Biology of Prostate-Specific Antigen

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Prostate-specific antigen (PSA) is an androgen-regulated serine protease produced by both prostate epithelial cells and prostate cancer (PCa) and is the most commonly used serum marker for cancer. It is a member of the tissue kallikrein family, some of the members of which are also prostate specific. PSA is a major protein in semen, where its function is to cleave semenogelins in the seminal coagulum. PSA is secreted into prostatic ducts as an inactive 244–amino acid proenzyme (proPSA) that is activated by cleavage of seven *N*-terminal amino acids. PSA that enters the circulation intact is rapidly bound by protease inhibitors, primarily alpha1-antichymotrypsin, although a fraction is inactivated in the lumen by proteolysis and circulates as free PSA. This proteolytic inactivation, as well as the cleavage of proPSA to PSA, is less efficient in PCa. Serum total PSA levels are increased in PCa, and PSA screening has dramatically altered PCa presentation and management. Unfortunately, although high PSA levels are predictive of advanced PCa, a large fraction of organ-confined cancers present with much lower total PSA values that overlap those levels found in men without PCa. Measurement of free versus total PSA can increase specificity for PCa, and tests under development to measure forms of proPSA may further enhance the ability to detect early-stage PCa. PSA is also widely used to monitor responses to therapy and is under investigation as a therapeutic target. Finally, recent data indicate that there may be additional roles for PSA in the pathogenesis of PCa.

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PROSTATE-SPECIFIC antigen (PSA) is an androgen-regulated serine protease and member of the tissue kallikrein family of proteases.[1] It is produced primarily by prostate ductal and acinar epithelium and is secreted into the lumen, where its function is to cleave semenogelin I and II in the seminal coagulum.[2] However, its major relevance in oncology is as a biomarker to detect prostate cancer (PCa) and to assess responses to treatment. The value and appropriate use of PSA screening remain controversial, but the success of primary therapy is certainly dependent on identifying tumors before they have spread outside the prostate. Unfortunately, standard serum total PSA tests lack the sensitivity and specificity to detect a large fraction of early-stage tumors. However, insights into PSA biology in normal prostate and PCa promise to improve PCa detection and lead to novel uses for PSA. This review outlines the basic biology of PSA, with a focus on aspects that are relevant to its uses in PCa and a possible role in PCa pathogenesis.

NORMAL STRUCTURE, EXPRESSION, AND FUNCTION

Gene Structure of PSA and the Tissue Kallikrein Gene Family

PSA is a member of the tissue kallikrein family, located on chromosome 19q13.4.[1] Kallikreins were initially defined as serine proteases that digest certain high-molecular-weight proteins to release bioactive peptides termed kinins. They are now divided into two families, the tissue and plasma kallikreins. Human plasma kallikrein on chromosome 4 (also termed Fletcher factor or KLKB1), which cleaves bradykinin from high-molecular-weight kininogen, is produced exclusively in the liver and is unrelated to the tissue kallikreins. Among the tissue kallikreins, the only enzyme with appreciable kallikrein activity is human kallikrein 1 (hK1; pancreatic-renal kallikrein).[3,4]

Until recently, there were only three identified human tissue kallikreins: hK1, hK2 (glandular kallikrein), and hK3 (PSA). However, cDNA cloning and sequencing of the human genome have revealed a total of 15 tissue kallikrein genes on chromosome 19q13.3-q13.4, which are all encoded by five exons of similar size and have 40% to 80% sequence homology (Fig 1).[1,5] The gene encoding hK1 (*KLK1*) is closest to the centromere and is followed by *KLK15*, *KLK3* (encoding PSA), and *KLK2*. The kallikrein genes are expressed in multiple tissues, and many are steroid hormone regulated. In addition to PSA, hK2 and hK4 seem to be expressed primarily in prostate and are androgen regulated.[6-9] The close linkage of *KLK2*, *KLK3*, and *KLK4*, in conjunction with their primary expression in prostate, indicate that common prostate-specific regulatory elements (in addition to androgen-responsive elements) may be controlling their expression. Studies indicate that hK2 and possibly hK4, although hK4 is expressed at much lower levels, may also be useful markers for PCa.[10]

Androgen Regulation of PSA Expression

Transcription of the PSA gene is positively regulated by the androgen receptor (AR), and PSA has been extensively studied as a model androgen-regulated gene. The AR is a steroid hormone receptor that binds as a homodimer to specific DNA sequences, termed androgen-responsive elements (AREs), and a

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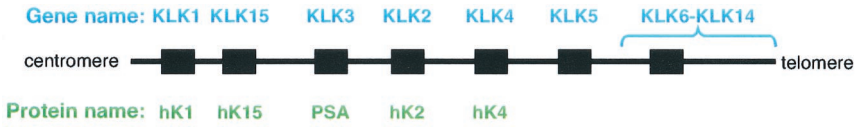

Fig 1. Map of the human tissue kallikrein locus. The gene and corresponding protein names are indicated.

consensus ARE is located at −156 to −170 from the transcriptional start site of the PSA gene (Fig 2).[11] The AR can also bind weakly to sites that differ from the strong consensus ARE, and such a weak nonconsensus ARE (termed ARR) has been identified at −365 to −400.[12] Further studies have mapped the region responsible for high-level androgen-stimulated PSA expression to a fragment of about 450 base pairs, located approximately 4.2 kb upstream of the transcriptional start site, termed the PSA distal enhancer (Fig 2).[13-15] This region contains a single strong consensus ARE (ARE III), but binding studies have demonstrated the presence of multiple additional weak nonconsensus AREs.[16] The cooperative binding of multiple ARs to this region likely accounts for its strong androgen-dependent activity.

PSA is consistently expressed in PCa, although its level of expression on a per cell basis is lower than in normal prostate epithelium.[17,18] This expression reflects AR transcriptional activity in the vast majority of PCa, although additional factors regulating the PSA promoter have also been identified.[19,20] Importantly, although the decline in PSA levels in response to androgen deprivation therapy is certainly caused in part by tumor cell death, it is also the result of decreased AR-stimulated PSA production by surviving tumor cells. As a result, androgen-deprivation therapies may in some cases have greater effects on PSA production than on tumor survival. In particular, complete androgen blockade by combined castration and AR antagonist treatment (versus castration alone) results in more rapid PSA declines and lower nadir levels, but this does not translate into a significant improvement in survival.[21] Therefore, although the rate and magnitude of PSA decline are predictive of clinical responses in patients receiving the same treatment, they must be used cautiously when comparing different therapies.

PSA Expression in Androgen-Independent PCa

PSA is also expressed in the majority of PCas that recur after androgen-deprivation therapies (hormone refractory or androgen-independent PCa). The factors regulating PSA expression in androgen-independent PCa are uncertain, but the AR is consistently expressed and seems to be transcriptionally active in most cases.[22-24] Current data indicate that androgen deprivation therapies select for a series of genetic and epigenetic changes in the AR and in AR-interacting transcriptional coactivator proteins, resulting in AR transcriptional activity despite castrate androgen levels.[25-27] This AR activity, which also seems to be relatively resistant to AR antagonists, likely contributes to the persistent PSA expression in androgen-independent PCa. However, the link between this PSA expression and tumor cell growth is more tenuous than in androgen-dependent PCa.

PSA Expression and Function in Prostate and Other Tissues

The majority of the glandular tissue in prostate is located in the peripheral zone, and seminal fluid produced by these glands empties into 12 to 20 excretory ducts and then into the urethra. PSA is a major protein in seminal fluid, with a concentration of 0.5 to 2.0 mg/mL.[28,29] It has a substrate specificity similar to chymotrypsin,[30] and its major physiological substrates are semenogelin I and II,[2] the proteins that mediate gel formation in semen. Prostate glands in humans consist of a single layer of secretory epithelial cells, which are surrounded by a continuous layer of basal cells and a basement membrane (Fig 3). PSA is produced by these secretory epithelial cells in the acini and ducts, and it is secreted directly into the lumen. A characteristic early feature of PCa is disruption of the basal cell layer and

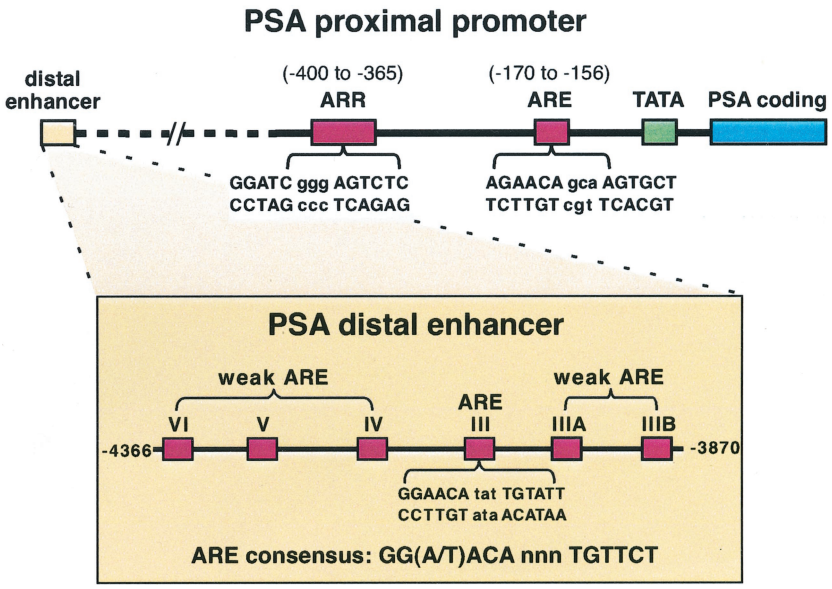

Fig 2. Major regions regulating prostate-specific antigen (PSA) gene expression. Above, PSA proximal promoter immediately upstream from the PSA coding region containing an androgen-responsive element (ARE) and an androgen-responsive region (ARR), with a weak nonconsensus ARE. The major region mediating high level PSA expression is the distal enhancer (detail below), which contains a central strong ARE closely related to the defined consensus ARE (shown), and multiple weak AREs.

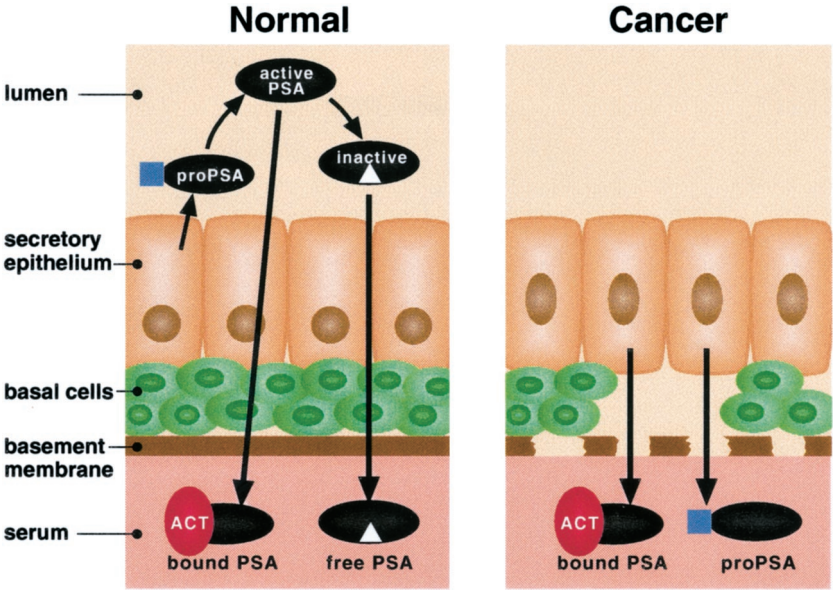

Fig 3. Model of prostate-specific antigen (PSA) biosynthesis in normal prostate epithelium versus cancer. Normal secretory epithelium, surrounded by basal cells and a basement membrane, secretes proPSA into the lumen where the propeptide is removed by hK2 to generate active PSA. A fraction of this active PSA can diffuse into the circulation, where it is rapidly bound by protease inhibitors (primarily alpha1-antichymotrypsin, ACT). The active PSA also undergoes proteolysis in the lumen to generate inactive PSA, which can enter the bloodstream and circulates in an unbound state (free PSA). In PCa, loss of basal cells, basement membrane, and normal lumen architecture results in a decrease in the luminal processing of proPSA to active PSA, and active PSA to inactive PSA, with relative increases in bound PSA and proPSA in the serum. Further truncated forms of proPSA in cancer are shown in Fig 4. Note that the partial basal cell loss as shown is found in prostatic intraepithelial neoplasia (PIN), while there is complete loss in PCA.

basement membrane, and this loss of the normal glandular architecture appears to allow PSA increased direct access to the peripheral circulation (Fig 3).[31,32]

PSA is normally found at much lower levels in paraurethral and perianal glands, apocrine sweat glands, breast, thyroid, and placenta.[29,33] These sites do not normally contribute measurable levels of PSA into the circulation, as PSA levels by standard assays fall to undetectable levels after radical prostatectomy. PSA production has also been reported in a variety of other cancers, including breast cancer. Its functions in these tissues are not yet clear, and its usefulness as a tumor marker outside of PCa remains to be established.

PSA Biosynthesis and Processing

PSA is synthesized with a 17–amino acid leader sequence (preproPSA) that is cleaved cotranslationally to generate an inactive 244–amino acid precursor protein (proPSA; Fig 4).[34] Cleavage of the *N*-terminal seven amino acids from proPSA generates the active enzyme, which has five intrachain disulfide bonds, a single asparagine-linked oligosaccharide, and a mass of 33 kda.[35-37] This proPSA cleavage normally occurs between the arginine at position 7 and isoleucine at position 8, with the isoleucine becoming the *N*-terminus of the mature active protein. This site can be readily digested by trypsin, but the major activating enzyme in vivo is hK2, which has a trypsin-like activity and is expressed predominantly by prostate secretory epithelium. PSA may also be activated by other prostate kallikreins, including prostase (hK4).[38]

Approximately 30% of PSA in seminal plasma is the intact proteolytically active enzyme, and approximately 5% is complexed with protein C inhibitor.[39,40] Additional forms are inactive because of internal cleavages (presumably by proteases in seminal fluid) between residues 85 to 86, 145 to 146, or 182 to 183 (Fig 4).[41] These cleaved PSA isoforms have also been termed BPSA as they have been identified in the prostate transition zone and found to be increased in benign prostatic hyperplasia (BPH).[40,42-44] Importantly, their concentrations are decreased in PCa tissue, presumably because of decreased exposure to proteases in seminal fluid.[42]

An additional form of PSA recently identified in PCa tissue is a truncated form of proPSA cleaved between leu 5 and serine 6 in the propeptide (Fig 4).[45,46] The resulting PSA protein, termed [-2]pPSA, has two extra amino acids relative to the active mature PSA and is catalytically inactive (and therefore circulates as free PSA). This form appears to be stable, as the additional two amino acids are not cleaved by trypsin or hK2. Immunohistochemical studies using a monoclonal antibody recognizing [-2]pPSA found increased staining in the secretions from malignant prostate glands.[47] Further truncated proPSA isoforms with one, four, or five extra *N*-terminal amino acids have also been found and may be elevated in PCa tissue.[45,46] The molecular basis for the elevation of these truncated proPSA isoforms in PCa is uncertain, but it likely reflects decreased cleavage of

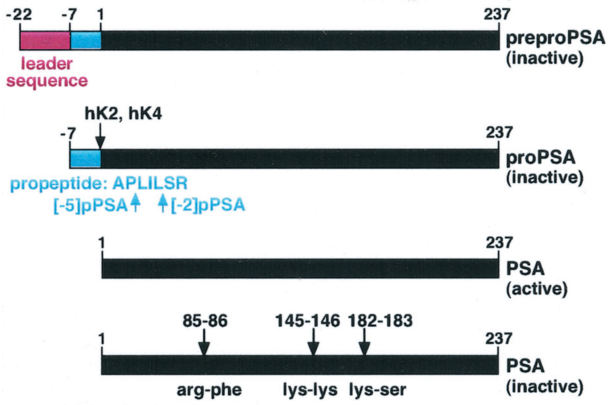

Fig 4. Structure of prostate-specific antigen (PSA) forms. The leader sequence on preproPSA is cleaved in the cell to generate proPSA, which is inactive. Cleavage of the propeptide in the lumen by hK2 generates the active mature PSA. Truncated forms of proPSA can also be generated by cleavage within the propeptide, and these truncated proPSA forms (including [-2]pPSA and[-5]pPSA) are inactive. Active PSA in the lumen of prostate ducts can be further cleaved at the indicated positions to generate inactive PSA. Active PSA that enters the bloodstream is rapidly bound to protease inhibitors, while all other forms circulate as free PSA.

proPSA by hK2 in PCa tissue. As outlined below, these isoforms can be found in peripheral blood and are under investigation as PCa markers.

PSA in Peripheral Blood

The majority (70% to 90%) of PSA that enters the peripheral blood is intact and circulates as an 80- to 90-kda complex with the protease inhibitor alpha1-antichymotrypsin (Fig 3).[48,49] Minor amounts are complexed with other protease inhibitors including alpha2-macroglobulin and alpha1-antitrypsin. The antibodies in most assays recognize both free and most complexed PSAs, but none recognize the complex with alpha2-macroglobulin as it completely surrounds the protein. PSA in peripheral blood that is catalytically inactive because of internal cleavages at residues 85 to 86, 145 to 146, or 182 to 183 does not form complexes with protease inhibitors or other proteins and circulates as free PSA (fPSA), comprising 10% to 30% of total PSA. As noted above, these internal cleavages occur in seminal plasma and are present at lower levels in PCa tissue, presumably because of disruption of the normal secretion of PSA into the ducts. Consequently, the ratio of free to total PSA (fPSA/tPSA, termed the PSA index) is lower in many patients with PCa and seems to aid in the discrimination between normal and PCa.

ProPSA and the various truncated forms of proPSA identified in prostate can also be detected in serum.[45] A recent study analyzing a small number of patients with biopsy-positive PCa and total PSA between 6 and 24 ng/mL found that [-2]pPSA comprised a high fraction of the fPSA (25% to 95%), which was greater than in biopsy-negative patients.[47] Further studies are clearly needed, but these truncated pPSA isoforms represent promising tools to increase the specificity of PSA testing.

USE OF PSA AS A TUMOR MARKER FOR PCa

Studies in the early 1990s confirmed that serum total PSA could be used to identify patients with PCa.[50-53] As a screening tool, serum PSA was clearly more sensitive than digital rectal examination, but it lacked specificity. When compared with prostatic acid phosphatase, serum PSA was demonstrated to be a more sensitive marker in PCa detection (although neither was highly specific).[54] These findings have led to wide use of PSA testing for early detection of PCa, although the optimal approach to PSA testing remains uncertain. The sections below do not attempt to detail the extensive literature on the use of PSA for PCa screening but, instead, focus on how PSA structure, processing, and regulation form the basis for diagnostic uses of PSA.

Total PSA for Early PCa Detection

Results of a large multicenter trial suggested the use of serum total PSA greater than 4 ng/mL as a threshold for performing prostate biopsies.[55] Unfortunately, using a single value for men of all ages results in the exclusion of an unacceptably high number of patients with clinically significant early-stage disease, as approximately 20% to 50% of clinically significant organ-confined PCa occurs in men with serum total PSA less than 4 ng/mL.[56-58] This lack of specificity reflects the substantial overlap between serum total PSA in normal versus PCa patients, which is not surprising, as PSA is made at comparable or higher levels by normal prostate epithelium. High levels of PSA in patients with advanced PCa clearly reflect the large number of tumor cells. In contrast, PSA increases in early-stage disease seem to be the result primarily of disruptions in the normal glandular architecture (with a larger fraction of the PSA produced going into the systemic circulation), and this increase is modest compared with the normal variability in the population.

Since the introduction of serum PSA and digital rectal examination as screening tools for PCa, the incidence of PCa has risen dramatically along with a shift toward organ-confined disease. However, although use of serum PSA clearly leads to earlier PCa detection, the survival benefit of PSA screening has not been adequately demonstrated in a randomized controlled trial, and recommendations for its use are mixed. For example, both the American Urologic Association and American Cancer Society recommend offering yearly PSA and digital rectal examination for men who are 50 and older and who have a life expectancy of greater than 10 years. A randomized study of screening for prostate, lung, colorectal, and ovarian cancer by the National Cancer Institute is currently under way and may provide additional insight in the future.[59] More extensive discussions addressing the value of PSA in PCa screening can be found in recent reviews.[60,61]

Adjusted Total PSA for Early PCa Detection

Given the limitations of using 4 ng/mL as the minimum threshold for detecting PCa, efforts have been made to improve the diagnostic accuracy of screening for total PSA. As serum PSA normally increases with age (reflecting in part BPH) and is influenced by race, both age- and race-specific normalized values have been established.[62-64] The use of age-specific ranges was suggested to have the advantage of increasing the sensitivity of PSA testing in younger men as well as enhancing its specificity in older men, resulting in the increased detection of curable cancers in the younger population and decreasing the detection of clinically insignificant cancers in older men. However, use of age-specific PSA ranges remains controversial, especially because more recent data indicate that using age-specific ranges would result in missing an unacceptable number of clinically significant cancers in older men.[65,66]

To more directly compensate for BPH and prostate size, transrectal ultrasound has been used to measure prostate volume. Serum PSA is then normalized by prostate volume to give a "PSA density," with densities greater than 0.15 more suggestive of PCa.[67] However, the utility of this method as a screen is limited because of variations in prostate shape and ratios of epithelium to stroma.[68] A multicenter study that compared the usefulness of PSA density versus PSA for the early detection of PCa found that almost half of the cancers would be missed if the cutoff of 0.15 was used to determine the need for a biopsy.[69] Another approach has been to assess the change in PSA over time ("PSA velocity"), with values more than 0.75 ng/mL per year being predictive of PCa.[70] However, the clinical usefulness of this test is limited by the need for prior values and by variations that can occur as a result of nonmalignant causes.[71]

Serum PSA Isoforms for Early PCa Detection

Although PCa does not produce more PSA than normal prostate epithelium, a larger fraction produced by PCa seems to

escape proteolytic processing (activation or degradation; Fig 3). In normal prostate, the majority of fPSA reflects the mature protein that has been inactivated by internal proteolytic cleavage (Fig 4). In contrast, in PCa, this cleaved fraction is relatively decreased, resulting in lower fPSA to total PSA ratios (PSA index). Studies in men with PSA between 4 and 10.0 ng/mL indicate a PCa risk of less than 8% with a fPSA/tPSA greater than 25%, versus a greater than 56% risk with a fPSA/tPSA less than 10%.[72-74] Importantly, given the need to detect PCa at lower PSA levels, several studies indicate that the PSA index may also increase the sensitivity and specificity of PSA testing in men with less than 4 ng/mL total PSA.[73,75,76]

As outlined above, the decreased PSA processing in PCa also results in a relative increase in proPSA and its cleaved forms, in particular, [-2]pPSA. These proPSA forms are catalytically inactive and therefore circulate as fPSA and may constitute a major fraction of the fPSA in PCa patients.[47] If this is confirmed in larger studies, then the ratio of proPSA isoforms versus internally cleaved mature PSA could become a strong discriminator between normal and PCa (although general use will require the development of further antibodies and more-sensitive assays).

PSA to Determine Responses to Primary Therapy

As there is no significant source of serum PSA outside of the prostate gland, serum PSA can be used to monitor the efficacy of definitive local therapy (radical prostatectomy or radiation therapy). A detectable serum PSA by standard assays after radical prostatectomy is inevitably followed by clinical disease recurrence (the significance of results with "super-sensitive" PSA tests being unclear).[77] In contrast, it has been more difficult to measure efficacy in patients treated with external beam radiation, as PSA is detectable, albeit at low values, after radiation. Therefore, the American Society for Therapeutic Radiology and Oncology (ASTRO) has defined failure as three consecutive rises in PSA after radiation therapy,[78] but nadir PSA levels are still useful predictors of relapse.[79,80] The ASTRO guidelines for defining PSA failure are also generally applicable to brachytherapy, but it is even more difficult to interpret PSA levels after this procedure. Several reports have shown substantial transient elevations in PSA as far as 3 years after the procedure that are not related to disease recurrence.[81,82]

PSA for Early Detection of Recurrent PCa

The prognostic value of PSA monitoring after primary radical prostatectomy or radiation therapy was demonstrated in early studies that found an elevation in PSA before clinical relapse in 92% of patients.[83,84] However, the time from first PSA recurrence to clinically evident recurrent PCa is variable and can be prolonged. In a large series from one urologist at Johns Hopkins, [85] the median time to clinical recurrence from first detectable PSA after radical prostatectomy, without hormonal or any other therapy, was 8 years. In addition, the median time from the recurrence of clinical disease to death was another 5 years. Nonetheless, a rapid rate of PSA rise (expressed as PSA doubling time $<$ 10 months) was predictive of earlier clinical relapse.

In addition to Gleason grade and surgical margin status, the time at which PSA becomes detectable after radical prostatectomy may be important in determining whether the relapse is local or distant.[86,87] On the basis of retrospective data, if the serum PSA becomes detectable in the first 2 years after surgery, the patient is more likely to have distant disease with little efficacy of radiation therapy to the prostate bed.[87] Similarly, in one large series, a PSA velocity of less than 0.75 ng/mL/yr was seen in 94% of those who developed local failure.[88]

PSA to Monitor Responses to Hormonal Therapy

In patients treated with androgen-deprivation therapy for metastatic PCa, serum PSA was found to fall dramatically in the majority of patients and paralleled improvements in clinical symptoms, with a PSA nadir of less than 0.4 ng/mL being predictive of the duration of remission.[89] However, the use of PSA decline as a surrogate marker for survival remains controversial. In the large randomized study of orchiectomy and flutamide versus orchiectomy and placebo, a significantly larger proportion of flutamide-treated subjects reached a PSA nadir of less than 4 ng/mL, but this was not associated with improved overall survival.[21]

Remarkably, PSA monitoring can also be used for early detection of recurrent androgen-independent PCa. As detailed above, PSA expression in this setting appears to reflect adaptations by the tumor cells that stimulate AR transcriptional activity. Unfortunately, these adaptations (which remain poorly defined) also markedly enhance malignant potential, with rapidly progressing clinical disease usually apparent within months after the initial rise in PSA.

PSA to Assess Responses to Therapy in Androgen-Independent PCa

Unlike other solid tumors or primary PCa, the majority of patients with androgen-independent PCa have disease primarily or exclusively in bone. Although this can be evaluated by bone scan, and progression documented as new sites of uptake, improvements in bone scans in response to therapy occur very slowly, if at all. Therefore, it is not possible to measure responses by standard radiological methods in most patients with androgen-independent PCa. This lack of measurable disease has made it difficult to rigorously determine therapeutic efficacy. PSA is attractive as a surrogate marker, but PSA declines may not consistently reflect tumor responses (particularly with secondary hormonal therapies that may inhibit AR-stimulated PSA expression without suppressing tumor growth). However, several retrospective analyses suggest that patients with a more than 50% PSA decline in response to therapy have an increase in survival[90-92] and a consensus panel has established guidelines for using PSA as a measurement of disease outcome.[93]

THERAPEUTIC USES OF PSA

Efforts are ongoing to exploit the enzyme activity and specificity of PSA production by PCa for therapeutic purposes. One approach is the construction of PSA cleavable prodrugs. As outlined above, PSA in the circulation is either inactive because of internal cleavage or is bound to protease inhibitors. Therefore, levels of enzymatically active PSA in tumor microenvironments are much higher than in the general circulation.[94] On the basis of this rationale, a 7-mer peptide (his-ser-ser-lys-leu-gln-leu) has

been designed that can be cleaved specifically by active PSA, but not by other proteases (including chymotrypsin).[95] Conjugation of this peptide to doxorubicin generates a largely inactivate prodrug that can be selectively activated by PSA-producing tumors both in vitro and in vivo.[95,96] Another prodrug under investigation links the same peptide to thapsigarin, a compound that when activated by removal of the peptide induces apoptosis even in nondividing cells.[97]

The delivery of toxic genes by replication competent adenovirus vectors regulated by the PSA promoter-enhancer is another promising approach. These vectors can replicate approximately 400 times more efficiently in PSA-expressing cells compared with PSA-negative cells and can selectively deliver toxic genes and kill PSA-expressing PCa in vitro and in vivo.[98-100] A potential concern with the use of regulatory elements from the PSA gene is that replication would be androgen dependent, although persistent PSA expression in androgen-independent PCa indicates that it may not be necessary to replete androgen in these patients. Indeed, a PSA promoter-enhancer-regulated adenovirus vector was shown to replicate in a PSA-producing androgen-independent subline of LNCaP PCa cells.[101]

A third approach is using PSA vaccines with viral vectors that have been shown to elicit both cellular and humoral immune responses to proteins expressed in their genome. A phase I trial of a vaccinia-based PSA vaccine found that most patients treated with the highest dose of vaccinia and concomitant GM-CSF generated PSA-specific T cells.[102] PSA levels were stable for at least 6 months in 14 of 33 men, indicating some biologic activity for this approach. Current studies are ongoing, comparing the effectiveness of fowlpox to vaccinia virus PSA vectors.

POSSIBLE ROLES FOR PSA IN THE PATHOGENESIS OF PCA

As discussed above, PSA in the circulation is largely inactive as a result of being bound to carrier proteins. However, the proteolytic capacity of PSA in tumor microenvironments has the potential to cleave a number of proteins that may influence PCa development or progression. One such protein that can be cleaved by PSA is insulin-like growth factor binding protein-3 (IGFBP-3), the major serum binding protein for insulin-like growth factor-1 (IGF-1).[103,104] IGF-1 is a growth factor for PCa, and increased serum levels of IGF-1 have been shown to be a risk factor for PCa.[105,106] IGFBP-3 is produced by prostate epithelial cells, and in vitro studies indicate that its cleavage by PSA decreases IGF-1 binding and can increase proliferation in response to added IGF-1.[104] The significance of IGFBP-3 cleavage by PSA in vivo is uncertain, but a recent study found an increase in IGFBP-3 levels after radical prostatectomy.[107] However, this increase did not clearly reflect an effect of PSA, as it did not correlate with preprostatectomy PSA levels.

PSA may also cleave proteins that affect cell migration and metastasis. For instance, PSA has the capacity to cleave extracellular matrix glycoproteins such as fibronectin and laminin, and in vitro studies have shown that microinvasion of LNCaP cells can be inhibited by PSA-neutralizing antibodies.[108] The extracellular matrix may itself have an affect on PSA production, as LNCaP cells grown on stromal extracellular matrix secrete increased levels of PSA compared with cells grown on plastic cell culture dishes.[109] PSA and hK2 can cleave and activate urokinase-type plasminogen activator, which may enhance tumor cell invasion, although hK2 is likely to have a much greater capacity for this activity than does PSA.[110]

PSA has potent mitogenic activity in vitro for osteoblasts, which may be mediated by activation of transforming growth factor-beta or by proteolytic modulation of osteoblast cell surface receptors.[111] These findings are significant, as they suggest a role for PSA in bone metastases and osteoblastic responses. However, PSA can also degrade parathyroid hormone-related protein (PTHrP), which abrogates the mitogenic effects of this protein on osteoblasts.[112,113]

In contrast to tumor-promoting activities, PSA may have antiangiogenic effects by cleavage of plasminogen to generate peptides with angiostatin-like activity[114] or by inactivation of the angiogenic inducers fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF).[115] Consistent with this hypothesis, a study of paraffin-embedded PCa specimens demonstrated an inverse relationship between PSA expression and microvessel density, a measure of tumor angiogenesis.[116]

Taken together, these studies indicate mechanisms by which PSA could have tumor-promoting or antitumor effects, but the in vivo significance of any of these mechanisms remains to be established. Assessing the tumor-promoting or tumor-inhibiting properties of PSA in the tumor microenvironment is particularly difficult, as systemic levels of cleavage products may not reflect those in the tumors. There is also a paucity of suitable animal models for studying in situ the effects of PSA on tumor development. Finally, the effects of other kallikreins with similar proteolytic capacities, such as hK2, further complicates the question of whether PSA is simply a tumor marker or has an intrinsic ability to alter tumor progression.

CONCLUSION

In conclusion, serum total PSA can be used as a biomarker of PCa responses to therapy and an early indicator of PCa recurrence. In contrast, PSA production by normal prostate epithelium limits the sensitivity and specificity of serum total PSA as an indicator of early-stage PCa. The decreased luminal proteolytic processing of PSA produced by tumor cells causes a decrease in the cleaved inactive form of mature PSA (which circulates as free PSA), resulting in a lower ratio of serum-free to total PSA. The decreased luminal processing further results in an increase in proPSA isoforms. As these proPSA isoforms also circulate as free PSA, measurement of proPSA versus cleaved mature PSA may provide a more specific screen for early PCa. Further studies of PSA processing, alternative splicing, and glycosylation may yield additional improvements in early PCa detection. The accuracy of PCa screening may also be enhanced by measurement of multiple markers, including other prostate-specific kallikreins.

Future studies of PSA screening need to focus on men with low serum-total PSA levels (< 4 ng/mL), as an unacceptably high fraction of men diagnosed with PCa at intermediate PSA levels (4 to 10 ng/mL) will eventually develop metastatic PCa, even when their disease appears to be organ confined at surgery. Indeed, these late recurrences after primary therapy demonstrate that longer follow-up is needed to determine the success of PSA screening in detecting early PCa that is truly confined to the

prostate, as there are no reliable methods to detect microscopic metastatic disease. Moreover, the high false-negative rate of prostate biopsies (particularly in the setting of low-volume disease) indicates both the need for longer follow-up to identify patients with false-negative biopsies who subsequently develop clinical PCa and the need for advances in prostate imaging and biopsy methods. Finally, further advances in molecular characterization of PCa are needed to better predict natural history from biopsy samples and to determine who is likely to benefit from therapy.

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