Review

The diagnostic and prognostic utility of prostate-specific antigen for diseases of the breast

Margot H. Black[1] and Eleftherios P. Diamandis[1,2]
[1]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital;* [2]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada*

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Summary

Although prostate-specific antigen (PSA) is the most valuable tumor marker for the diagnosis and management of prostate carcinoma, it is widely accepted that PSA is not prostate specific. Numerous studies have shown that PSA is present in some female hormonally regulated tissues, principally the breast and its secretions. In this review, we summarize the findings of PSA in the breast, and focus on its potential for clinical applications in breast disease. PSA is produced by the majority of breast tumors and is a favorable indicator of prognosis in breast cancer. Low levels of PSA are released into the female circulation, and while the level of serum PSA is elevated in both benign and malignant breast disease, the molecular form of circulating PSA differs between women with and without breast cancer. These findings indicate that PSA may have potential diagnostic utility in breast cancer. PSA may also have a clinical application in benign breast disease, as both the level and molecular form of PSA differ between Type I and II breast cysts. High levels of PSA have been reported in nipple aspirate fluid (NAF) and recent studies have shown that the concentration of PSA in NAF is inversely related to breast cancer risk, indicating that NAF PSA may represent a clinical tool for breast cancer risk assessment. Thus, PSA represents a marker with numerous potential clinical applications as a diagnostic and/or prognostic tool in breast disease.

Introduction

"The mind likes a strange idea as little as the body likes a strange protein and resists it with similar energy. It would not perhaps be too fanciful to say that a new idea is the most quickly acting antigen known to science. If we watch ourselves honestly we shall often find that we have begun to argue against a new idea even before it has been completely stated." Wilfred Batten Lewis Trotter (1872–1939), English Surgeon.

Biological research involves the perpetual exchange of novel thoughts and ideas, progressing toward a greater understanding of physiological phenomena. However, the knowledge we gain from new discoveries is inevitably coupled with insight into how much we do not know. About 20 years ago, we were introduced to a protein named prostate-specific antigen (PSA). Discoveries over the past decade have provided every indication that we were exposed to only one facet of this protein's functionality.

PSA is a well-established tumor marker for the diagnosis and management of prostate cancer. With the advent of more sensitive methodologies for PSA detection and measurement came the finding that PSA is not prostate specific, but is present in female tissues, predominantly the breast and its secretions. Furthermore, PSA has numerous potential clinical applications in breast disease as a predictive indicator for prognosis, diagnosis, and response to treatment.

Throughout the course of this review, evidence that PSA is a misnamed, multifunctional protein with diverse potential applications will be presented.

The human kallikrein family

The gene encoding PSA is a member of the human glandular kallikrein gene family, the locus of which is comprised of three genes and spans a 60–70 kb region on chromosome 19q13.3-q13.4 [1, 2]. The classical

members of the human kallikrein family are hKLK1, hKLK2, and hKLK3, which encode three extracellular serine proteases hK1, hK2, and PSA (hK3), respectively [1, 2]. More recently, a large area of this locus has been characterized and evidence was provided that the kallikrein gene family may be comprised of at least 13 genes in humans [3].

Originally, the kallikreins were defined as proteases which cleave vasoactive peptides (kinins) from kininogen precursors [4]. Human tissue kallikrein (hK1, also referred to as urinary kallikrein or pancreatic/renal kallikrein) is the only member of the glandular kallikrein family known to possess true kininogenase activity [5] and was the first kallikrein to be discovered [6]. The term 'kallikrein' was extended to hK2 and PSA based on their structural similarities to hK1 [7]. hK1 exhibits trypsin-like specificity and plays a role in the maintenance of blood pressure by regulating the liberation of vasoactive bradykinin from kininogen [6] and is primarily expressed in human renal, pancreatic tissue, and salivary glands [8].

Human glandular kallikrein (hK2) was discovered relatively recently [9], and its expression has been reported primarily in the prostate gland [10]. Like hK1, hK2 manifests trypsin-like serine protease activity [9]. It was recently reported that the function of hK2 is to proteolytically activate PSA following its secretion into the ductal system of the prostate gland [11–13].

PSA is a serine protease [14] that is unique, amongst the human kallikreins, in its physiological substrate specificity, which is similar to that of chymotrypsin [15]. PSA is expressed by the epithelial cells lining the acini and ducts of the prostate gland [16, 17]. Following its secretion into the lumen of the prostate gland, PSA becomes a constituent of seminal fluid. Present at concentrations of 0.5–5 mg/ml [18], PSA is one of the major proteins in seminal plasma. The urological function of PSA is to liquefy the seminal coagulum formed following ejaculation. Dissolution of the gel structure occurs by proteolytic degradation of its major structural constituents, namely fibronectin and the seminal vesicle proteins semenogelin I and II [19]. The generation of soluble semenogelin and fibronectin fragments results in the release of motile spermatozoa [19]. Other reported seminal substrates for PSA are parathyroid hormone-related protein (PTHrP) and secretory leucocyte protease inhibitor (SLPI), although the significance of these relationships is unknown [20]. In seminal fluid, approximately 70% of PSA is proteolytically active [21]. Fifteen to 30% of seminal PSA

is devoid of enzyme activity and does not bind to protease inhibitors. This form of PSA, referred to as 'clipped' or 'nicked', is internally cleaved at the peptide bond between lysine$_{145}$ and lysine$_{146}$ [21] by an unidentified endopeptidase.

PSA as an oncologic marker

Low concentrations of PSA are normally released into the blood [22]. The enzymatic activity of serum PSA is regulated mainly by protease inhibitors. PSA forms stable complexes with two major extracellular hepatically-produced serine protease inhibitors, alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin (A2M) [21]. PSA complexed to ACT is the predominant form of PSA in serum [23].

A small percentage (<30%) of the total PSA occurs in a noncomplexed 'free' form, despite molar concentrations of ACT and A2M which are 10^4 to 10^5-fold higher than PSA [24]. While the covalent bonding of serine protease inhibitors to PSA diminishes PSA enzyme activity [21], it is not yet clear whether the free PSA in serum is enzymatically active. It is unlikely that the free form of serum PSA manifests enzyme activity since there is a considerable molar excess of both unreacted ACT and A2M. The free form likely represents zymogenic [25] or 'nicked' [26] PSA.

The clinical utility of PSA as a marker for prostate cancer emerged in 1980 with the initial report of elevated PSA levels in the serum of prostate cancer patients [27]. Since this time, the use of PSA as a tumor marker has flourished and PSA has proven to be the most useful marker in urologic oncology [28]. PSA immunoassays are widely used to detect early stage prostate cancer, to evaluate disease progression, and to assess therapeutic response [29]. Furthermore, PSA levels may be utilized to identify postsurgical residual disease or tumor recurrence [30].

Shortly following the discovery of different molecular forms of PSA in the serum [21], it was demonstrated that PSA–ACT accounted for a higher fraction of serum PSA in prostate cancer patients than in those with BPH [31], while free, noncomplexed PSA constituted the dominant form in patients with BPH. The difference in molecular forms between benign and malignant disease has become a promising tool for the differential diagnosis between BPH and prostate cancer. While the total PSA level alone is neither sensitive nor specific enough for the early diagnosis of prostate

Table 1. Reports of extraprostatic PSA in chronological order

Female periurethral gland	[38–40]	Breast	[62, 74]
Urethra/paraurethral		Lung neoplasm	[75–77]
gland neoplasm	[41–44]	Milk of lactating women	[71, 78]
Apocrine sweat gland/		Ovarian neoplasm	[79]
sweat gland neoplasm	[45]	Pancreas/pancreatic	
Urachus	[46]	neoplasm	[49, 80]
Bladder neoplasm	[47–49]	Breast carcinoma sera	[60, 81–85]
Cloacogenic glandular		Female sera	
epithelium/anal gland	[50]		[61, 71, 86–89]
Ovarian cystic teratoma	[51, 52]	Broanchoalveolar lavage	
Male periurethral gland	[53]	fluids	[71]
Salivary gland/		Breast cystic disease	[61, 62, 65, 71, 90–93]
salivary gland neoplasm	[45, 54, 55]		
Male accessory sex glands	[56]	Nipple asprirate fluid	[94–96]
Breast neoplasm	[57–66]	Ascitic fluid	[97]
Uterus/endometrium	[49, 67]	Cerebrospinal fluid	[98]
Extramammary paget disease	[68]	Pleural efflusions	[99]
Adrenal/colon/kidney/		Hirsute female sera	[100]
liver/parotid neoplasm	[69]	Thyroid/thyroid neoplasm/	
Amniotic fluid	[70–72]	bile duct neoplasm	[49]
Bone marrow	[73]	Neuroblastoma cell lines	[101]

cancer, the ratio of free to total PSA may improve both sensitivity and specificity [32].

The application of hK2 as an additional serum marker for the detection, prognosis, and monitoring of prostate cancer patients has recently emerged [33–37]. The measurement of both kallikreins introduces the possibility of multiple parameter testing for prostate cancer.

Extraprostatic PSA

The name 'prostate-specific antigen' reflects the initial widespread belief that expression of this protein was restricted to the prostate gland. The notion that PSA is prostate specific has, however, clearly been dispelled. Numerous studies have shown that PSA is expressed extraprostatically, suggesting that PSA may be functional outside the prostate gland. Table 1 summarizes the published reports of extraprostatic PSA.

Even more remarkable than the discovery of nonprostatic PSA were the findings that hormone-dependent tissues in females, such as the breast, produce PSA. The periurethral (Skene's) gland was the first female tissue that was reported to produce PSA [39]. This tissue has been referred to as the 'female prostate', as its developmental origin is common to

that of the male prostate [40, 111]. It may therefore not be surprising that such a gland produces PSA, regardless of the gender in which it is found.

Since the initial discovery of PSA in females, numerous normal and pathological tissues and bodily fluids have been reported to have PSA immunoreactivity. PSA is detectable in healthy breast tissue [62, 74], and is present in breast tumors [57–66] and breast cystic disease [61, 62, 65, 71, 90–93]. Various breast secretions contain PSA, including nipple aspirate fluid (NAF) [94–96], the milk of lactating women [71, 78], and breast cystic fluid [90–93]. Endometrial tissue produces PSA [67], as do ovarian tumors [79], and PSA is present in amniotic fluid [70–72]. Low levels of circulating PSA are detectable in female sera [61, 71, 81–89].

In retrospect, it seems logical that hormonally-regulated tissues such as the breast, ovaries, and endometrium would produce PSA in response to steroid hormone stimulation. It has been established that the proximal promoter of the PSA gene contains three functionally active androgen responsive elements (AREs), the activation of all of which is required for optimal levels of PSA transcription [102]. Extensive study of these AREs revealed a number of nonandrogenic PSA gene regulatory mechanisms. Androgen, progestin, glucocorticoid, and mineralo-

corticoid receptors utilize the same steroid response elements [103], and the ability of glucocorticoids and progestins to activate these elements in the PSA promoter has been demonstrated *in vitro* [102, 104, 105] in a variety of cell lines.

There are other indications that PSA may be of significance extraprostatically. A number of PSA substrates exist in addition to those in seminal plasma. PSA can proteolytically cleave and activate latent TGF-β [106]. PSA has been shown to activate urokinase-type plasminogen activator [107], the enzyme responsible for the conversion of plasminogen to plasmin, yet this relationship is questionable [108]. The extracellular matrix protein laminin, in addition to fibronectin, is proteolytically modified by PSA [109]. PSA has been reported to hydrolyze insulin A and B chain, gelatin, recombinant interleukin 2, and, to a lesser extent, myoglobulin, ovalbumin, and fibrinogen [14].

Furthermore, PSA shows extensive amino acid sequence identity with a number of growth factors, such as γ-nerve growth factor (56%), tonin (54%), epidermal growth factor-binding protein (53%), and α-nerve growth factor (51%) [14]. PSA can enzymatically digest insulin-like growth factor binding protein 3 (IGFBP-3), thereby decreasing the binding capacity for and increasing the availability of insulin-like growth factors (IGFs) [110]. Therefore, the function of PSA may extend beyond the realm of seminal dissolution to growth regulation.

The levels of PSA in females are generally quite low. However, the study of PSA expression in non-prostatic tissues has been greatly improved by the introduction of ultrasensitive PSA assays and PCR-based assays that facilitate the measurement of PSA protein and mRNA levels with extreme sensitivity. The measurement of PSA in female tissues, particularly the breast, has potential prognostic and diagnostic utility in a number of areas, as described below.

PSA as a prognostic indicator in breast cancer

PSA immunoreactivity in breast cancer cytosolic extracts was first identified in 1994 [57–59]. Recent studies with newer, ultrasensitive immunoassays demonstrated that approximately 70% of breast tumor cytosolic extracts contain immunoreactive PSA [112]. The localization of breast tumor PSA was accomplished using immunohistochemistry with anti-PSA monoclonal antibodies [64, 65]. The presence of PSA

in breast tumors as identified by immunoassays correlated with that detected immunohistochemically [65]. Western blotting and high performance liquid chromatography (HPLC) demonstrated that breast tumor PSA was of the same molecular weight as seminal PSA [57]. Molecular analysis verified that the mRNA of breast tumor PSA was identical in sequence to prostatic PSA [58]. DNA sequencing confirmed that no mutations were present in the coding region of the PSA gene in breast tumors [113, 114].

PSA positivity in breast tumors was associated with estrogen and progesterone receptor positivity [59], not surprisingly, since the PSA gene is steroid hormone responsive. It remains elusive which steroid hormones are involved in PSA gene regulation as the apparent association between estrogen receptors and PSA may be due to the mediation of progesterone receptor expression by estrogen receptors. *In vitro* experiments have confirmed the association between mammary PSA production and hormonal stimulation. The steroid hormone receptor-positive breast cancer cell lines T-47D and BT-474 can be induced by androgens, glucocorticosteroids, mineralocorticosteroids, and progestins to produce PSA [105, 115], whereas estrogen failed to induce any significant PSA production in either cell line [115]. The stimulation of PSA in all cases was found to be dose- and time-dependent. The molecular weight, mRNA sequence, and DNA coding sequence of PSA produced by breast cancer cells *in vitro* are identical to that of seminal/prostatic PSA [105, 113–115]. The production of PSA was observed exclusively in breast cancer cell lines that have steroid hormone receptors.

An *in vivo* model for the induction of mammary PSA expression was established to complement *in vitro* studies. This system consisted of human breast tumors growing as xenografts in severe combined immunodeficient (SCID) mice. Tumors stimulated with a progestin produced significantly more PSA than nonstimulated tumors [116]. The association between steroid hormone receptors and PSA production was also demonstrated *in vivo* in breast tissue. PSA is expressed at low levels by healthy mammary tissue [62, 74]. Breast cytosolic extracts from women receiving progestin-containing oral contraceptives had considerably more PSA immunoreactivity [74], confirming the hormonal dependency of PSA production.

Breast cancer is a heterogeneous disease. Given the relationship between PSA and steroid hormone receptor positivity, the presence of PSA in breast tumors is not a random event. However, the complete

molecular and physiological mechanisms behind its production have not yet been elucidated. The majority of PSA-producing breast tumors are steroid hormone receptor positive; however, not all steroid hormone receptor-positive tumors produce PSA. Similarly, PSA is produced *in vitro* exclusively by steroid hormone receptor-positive breast cancer cell lines, but not all of these lines can produce PSA. There are two hypotheses which may justify the absence of PSA expression in certain steroid hormone receptor-positive breast tumors or breast cancer cell lines. Firstly, these breast cancer cells may lack essential components of the PSA production pathway. PSA production is dependent upon an intact steroid hormone receptor pathway where receptor positivity is necessary but not sufficient. Although the steroidal machinery is present, certain factors (e.g. co-activators, co-repressors) necessary for PSA transcription and translation may be absent or dysfunctional. The second postulation stems from reports of multiple mutations in the 5′-flanking region of the PSA gene in breast tumors and breast cancer cell lines [113, 114]. Sequencing of the PSA gene identified no mutations in the coding sequence of any tumors or cell lines, but multiple mutations/polymorphisms were detected in the core promoter and enhancer region. These mutational or polymorphic events may alter the steroidal regulation of the gene, subsequently affecting the PSA expression level.

The fact that not all breast tumors produce PSA prompted studies of the utilization of PSA as a prognostic indicator in breast cancer. PSA positivity in primary breast tumors was found to be significantly associated with smaller tumors, steroid hormone receptor positivity, low cellularity, diploid tumors, low S-phase fraction, less advanced disease stage, younger patient age, lower risk of relapse, and longer overall survival [112, 117]. Similarly, low levels were more often found in larger tumors, tumors of older and postmenopausal patients, and in steroid hormone receptor-negative tumors, parameters associated with poor prognosis. These data are not surprising, given the correlation between the presence of steroid receptors and a positive prognosis. The presence of PSA immunoreactivity in breast cancer cells may be a marker of a functional steroid hormone receptor system. Therefore, PSA positivity may not only be utilized for the identification of breast cancer patients with improved prognosis, but may predict their response to adjuvant therapy by providing insight into the hormonal status of the tumor.

In accordance with the regulation of the PSA gene principally by androgens and progestins, PSA production is associated with breast tumors which express progesterone [59, 112] and/or androgen [118] receptors. Furthermore, PSA is useful as a prognostic indicator only in patients with tumors showing progesterone receptor positivity and estrogen receptor negativity [117]. This parameter is not useful in patients with estrogen receptor-positive tumors. However, PSA has shown utility in estrogen receptor-positive breast tumors as a predictive parameter for determining the response of metastatic breast cancer patients to hormonal adjuvant therapy [119]. High levels of PSA in primary breast tumors were related to poor response, short duration of response, and poor overall survival after tamoxifen therapy in recurrent disease. The level of PSA in cytosols of primary breast tumors may have clinical potential as a marker to select breast cancer patients who may or may not benefit from systemic tamoxifen therapy. Given that PSA is a favorable marker of prognosis in breast cancer, the finding that PSA is elevated in tumors and disease which are refractory to tamoxifen therapy was unexpected, with no obvious explanation at present.

PSA is a novel indicator of favorable prognosis in breast cancer patients. This finding is significant because an ongoing goal in cancer research is to define biological indicators of prognosis, and to identify predictive parameters which facilitate treatment strategies based on the likelihood of response. Furthermore, insight into the mechanisms behind malignant pathology may be gained through the investigation of cell biological prognostic factors. PSA is emerging as a potential tool for the prediction of breast cancer prognosis and response to endocrine therapy.

PSA in breast cyst classification

It has been demonstrated throughout the course of several studies that most breast cysts contain immunoreactive PSA, and large amounts of PSA (up to $82 \, \mu g/l$) may accumulate in breast cystic fluid [71, 90, 92]. Cystic fluid proteins are secretory products of epithelial cells surrounding the cysts [120], suggesting that PSA is secreted by the breast epithelium. Breast cysts have been divided into two subtypes: Type I (secretory/apocrine) and Type II (transudative/flattened). Recent studies have shown that the production and intracystic accumulation of PSA was

associated more with the Type 1 cysts [61, 92, 93]. Furthermore, it has recently been reported that the ratio of free PSA to PSA–ACT differs between Type I and II cysts. Type I cysts have a significantly higher proportion of free PSA than Type II cysts. An elevated free/bound ratio is directly correlated with a high K^+/Na^+ ratio in cyst fluid [93], a parameter currently used to identify Type I cysts [121]. Therefore, the concentration of PSA in breast cystic fluid and the free/bound ratio may offer utility in cyst subclassification. This finding is significant because women with Type I breast cysts are at a higher risk for subsequent breast cancer development [121]. Furthermore, the composition of cyst fluid may provide insight into the mechanism behind cyst formation and the evolution from a benign to a malignant condition.

PSA in nipple aspirate fluid and breast cancer risk

The majority (>75%) of nipple aspirate fluid (NAF) samples contain immunoreactive PSA [94, 95]. Concentrations of up to 11 mg/l have been reported, making NAF the biological fluid with the second highest concentration of PSA (after seminal fluid). The mean, median, and peak concentration of NAF PSA were higher in pre- than in post-menopausal women [95, 96], presumably due to the higher levels of circulating steroid hormones before menopause. Hyperplasia (with and without atypia) was identified in the NAF of a subset of patients, and was correlated with low NAF PSA concentrations in comparison with that obtained from subjects with normal cytology [96]. Another study demonstrated that the concentration of PSA in NAF is inversely associated with breast cancer risk [95]. NAF from women with no risk factors for breast cancer had relatively high NAF PSA concentrations, while women with breast cancer were more likely to have a low NAF PSA measurement. This finding, in addition to the attractiveness of NAF as a clinical medium due to its accessibility, render NAF PSA a potential tool in breast cancer risk assessment.

Serum PSA as a marker in breast disease

In males, a small fraction of the PSA secreted into the prostatic ductal system enters the general circulation. With the use of ultrasensitive PSA immunoassays, it has been demonstrated that at least 50% of normal female sera contain detectable PSA [89]. The likely source of circulating PSA in females is the mammary ductal system, as PSA is expressed predominantly in breast tissue and enters its secretions. The concentration of PSA in female sera is approximately 1000-fold lower than that of the breast, a ratio similar to that of PSA in male serum versus seminal fluid. Female serum PSA is also approximately 100–500 times lower than male serum PSA.

Not surprisingly, there are numerous examples demonstrating the association between serum PSA concentrations and circulating steroid hormone levels. Firstly, serum PSA in women is inversely correlated with age, that is, post-menopausal women have lower serum PSA concentrations than pre-menopausal women, presumably due to the plunge in hormonal levels at menopause [88]. Secondly, PSA is differentially expressed during the menstrual cycle, as a rise in serum PSA levels follows the progesterone concentration peaks [88]. PSA levels were found to increase in the sera of pregnant women [72] and, furthermore, serum PSA is elevated in both male and female newborns [122]. Steroid hormones are present at high concentrations during fetal life and around the time of birth [123], and serum PSA is likely derived from hormonally responsive tissues under the influence of these hormones.

Serum PSA levels are elevated in most endocrine-dependent disorders, including breast cancer, breast cystic disease, and uterine leiomyoma [84, 85]. Furthermore, women with high levels of circulating androgens, consequently exhibiting hirsutism, have augmented serum PSA measurements [100]. The PSA increase is one result of a disrupted hormonal balance in these women, triggering the aberrant expression of hormone-dependent genes such as PSA. Although the concentration of circulating PSA in breast cancer patients is not always directly correlated with that in the breast tumor, it is likely that the tumor is the source of PSA in the serum. A recent case study demonstrated that breast cancer patients receiving oral contraceptives at the time of diagnosis and surgery experienced a 10-fold increase in serum PSA levels in comparison to pre-treatment values [124]. The concentration of circulating PSA decreased to the original level 2–3 days after tumor removal. This data indicates that PSA secreted by the tumor diffuses into the general circulation.

The measurement of circulating PSA in breast cancer patients was taken to the molecular level by Leher et al. [82]. Using RT-PCR, this group detected PSA mRNA in the blood of approximately 25% of breast

Table 2. Rationale for PSA molecular forms in breast tissue and serum

Patient group	Breast tissue		Serum
	PSA Gene	PSA Protein	
Healthy females	Normal regulation by steroid hormones	Translation of normal PSA capable of binding to ACT	Low levels of circulating PSA PSA–ACT is predominant form
Benign breast disease Uterine leiomyoma Androgen excess	Higher levels of expression due to alteration of hormonal status	Higher levels of translation of normal PSA capable of binding to ACT	Elevated levels of circulating PSA PSA–ACT is predominant form
Breast cancer	Higher levels of expression due to alteration of hormonal status	Tumor-specific post-translational modification prevents binding to ACT	Elevated levels of circulating PSA Free PSA is predominant form

cancer patients, demonstrating the potential use of PSA to detect circulating breast cancer cells.

Serum PSA has been shown to be a potential prognostic indicator in breast cancer patients receiving adjuvant treatment, such as megestrol acetate (MA) [125]. MA treatment induces PSA production in approximately 50% of breast cancer patients in a dose-dependent manner and this induction is associated with patient mortality. The subgroup of patients whose PSA increases post-MA treatment are at increased risk for death and alternative treatment may be more beneficial in these patients. Again, the reason why PSA induction is associated with an unfavorable prognosis remains unexplained and contradicts the reports of favorable outcome in breast cancer patients whose tumors showed PSA positivity and who received no drug therapy.

Serological PSA circulates in the presence of serine protease inhibitors with which it may complex. PSA is able to bind to ACT and A2M, which are normally present in the serum [21]. The quantification of free PSA versus PSA–ACT in the sera of males has proven to be valuable in distinguishing between malignant and benign prostatic disease [32]. Therefore, the molecular forms of PSA in female sera were investigated using high performance liquid chromatography (HPLC) [81, 83, 84] and ultrasensitive immunoassays which could differentiate between free PSA and PSA–ACT [85]. The results of these studies were intriguing. PSA in the sera of healthy women is able to bind to ACT. Consequently, analysis of the PSA fractions in normal female sera using HPLC showed that PSA–ACT was the major fraction [81, 83, 84]. Free PSA was undetectable in the vast majority of sera

from normal females using an ultrasensitive free PSA immunoassay [85].

Women afflicted with endocrinopathies, such as benign breast disease, uterine leiomyoma, or androgen excess, also had PSA–ACT as the major form in sera. [81, 83–85]. Free PSA was detectable in the sera of these patients, but the majority of the serological PSA occurred in a complex with ACT.

The development of breast cancer has an interesting effect on the molecular forms of PSA in the serum. The predominant serological form (>50%) of PSA in a significant proportion of females with breast cancer is free PSA [81, 83–85]. Moreover, the concentration of free PSA in serum decreases in breast cancer patients following surgery while total PSA is only slightly decreased, indicating that a major component of total PSA (in this case PSA–ACT) is not produced by tumor cells, but more likely by normal breast tissue. These findings are summarized in Table 2.

If serum PSA originates from breast tissue, the high proportion of free PSA in breast cancer patients likely originates from the tumor itself. It is likely that the free PSA produced by breast tumors is incapable of binding to serine protease inhibitors such as ACT, given the large molar excess of serum ACT in comparison to PSA. Given that the PSA coding and mRNA sequences are not modified in breast tumors, PSA likely undergoes a post-translational modification which prevents complex formation with ACT, thereby increasing the proportion of free PSA. Alternatively, free PSA may circulate in the 'nicked' or zymogenic form, as is postulated in prostate cancer patients.

Since free PSA is highly specific for breast cancer in comparison to post-surgical breast cancer patients,

women with benign breast disease, and healthy women, the measurement of the relative forms of PSA in the sera of women may have clinical potential. Free PSA may have potential clinical applicability as a circulating tumor marker either alone or in combination with other diagnostic markers. Numerous attempts are presently being made to identify serological markers of breast tumors. Immunological tests based on the measurement of a biochemical parameter in the blood, the concentration of which is altered in breast cancer but not in normal situations or benign breast diseases, represent a noninvasive, cost-effective, time-efficient diagnostic tool. These characteristics are lacking in other diagnostic modalities such as mammography. However, there are no known serum markers which could be used to diagnose breast cancer with good sensitivity and specificity and hence, no such test has yet been developed. A considerable number of tumor markers and other biochemical tests are used for the monitoring of breast cancer patients, but no such tests exist for diagnosis.

The major difficulty in using free PSA for breast cancer diagnosis is its very low concentration in serum. Many patients have undetectable PSA while others have concentrations close to the detection limit of the most sensitive free PSA immunoassay [126]. Clearly, immunoassays for free PSA with a detection limit of <0.1 ng/l are needed for such applications, but they do not exist at present.

PSA in amniotic fluid

Human amniotic fluids contain detectable PSA [70–72]. The concentration of PSA increases with gestation from week 11 to 21, at which time it plateaus or slowly drops. The presence and change of PSA in amniotic fluid have an impact on the concentration of PSA in the serum of pregnant women, as it parallels that of amniotic fluid.

Recent investigations have suggested that PSA in maternal serum may be a useful screening parameter in order to identify mothers at risk of carrying fetuses affected with abnormalities. High PSA concentrations in maternal serum are associated with the incidence of Down's syndrome [127] and the utility of PSA in diagnosing this, and other, fetal abnormalities is being investigated. However, these findings have not been confirmed in subsequent studies [128] despite the fact that amniotic fluid PSA was detected and found to be lower in patients affected with Down's syndrome.

Physiological role of extraprostatic PSA

A multitude of unanswered questions exists regarding the functional role of nonprostatic PSA. In fact, it remains elusive whether mammary PSA is even enzymatically active. One hypothesis is that breast tumor PSA is devoid of enzymatic activity. The rationale behind this postulation is that PSA produced by breast tumors and released into the sera of breast cancer patients often circulates in the free form, despite the large molar excess of serine proteases with which it may bind. The small fraction of free PSA in male sera has been shown to be the zymogen proPSA or nicked PSA, both of which are enzymatically inactive. Similarly, PSA expressed in breast tumors and released into the circulation may be zymogenic or nicked. Alternatively, PSA produced by malignant breast tissue may undergo a tumor-specific post-translational modification such that its enzymatic activity is lost. The tumor specificity of this mechanism is stressed since female breast cancer patients have a higher proportion of circulating free PSA.

The next logical question is that if breast tumor PSA is enzymatically active, does it play a stimulatory or inhibitory role in tumor progression? Some findings support the notion that PSA is involved in the promotion of tumor growth and metastasis. PSA is a protease, a family of proteins known to be involved in tumor progression. In fact, it is known that enzymatically active PSA is mitogenic, stimulates cell detachment and metastasis [106], and degrades the extracellular matrix proteins, facilitating local invasion [109]. PSA converts latent TGF-β to its active form [106], high levels of which may be associated with metastasis [129]. PSA is an IGFBP-3 protease [110]. IGFBP-3 modulates the levels of IGF-1 in the blood and degradation of IGFBP-3 results in a loss of affinity of the binding protein for IGF-1, and a concomitant increase in circulating IGF-1 [110], a potent mitogen for breast cancer cells [130], and decrease in IGFBP-3, a potential apoptosis mediator [131]. The hypothesis that PSA promotes tumor progression supports the rationale that PSA is associated with a positive prognosis simply because it is a product of steroid hormone regulatory mechanisms present in the well-differentiated cells that constitute the most treatment-responsive tumors. However, this school of thought maintains that although it is produced by well-differentiated cells, PSA plays a detrimental role in cancer progression.

An alternative hypothesis is that PSA plays a functional role in the inhibition of tumor progression. This

scenario upholds the notion that relatively high levels of PSA are present in tumors with the most favorable prognosis not only because of the association with steroid hormone receptors, but because PSA represses tumor growth and metastasis. It has been suggested that the expression of PSA may occur in response to androgens, which interfere with the estrogenic effect on the promotion of breast cancer cell growth [132]. Lai et al. [91] reported that PSA stimulates the conversion of the potent estradiol (E_2) to the less potent estrone (E_1), thereby inhibiting the growth of certain breast cancer cell lines *in vitro*. A recent study reported that the expression of induction of PSA expression in a PSA-negative prostatic carcinoma cell line supressed tumorigenicity and metastasis due to an increase in apoptosis [133]. PSA proteolytically cleaves parathyroid hormone-related protein (PTHrP) [134], present in both seminal fluid and breast tissue. The cleavage of this protein by PSA may represent an inhibitory role in breast cancer progression as PTHrP stimulates breast cancer cell proliferation *in vivo* and *in vitro*, and a role in breast cancer metastasis has been suggested as PTHrP is expressed in most osseous metastases [135]. Finally, it has been suggested that PSA may proteolytically generate growth inhibitory peptides from the BRCA1 gene product [136].

Mammary hK1, hK2, and other kallikrein-like proteins

hK1 has been identified in normal and malignant breast tissue and in breast milk [137, 138] using Western blotting and immunohistochemistry with a monoclonal antibody specific for hK1.

Recently, Hsieh et al. [139] demonstrated that the breast carcinoma cell line T47-D produces immunoreactive hK2 and that hK2 production is under the control of androgens, progestins, glucocorticoids, and mineralocorticoids. Using an ultrasensitive hK2 immunoassay developed in our laboratory [35], we demonstrated that over half of breast tumor extracts contain immunoreactive hK2, and that the hK2 concentration was correlated with that of PSA [140]. Furthermore, tumors expressing steroid hormone receptors contained higher concentrations of both PSA and hK2. Like PSA, hK2 is present in benign breast disease as measurable quantities exist in breast cyst fluid [141]. hK2 is detectable in NAF [140] and the milk of lactating women [141], indicating that diseased breast tissue and normal breast secretions contain both PSA and hK2. The presence and possible function of these kallikreins in normal or diseased breast should be further investigated.

Two other serine proteases that are hormonally regulated map to the same region as, and have significant homology with, PSA may represent additional markers of the human kallikrein gene family. Zyme (protease M/neurosin) and NES1 were found to be downregulated in breast cancer, in comparison to breast tissue, in striking similarity to PSA [142, 143]. It is possible that all of these kallikreins may have an integrated role in breast cancer.

Conclusions

The term prostate-specific antigen is a biological misnomer. As is the case with many physiological phenomena, novel discoveries regarding PSA have clouded and complicated our understanding of this protein. It is clear that PSA is produced in extraprostatic, hormonally regulated glandular tissues such as the female breast and, consequently, can no longer be regarded as a marker specific to prostatic tissue, but as a ubiquitous molecule which can be produced by cells bearing steroid hormone receptors under conditions of steroidal stimulation.

The potential applications of PSA in breast cancer are shown schematically in Figure 1. PSA is normally produced in hormonally dependent female tissues, including the breast and endometrium. One manifestation of diseases such as breast cancer or benign breast disease is an alteration in hormonal status. This phenomenon causes an increase in PSA expression, and a subsequent rise in the levels of PSA released into the serum. This condition alone is an indication of the importance of hormone-responsive genes such as PSA. In breast cancer there is not only an increase in serum PSA, but the malignant transformation triggers an alteration in the molecular form of PSA such that complex formation with serine protease inhibitors in the serum is no longer possible, causing an increase in the concentration of circulating free PSA. The study of tumor-specific molecules such as free PSA is important for two reasons. Firstly, a greater understanding of tumor-specific proteins may provide insight into the processes behind malignant transformation. Further investigation is required into the function of extraprostatic PSA, and the molecular mechanisms by which it is produced. Secondly, circulating tumor-specific mo-
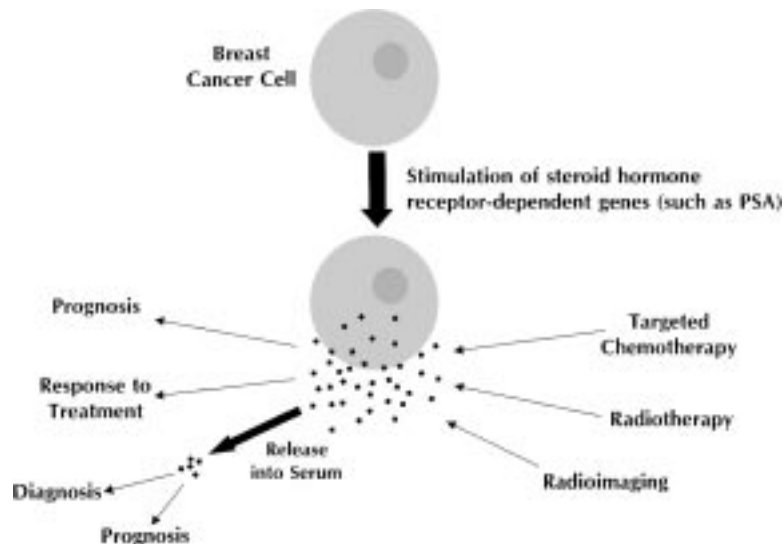
Figure 1. Clinical applications of a secreted, tumor-specific biochemical marker. Breast cancer cells containing active steroid hormone receptors can express hormonally regulated genes, such as PSA. PSA is a secreted gene product which can accumulate within the tumor milieu, and may eventually enter the circulation. Breast tumor PSA may serve as a target for treatment such as chemotherapy, and may be used to predict prognosis or to monitor response to treatment. PSA released into the serum is an easily measurable diagnostic/prognostic biochemical marker.

lecules represent potential screening tools which may facilitate early detection and diagnosis of cancer. The level and, more recently, the molecular form of circulating PSA have proven to be invaluable in prostate cancer early detection, and a similar serum marker for breast cancer would reform gynecologic oncology. Given the dire need for tumor markers, further studies are essential to potentiate its diagnostic use. Mammary PSA also has applicability at the treatment stage of breast cancer. PSA may be used as a prognostic tool, and applied in the prediction of response to treatment.

PSA not only has diagnostic potential in breast cancer, but represents a tool for risk assessment. NAF, like sera, represents a relatively accessible bodily fluid. By examining NAF PSA levels concomitantly with other risk factors, those individuals at greatest risk for breast cancer development may be better identified.

PSA has potential utility not only in breast cancer, but in benign breast disease. The concentration and form of PSA in breast cysts may be used, in addition to other components of breast cyst fluid, to classify cysts as Type I or II. This classification is important given the association of breast cancer development with certain breast cysts. PSA is the most important marker in prostatic disease, but represents a protein with a wide variety of applications in a number of disorders. The literature on extraprostatic PSA is accumulating at an alarming rate, in terms of both original papers and review articles, by a variety of researchers [144–146]. Further study will potentiate the utility of this molecule in the detection and treatment of breast disease.

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Address for offprints and correspondence: Eleftherios P. Diamandis, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5; *Tel.:* (416) 586-8443; *Fax:* (416) 586-8628; *E-mail:* ediamandis@mtsinai.on.ca