley, J. L., Borre, M., Vogelzang, N. J., Ng, S., Agarwal, N., Parker, C. C., et al. (2019). Phase III Trial of PROSTVAC in Asymptomatic or Minimally Symptomatic Metastatic Castration-Resistant Prostate Cancer. *Journal of Clinical Oncology, 37*(13), 1051–1061. https://doi.org/10.1200/JCO.18.02031.
148. Joniau, S., Abrahamsson, P. A., Bellmunt, J., Figdor, C., Hamdy, F., Verhagen, P., et al. (2012). Current vaccination strategies for prostate cancer. *European Urology, 61*(2), 290–306. https://doi.org/10.1016/j.eururo.2011.09.020.
149. Geary, S. M., & Salem, A. K. (2013). Prostate cancer vaccines: update on clinical development. *Oncoimmunology, 2*(5), e24523. https://doi.org/10.4161/onci.24523.