New diagnostic applications of prostate-specific antigen

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Introduction

PSA was first discovered in seminal plasma [1] isolated from prostatic tissue [2] and found elevated in the serum of patients with prostate cancer [3]. It is a 33-kDa single-chain glycoprotein. The PSA gene has extensive homology with the genes encoding proteases of the kallikrein family [4,5], serine proteases which are crucial to the processing of various polypeptide precursors to their bioactive forms. Other members of this family include toxin, which cleaves angiotensinogen to angiotensin II in the rat. γ-renin and the growth factor-processing enzymes γ-nerve growth factor and epidermal growth factor-binding protein in the mouse [6].

It is thought that, in the prostate gland, PSA is produced exclusively by the epithelial cells, and is present in semen at concentrations of around 1 g/L [7]. In the seminal fluid, PSA is involved directly in the liquefaction of the seminal coagulum by cleaving seminal vesicle proteins [8]. PSA has chymotrypsin-like enzymatic activity [7].

PSA molecular forms

PSA is present in normal, benign hyperplastic and malignant prostatic tissue and in metastatic prostatic carcinoma. In the serum of normal men, PSA levels are usually < 4 ng/mL. The serum PSA is present in two molecular forms, as free PSA with a molecular weight of ≈ 33 kDa and as PSA bound to proteinase inhibitors, predominantly α1-antichymotrypsin (ACT) (≈ 100 kDa) and α2-macroglobulin (A2M) (≈ 800 kDa) [9,10]. Current PSA immunoassays only measure the free PSA and the PSA-ACT complex: in serum, the ratio free PSA : PSA-ACT is about 1 : 4. The molecular forms of PSA in serum can be detected after separation on gel filtration columns [11]. Free PSA can now be measured directly with specific immunoassays [12,13].

Tissue-specificity of PSA – early reports

PSA is one of the most useful tumour markers because of its tissue-specificity. It is widely used for screening, diagnosis and monitoring of patients with prostate cancer. In the last few years, several reports have challenged the absolute tissue-specificity of PSA. Papotti et al. [14] reported in 1989 that some apocrine sweat gland carcinomas and some rare apocrine breast carcinomas stained positively for PSA when polyclonal antibodies were used in immunohistochemical stains. However, none of these tumours stained positively when monoclonal PSA antibodies were used and the authors concluded that the positive staining was due to cross-reactivity of the polyclonal antibodies. Importantly, none of the most common invasive breast ductal carcinomas was found positive by either antibody [14]. More recently, McLachlin and Srigley reported two cases of mature cystic teratomas of the ovary in females that contained prostatic tissue and stained positively for PSA [15]. Pummer et al. [16] reported increased levels of immunoreactive PSA in the sera of women with renal cell carcinoma, but these findings were explained as artefacts of the polyclonal antibody-based immunoassay used, as a more specific monoclonal antibody-based assay was free of such apparent interference. More recently, Van Krieken presented evidence that PSA is rarely produced by salivary gland neoplasms [17] and we confirmed this finding [18].

Several other groups have shown PSA to be present in the perirethral and perianal glands [19–21]. Considered together, these results suggest that certain tumours or tissues other than the prostate can produce PSA, although this event is extremely rare.

PSA in female serum

As women have no prostate, it was assumed for many years that women do not produce PSA in any tissue and that PSA is not present in the female blood circulation. However, PSA has been immunohistochemically localized to the female per urethral glands. This finding led Wernert et al. to propose that the ‘female prostate’ is the per urethral glands, as 67% of them stained positively for PSA and appeared histologically similar to the prostate gland before puberty [22]. With newly developed, highly sensitive assays for PSA, it was found that some females have measurable serum levels of PSA. However, the tissue origin of this PSA is still not known. We currently believe that female serum PSA arises from the breast (see below). In a study of 1061 sera from healthy
and hospitalized women, we have shown that 1.5% of them had serum PSA levels >0.10 ng/mL [23]. These PSA-positive sera were associated with women aged >50 years. We propose that steroid hormones (e.g. androgens, progestins or glucocorticoids) stimulate the target organ (female breast, periurethral glands or an unknown organ) to produce and release PSA into the circulation.

Nonprostatic PSA

The reports which challenged the absolute tissue-specificity of PSA attracted little interest because of their rarity. However, using ultrasensitive immunological assays for PSA, we recently reported that PSA is very often present in female breast tumour cytosolic extracts [24,25]. At the threshold level of 0.015 ng of PSA per mg of total protein, half of breast tumours were positive for PSA [25]; at 0.030 ng/mg, the positivity was 30% and some female breast tumours contained PSA levels > 50 ng/mg. The molecular weight of PSA in the female breast tumours was identical to that of seminal PSA and free serum PSA (≈ 33 kDa). The enzymatic activity of PSA has not as yet been tested in breast tumour extracts. Molecular characterization of breast-tumour PSA mRNA with RT-PCR and nucleic-acid sequencing has shown that this mRNA is identical in sequence with PSA mRNA from prostatic tissue [26]. Association analysis for 1275 tumours has shown that PSA was associated with the presence of progesterone receptor (PR) but not oestrogen receptor (ER) [25]. Premenopausal women and women with early stage cancer are more frequently PSA-positive than postmenopausal women or women with late-stage disease. Survival analysis has shown that patients with breast tumours producing PSA live longer and relapse less frequently [27,28]. Thus, PSA is a new favourable prognostic indicator in female breast cancer. A tissue culture system that was developed with female breast cancer cell lines has shown that PSA production in these cell lines was mediated through the action of PR and androgen receptors (AR), mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), but not ER [29]. These data are in accord with the mechanism of gene regulation by steroid hormone receptors [30].

Subsequent to our studies on breast cancer, Clements and Mukhtar reported that PSA is present in normal endometrial tissue [31]. These authors speculated that PSA may play a role as a local regulator of uterine function but a substrate for this enzyme is currently unknown. Further studies by our group have shown that PSA is a very ubiquitous enzyme. For example, we have found that a normal female breast can produce large amounts of PSA in subjects who receive progestin-containing oral contraceptives [32]. Moreover, after pregnancy, the normal breast produces PSA and secretes it into the milk of lactating women [33]. Some milk contains > 300 ng/mL of PSA while some contains only traces. Breast discharge fluid contains more PSA than any other female or male fluid except for seminal plasma; we have found levels up to 4000 ng/mL (unpublished data). We also found PSA in breast cystic fluid [34] and in amniotic fluid [35]. In amniotic fluid, PSA concentration increases with gestation from weeks 11–21 and levels off or declines slowly afterwards [35]. Cases of fetuses with various congenital abnormalities associated with abnormal PSA levels in amniotic fluid have been identified recently by our group [35].

A more recent survey of various tumours has revealed that at least some ovarian, liver, kidney, adrenal, colon, parotid and lung tumours produce PSA [36,37]. As many of these tissues are known to contain steroid hormone receptors, these findings have led us to speculate that any tissue which contains steroid hormone receptors has the ability to produce PSA, provided that the cognate-stimulating steroid hormones are also available. The stimulating hormones could be exogenously administered or endogenously released by the adrenals or the ovaries. The AR, GR, MR and PR have the ability to regulate PSA gene expression through the same hormone-response element present in DNA [29,30].

Physiological role of PSA in nonprostatic tissue

The physiological role and importance of the presence of PSA in many normal, benign or malignant tissues, extracts and fluids is currently unknown. PSA in all tissues and fluids examined, except serum, is predominantly present as a 33-kDa monomer. Complexes with proteinase inhibitors also exist, but at much lower concentrations. We propose that PSA acts enzymatically upon one or more substrate(s) and modifies their action in a fashion similar to the function of the other proteinases of the kallikrein family. Such substrates remain to be identified. Recent data on prostatic tissue could be extrapolated to support the view that PSA may be involved in growth regulation of mammary and other tissues. The sequence of PSA shows extensive homology with γ-nerve growth factor (56%), epidermal growth-factor-binding protein (53%) and α-nerve growth factor (51%) [38]. In addition, PSA can enzymatically digest IGF binding protein III (IGFBP-3). This activity is thought to regulate IGF-1 concentration because digestion of IGFBP-3 by PSA releases biologically active IGF-1 [39]. Findings by other groups further support the hypothesis that PSA is a regulator of IGFBP-2 and IGFBP-3 in patients with prostate cancer [40]. Killian et al. have recently found that PSA has mitogenic activity, presum-
ably due to activation by PSA of latent transforming growth factor β (TGF-β) and through the modulation of cell adhesion [41]. Others have shown that PSA binds and inactivates protein-C inhibitor [42].

Recently, Fichtner et al. [43], using an in vivo rat bladder model, found that PSA released kinin-like substance(s) after proteolysis of seminal vesicle fluid, and this substance(s) was able to stimulate smooth muscle contraction. This finding supplements previous investigations which showed that PSA has chymotrypsin-like activity and is able to digest seminal vesicle proteins like semenogelins and fibronectin, thus causing seminal liquefaction.

Our detection of PSA in breast, colon, ovarian, parotid, kidney, lung and liver tumours, stimulated normal breast, amniotic fluid and breast milk, and data presented by others for normal endometrium suggest that PSA can no longer be regarded as a specific prostatic marker and as a physiological molecule associated only with semen liquefaction. Instead, PSA should be regarded as a molecule which could be produced by cells bearing steroid hormone receptors under conditions of steroid hormone stimulation. The biological role of PSA in normal tissues, in tumours and during pregnancy raises numerous questions which will only be answered by further investigation.

**Diagnostic value of serum PSA in women**

In a recent paper [44], we examined whether total PSA in female serum has any diagnostic value for breast cancer. We concluded that there were no substantial differences in total serum PSA between normal women and women with breast cancer. However, more recent data [45 and patent pending] have shown that PSA subfractions may have diagnostic value. In normal women, most serum PSA is in the complexed form (PSA-ACT). In women with breast cancer, a substantial proportion exists as free PSA. Based on these findings, we developed a model for PSA regulation by breast cancer cells (Fig. 1).

**Ultrasonic PSA assays and PCR**

Most of the new data on PSA have been generated with assays that have detection limits of <0.01 ng/mL [11,46]. Currently, the most sensitive PSA assay available commercially is the IMMULITE® assay [46].

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Fig. 1. PSA is produced by steroid hormone receptor-positive breast epithelial cells under regulation by progestins/androgens. Normal epithelial cells produce and secrete enzymatically active PSA which binds to $\alpha_1$-antichymotrypsin when it enters the general circulation. Breast tumours seem to produce and secrete enzymatically inactive PSA (either internally ‘nicked’ PSA, pro-PSA or KLK-2) which cannot bind to $\alpha_1$-antichymotrypsin when it enters the general circulation.

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with a functional sensitivity of $\approx 0.005–0.01 \text{ ng/mL}$ and our latest PSA assay, which has a functional sensitivity of 0.001 ng/mL [46]. Using these assays, we and others, have shown that prostate cancer relapse after radical prostatectomy can be detected much earlier than by using less sensitive assays [47,48]. RT-PCR for PSA mRNA can detect a single prostate cell [49–52], and is useful for detecting micrometastases in lymph nodes [51] and cancer cells in the blood [50,52,53]. I anticipate that such assays will be applied to breast cancer in the near future.

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