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Minireview

Prostate-specific Antigen: A Cancer Fighter and a Valuable Messenger?

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Background: **Prostate-specific antigen (PSA) is a valuable prostatic cancer biomarker that is now widely used for population screening, diagnosis, and monitoring of patients with prostate cancer. Despite the voluminous literature on this biomarker, relatively few reports have addressed the issue of its physiological function and its connection to the pathogenesis and progression of prostate and other cancers.**

Approach: **I here review literature dealing with PSA physiology and pathobiology and discuss reports that either suggest that PSA is a beneficial molecule with tumor suppressor activity or that PSA has deleterious effects in prostate, breast, and possibly other cancers.**

Content: **The present scientific literature on PSA physiology and pathobiology is confusing. A group of reports have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule, whereas others suggest that PSA may, through its chymotrypsin-like activity, promote tumor progression and metastasis.**

Summary: **The physiological function of PSA is still not well understood. Because PSA is just one member of the human kallikrein gene family, it is possible that its biological functions are related to the activity of other related kallikreins. Only when the physiological functions of PSA and other kallikreins are elucidated will we be able to explain the currently apparently conflicting experimental data.**

Prostate-specific antigen (PSA)[1] was introduced to urological practice ~15 years ago, and it currently is widely used for the diagnosis and management of prostatic carcinoma *(1)*. PSA is the first tumor marker that has been approved by the Food and Drug Administration as an aid for diagnosing prostate cancer in population screening programs. The paramount position of PSA as a urological tumor marker is well accepted and indisputable. A few thousand reports on this molecule have already been published. One of the major advantages of PSA as a tumor marker is its tissue specificity. However, more recently, many publications have confirmed that PSA is widely expressed, at lower concentrations than in prostate, in many tissues, especially in the female breast *(2, 3)*. PSA has been detected in all nonpathological and pathological breast secretions, tissue extracts, and fluids (milk, breast cyst fluid, nipple aspirate fluid). These new findings do not limit the value of PSA in prostate cancer diagnostics but may expand its applications to breast cancer.

Despite the extensive literature on this molecule, only a handful of reports have addressed the issue of PSA's physiological function in the prostate, breast, and other tissues. Although PSA is produced by the prostatic epithelial cells in relatively enormous amounts and its regulation is under the control of androgens and progestins, we do not have a good understanding of why this molecule is so abundantly expressed and what role it plays in prostatic physiology. Rodent animal models did not help much in this regard because PSA is a molecule restricted to primates *(4)* and spontaneous prostate cancer is exceedingly rare in animals.

Among the major recent advances in this field is the discovery of a whole new human gene family, of which PSA is a member *(5)*. Until recently and in contrast to the findings in rodents (which usually possess 10–25 different kallikrein genes), it was thought that in humans the kallikrein gene family consists of only three members *(6)*. We now know that the human kallikrein gene family consists of at least 14 genes *(7)*, all of which encode for serine proteases that have many structural similarities and significant homologies between them. Several of

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[1] Nonstandard abbreviations: PSA, prostate-specific antigen; hK2, human glandular kallikrein 2; IGFBP-3, insulin-like growth factor-binding protein 3; and IGF-I, insulin-like growth factor I.

these proteases are now known to be expressed in prostate, breast, testis, brain, salivary glands, skin, and other tissues. Especially, PSA, human glandular kallikrein 2 (hK2), and prostase/KLK-L1 appear to be relatively prostate-specific *(1, 4, 8–11)*, although as already mentioned, the female breast also expresses these proteins.

There is now an enormous body of literature on PSA as a prostatic biomarker *(12–17)*. The impact of PSA screening on prostate cancer mortality is now starting to become clearer. For the first time in many years, prostate cancer mortality in the United States dropped substantially in 1999, and this favorable trend is expected to continue. Because this short review does not focus on the clinical aspects of PSA diagnostics, the reader is referred to more specialized publications *(1, 12–17)*.

What Are the Physiological Functions of PSA and the Other Kallikreins Expressed by Prostatic Tissue?

The currently most widely accepted physiological function of PSA relates to its ability to digest the seminogelins and fibronectin present in high concentrations in seminal plasma (produced by the seminal vesicles), thus liquefying the seminal clot shortly after ejaculation *(18)*. The physiologic consequences of the cleavage of seminogelins are not known, although this process does increase sperm cell motility. Other investigators have reported that PSA can release a kinin-like substance that stimulates smooth muscle contraction by digesting a glycoprotein present in seminal vesicle fluid *(19)*. Of the ~20 additional papers published to address the question of PSA's physiological function, one-half portray PSA as a cell growth inhibitor, an anticarcinogenic/antiangiogenic molecule, or as an inducer of apoptosis. These activities (summarized with specific references in Table 1) qualify PSA as a molecule with tumor suppressor activity. Interestingly, among the newly discovered members of the kallikrein gene family, two other proteins, the normal epithelial cell-specific 1 gene product, NES1 *(20, 21)*, and protease M, also known as zyme or neurosin *(22)*, also appear to behave as tumor suppressors in breast and other tissues. Additionally, and contrary to what is widely understood by nonspecialists, PSA expression in malignant prostatic and breast tissues is generally reduced in comparison with healthy or benign hyperplastic tissues *(23–28)*. In fact, in a very recent report, patients with low tissue PSA concentrations had more rapidly progressing disease and died more frequently from prostate cancer than patients who had relatively high tissue PSA concentrations *(29)*. The increased serum concentration of PSA in prostate cancer is attributable to increased cell numbers, destruction of the prostatic architecture, and diffusion of higher amounts of PSA into the general circulation. These findings, collectively, suggest that PSA is a molecule whose expression may be beneficial to breast and prostate, and its down-regulation may predispose to cancer or may be associated with more aggressive disease. It has thus been suggested that efforts to produce cancer vaccines or other therapies targeting PSA expression may be the wrong strategy and that treatment approaches to treat prostate, and possibly breast, cancer should be directed toward overexpression of PSA at the tissue (tumor) level *(30, 31)*. In other words, PSA should be considered as a "cancer fighter" at the tissue level and as a "valuable messenger" (indicator) at the level of the systemic circulation, which can be used to either detect or monitor cancer. The proverb "Do not kill the messenger" may be applicable in this case.

Table 1. Evidence that PSA may have beneficial properties.

	References
• PSA is down-regulated in prostate cancer tissues in comparison with healthy or hyperplastic prostatic tissues.	*(28)*
• Lower tissue PSA is associated with more aggressive forms of prostate cancer.	*(29)*
• PSA is down-regulated in cancerous breast tissues in comparison with healthy or hyperplastic breast tissues and in more aggressive forms of breast cancer (in tumors that are steroid hormone receptor-negative, tumors with high S-phase fraction, larger tumors, aneuploid tumors). Patients with PSA-positive tumors have earlier disease stage, live longer, and relapse less frequently.	*(3, 25–27)*
• Lower PSA concentrations in nipple aspirate fluid of women are associated with higher risk for developing breast cancer.	*(24)*
• PSA inhibits growth of MCF-7 breast carcinoma cell lines and stimulates conversion of estradiol to estrone in this cell line. No effect on the MDA-MB-231 cell line.	*(43)*
• Transfection of PSA cDNA in PC-3 prostate carcinoma cells prolongs their doubling time, reduces their tumorigenicity and metastasis in nude mice (and improves the survival of such animals), and induces apoptosis.	*(44)*
• PSA inhibits endothelial cell proliferation, migration, and invasion. PSA inhibits endothelial cell responses to angiogenic stimuli (FGF-2[a] and VEGF). PSA reduces lung tumor numbers in mice. PSA is a new antiangiogenic molecule.	*(30)*
• PSA releases antiangiogenic fragments (angiostatin-like) by digestion of plasminogen.	*(23)*
• PSA cleaves PTHrP and abolishes its biological function. PSA may inhibit bone resorption and modulate prostatic growth.	*(39, 40)*

[a] FGF-2, fibroblast growth factor-2; VEGF, vascular endothelial growth factor; PTHrP, parathyroid hormone-related peptide.

Another set of publications pinpoints the fact that PSA may be deleterious to breast, prostate, and other tissues. For example, in one clinical study, it was found that breast tumors that produced higher PSA concentrations appeared to be more resistant to tamoxifen treatment when compared with tumors negative for PSA *(32)*. In another clinical trial of metastatic breast cancer patients to whom medroxyprogesterone acetate (a synthetic progestin) was given, it was found that the patients who responded with higher PSA production in their tumors, as assessed by measuring serum PSA, died more frequently than those patients who did not produce any PSA *(33)*. This study

Table 2. Evidence that PSA may be deleterious in cancer.

	References
• Breast tumors with higher PSA content do not respond well to tamoxifen therapy.	*(32)*
• Patients with breast tumors that produce PSA after stimulation by medroxyprogesterone acetate have worse prognosis than patients with tumors that do not produce PSA.	*(33)*
• PSA may cleave IGFBP-3, thus liberating IGF-I, which is a mitogen to prostatic stromal and epithelial cells.	*(35–37)*
• PSA may activate latent TGF-β,[a] stimulate cell detachment, and facilitate tumor spread.	*(38)*
• PSA may proteolyse basement membrane and mediate invasion and metastasis.	*(34)*

[a] TGF-β, transforming growth factor-β.

suggested that PSA production in response to medroxyprogesterone acetate treatment is an indicator of failure of this therapy in metastatic breast cancer. Webber et al. *(34)* put forward the idea that like other proteases, PSA may facilitate prostate cancer cell invasion and metastasis. These authors suggested that targeting of such activity may be a strategy for prevention or early intervention in prostate cancer patients. A few other reports have further suggested that PSA is an insulin-like growth factor binding protein-3 (IGFBP-3) protease that, through its proteolytic action, releases free, bioactive insulin-like growth factor I (IGF-I) previously bound to IGFBP-3 *(35)*. IGF-I is a known mitogen of many cell types and a risk factor for prostate and breast carcinoma development *(36)*. Furthermore, a weak stimulation of growth of prostatic stroma cells and prostatic epithelial cells was identified for PSA, despite the fact that a receptor for this molecule has not yet been discovered *(37)*. Others have suggested that PSA may activate latent transforming growth factor-β *(38)* or may cleave parathyroid hormone-related peptide *(39, 40)*. The data suggesting that PSA may be a deleterious molecule in cancer are summarized in Table 2.

Why Do These Studies Produce Seemingly Equivocal Results?

One reason that these studies have produced equivocal results is that the experiments performed are not directly comparable because different cell types were used (healthy vs malignant cells) and the experimental conditions in the in vitro and in vivo carcinogenesis models were different. In addition, some data were produced using plasmid transfections, which do not represent physiological situations, and many of the other experimental systems utilized use isolated conditions, with only one or two components of the possible biological network of PSA. Of course, none of these experimental systems accurately resembles the prostatic architecture and the interactions between the various cell components (e.g., epithelium, stroma, neural networks, and paracrine actions). Moreover, an important technical issue is that the PSA used in such studies was purified from seminal plasma, and it was very likely contaminated with other homologous serine proteases/kallikreins (e.g., hK2 and prostase/KLK-L1), which have similar molecular weights and other physiological properties. hK2 has already been shown to have thousands of times higher enzymatic activity than PSA *(4)*. Therefore, minor impurities of hK2 in PSA preparations may lead to erroneous conclusions regarding enzymatic activity and function. Only the use of recombinant proteins guarantees that these preparations are free of contamination by other serine proteases, and recombinant proteins have not been generally used in the cited experiments. Additionally, a significant portion of purified PSA from seminal plasma is internally clipped and thus biologically inactive *(41)*. The presence of various proteinase inhibitors in seminal plasma (including

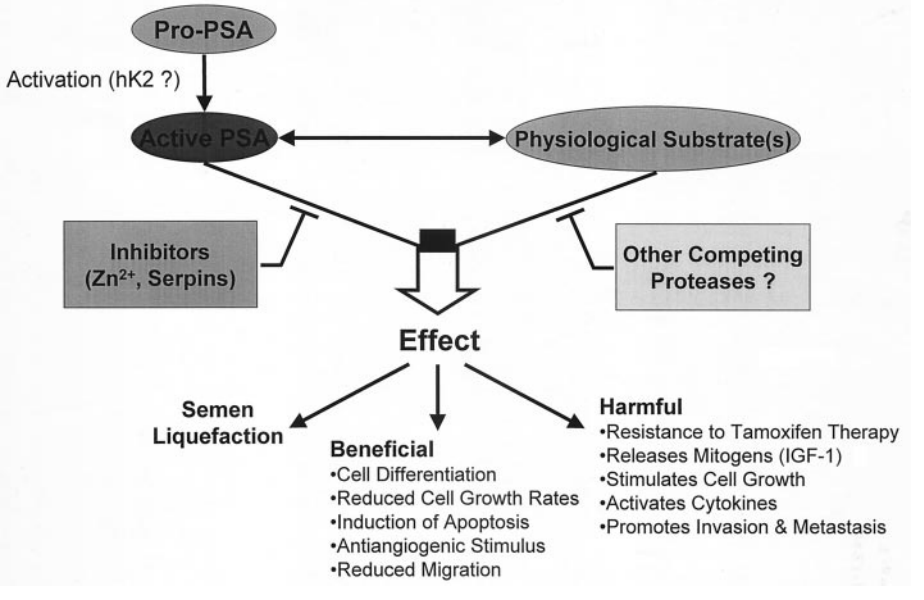

Fig. 1. Simplified network of PSA physiology.

Many components of this system have been identified in the prostate, but the final effect (if any) regarding initiation and progression of prostate cancer is not known. The PSA-activating enzyme may be hK2 *(40)*. Known physiological substrates include seminogelins and fibronectin *(18)*. Other competing proteases may include hK2, prostase/KLK-L1, cathepsin D, and others. Inhibitors include Zn^{2+}, α_2-macroglobulin, protein C inhibitor, and α_1-antichymotrypsin. See text discussion.

α_1-antichymotrypsin, α_2-macroglobulin, and protein C inhibitor) may further complicate the interpretation of the data.

A simplified physiological network for PSA, which includes only minimal components, is shown in Fig. 1. PSA is secreted as a pro-enzyme (pro-PSA), which must be activated by removal of the first seven amino acids (activation) *(6)*. This process may be mediated by hK2 *(42)*. Active PSA can then act on and hydrolyze its physiological substrate(s) and mediate its biological effect. Competing with this process are other proteases in prostatic, breast, or other tissues, or in seminal plasma and other fluids (e.g., milk and nipple aspirate fluid), which may also act on the same substrate(s), and several PSA inhibitors (including Zn^{2+}, α_2-macroglobulin, protein C inhibitor, and α_1-antichymotrypsin). In many of the currently published reports, the biological function of PSA is examined without considering the surrounding environment, which no doubt is likely more complex than the one shown in Fig. 1. Thus, the generation of apparently conflicting data should not be surprising.

In conclusion, there is no doubt that PSA is a valuable biomarker of prostatic carcinoma. Despite its widespread clinical use, we do not as yet understand its physiological function and its relation to the initiation and progression of prostate, breast, or other cancers. The newest published discoveries *(29–31)* point to the fact that PSA may act as an anticancer, antiproliferative, antiangiogenic agent that may confer protection to the prostate gland (and possibly the breast) against developing cancer. Other new data further suggest that additional members of the expanded kallikrein gene family may also behave as putative tumor suppressors *(7, 20–22)*. It will be important to undertake more research toward further understanding the physiology of this gene family in various tissues. These approaches may lead to the application of PSA and other kallikreins, not only as valuable biochemical markers but also as promising therapeutic targets.

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