# Prostate Specific Antigen - New Applications in Breast and Other Cancers

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**Abstract.** *Prostate specific antigen was, until recently, thought to be a highly specific biochemical marker of prostatic epithelial cells which is not produced by any female tissue. We have used immunological and molecular techniques to demonstrate the presence of PSA protein or mRNA in various non-prostatic tissues. We have recently found that PSA is present in 30-40% of breast tumors and at a lower percentage in other tumors including lung, colon, ovary, liver, kidney, adrenal and parotid tumors. Others have found PSA in skin and salivary gland tumors and in normal endometrium. We found PSA in the normal breast, the milk of lactating women, breast discharge fluid and in amniotic fluid. We developed a tissue culture system that reproduces the phenomenon of PSA production by breast tumors. Using this system, we showed that PSA regulation is under the control of steroid hormones and their receptors. PSA is produced by normal, hyperplastic and malignant breast tissue and is present in all breast secretions and some other tumors. The physiological role of PSA in these tissues, fluids and tumors is currently unknown. A substrate for PSA in these tissues has not as yet been identified.*

Prostate specific antigen (PSA) was first discovered in seminal plasma by Hara *et al* in 1971 (1). In 1979, PSA was isolated from prostatic tissue (2) and in 1980 it was found that it is elevated in the serum of prostate cancer patients (3). Biochemically, PSA is a single chain glycoprotein with a molecular weight of ~33 KDa. The PSA gene has been cloned and sequenced and found to have extensive homology with the genes encoding proteases of the kallikrein family (4,5). Kallikreins are serine proteases which are crucial to the processing of various polypeptide precursors to their bioactive forms. Other members of this family include tonin 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).

PSA is a kallikrein-like serine protease that is thought to be exclusively produced by the epithelial cells lining the acini and ducts of the prostate gland. PSAis present in the semen at concentrations around $10^6$ μg/L (7). In the seminal fluid, PSA is involved directly in the liquefaction of the seminal coagulum that is formed at ejaculation by cleaving a number of seminal vescicle proteins (8). PSA has chymotrypsin-like enzymatic activity (7).

## Molecular Forms of PSA

PSA is present in normal, benign hyperplastic, malignant prostatic tissue and in metastatic prostatic carcinoma. In the serum of normal men PSA levels are usually below 4 μg/L. The serum PSA is present in two molecular forms: (a) as free PSA with a molecular weight of ~33 KDa (b) as PSA bound to proteinase inhibitors, predominantly $a_1$-antichymotrypsin (ACT) with a molecular weight of ~100 KDa and $a_2$-macroglobulin (A2M) with a molecular weight of ~800 KDa (9,10). Current commercial PSA immunoassays can measure only the free PSA and the PSA-ACT complex; the PSAA2M complex is not recognized by anti-PSA antibodies. In serum, the ratio of 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).

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## Tissue Specificity of PSA

PSA is one of the most useful tumor markers because of its tissue specificity. It is thus widely used for screening, diagnosis and monitoring of patients with prostate cancer. In the last few years a number of reports have challenged the absolute tissue specificity of PSA. Papotti *et al* reported in 1989 that some apocrine sweat gland carcinomas and some rare apocrine breast carcinomas stained positive for PSA when polyclonal antibodies were used in immunohistochemistry. However, none of these tumors stained positive 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 were found to be positive to either antibody (14). More recently, McLachlin and Srigley reported two cases of mature cystic teratomas of the ovary in females which contained prostatic tissue and stained positive 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 artifacts of the polyclonal antibody-based immunoassay used since a more specific monoclonal antibody-based assay was free of such interference. More recently, Van Krieken presented evidence that PSA is rarely produced by salivary gland neoplasms (17).

A number of other groups have demonstrated PSA's presence in the periurethral and perianal glands (18-20). All these data collectively considered, suggest that certain tumors or tissues other than the prostate can produce PSA, but that 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 was immunohistochemically localized to the female periurethral glands. This finding has led Wernert *et al* to propose that the 'female prostate' are the periurethral glands, since 67% of them stained positive for PSA and haihistological appearance similar to that of the prostate gland before puberty (21). With newly developed, highly sensitive assays for PSA, it was identified 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 comes the breast (see below). In a study of 1,061 female sera from healthy and hospitalized women we have shown that 1.5% of them had serum PSA $\geq$ 0.10 μg/L (22). We have identified for the first time that these PSA-positive sera were associated with women over the age of fifty. It is reasonable to propose that steroid hormones (e.g. androgens, progestins or glucocorticoids) stimulate the target organ (female breast, periurethral glands or an unknown organ) for PSA production and release it into the circulation.

## PSA in Non-Prostatic Tissue

The isolated reports which challenged the absolute tissue specificity of PSA did not attract much interest because of their extreme rarity. However, using newly developed ultrasensitive immunological assays for PSA, we have recently reported that PSA is frequently present in female breast tumor cytosolic extracts (23,24). At the cutoff level of 0.015ng of PSA *per* mg of total protein, which is easily measurable by these ultrasensitive PSA assays, 50% of the breast tumors are positive for PSA (24). At the cutoff level of 0.030ng/mg, the positivity rate is 30%. Some female breast tumors contained PSA levels > 50 ng/mg. The molecular weight of PSA in female breast tumors was identical to the molecular weight of seminal PSA and free serum PSA (~33 KDa). The enzymatic activity of PSA has not as yet been tested in breast tumor extracts. Molecular characterization of breast tumor PSA mRNA with reverse transcription-polymerase chain reaction and nucleic acid sequencing techniques has shown that this mRNA has an identical sequence to PSA mRNA from prostatic tissue (25). Association analysis between PSA levels and levels of progesterone and estrogen receptors in female breast cancer for 1,275 tumors has clearly shown that PSA was associated with the presence of the progesterone receptor but not the estrogen receptor (24). 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 demonstrated that patients with breast tumors producing PSA live longer and relapse less frequently than patients with tumors which do not produce PSA (26,27). 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 the progesterone (PR), androgen (AR), mineralocorticoid (MR) and glucocorticoid (GR) receptor but not the estrogen receptor (ER) (28). These data are in accordance with the mechanism of gene regulation by steroid hormone receptors; PR, AR, MR and GR bind to the same hormone response element on DNA which is different from the hormone response element of the ER (29).

Subsequent to our studies on breast cancer, Clements and Mukhtar recently reported that PSA is present in normal endometrial tissue (30). The authors have speculated that PSA may play a role as a local regulator of uterine function but the substrate of this enzyme is currently unknown. Further studies by our group have shown that PSA is a ubiquitous enzyme, and that breast cancer and endometrial cells are not the only tissues that can produce it. For example, we have found that normal female breast can produce large amounts of PSA in subjects who receive progestin containing oral contraceptives (31). Moreover, post-pregnancy, the normal breast produces PSA and secretes it in large amounts into the milk of lactating women (32). Some milk contains more than 300 μg/L of PSA while others contain only traces.

Breast discharge fluid contains more PSA than any other female or male fluid with the exception of seminal plasma, levels up to 4,000 μg/L have been found by us (unpublished data). PSA was also found by us in breast cystic fluid and in amniotic fluid (33). In amniotic fluid, PSA concentration increases with gestation from week 11 to 21 and levels-off or drops slowly afterwards (33). Cases of fetuses with various congenital abnormalities associated with abnormal PSA levels in amniotic fluid have been recently identified by our group (33,34).

A more recent survey of various tumors has revealed that at least some ovarian, liver, kidney, adrenal, colon, parotid and lung tumors produce PSA (34,35). 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. As already mentioned, the AR, GR, MR and PR have the ability to regulate PSA gene expression through the same hormone response element present in DNA (28,29).

## Physiological Role of PSA in Non-Prostatic Tissue

What is the physiological role and importance of the presence of PSA in many normal, benign or malignant tissues, extracts or fluids? . At present we can only hypothesise. PSA is a serine protease and in all tissues and fluids examined, except serum, the predominant form was the non-complexed, 33KDa free PSA monomer. Complexes with proteinase inhibitors also exist but at much lower concentration. It would be reasonable to propose that PSA enzymatically acts upon one or more substrate(s) and modifies 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%) (36). In addition, PSA can enzymatically digest insulin growth factor binding protein III (IGFBP-3). This activity is thought to regulate insulin growth factor-1 (IGF-1) concentration because digestion of IGFBP-3 by PSA releases biologically active IGF-1 (37). Findings by other groups further support the hypothesis that PSA is a regulator of IGFBP-2 and IGFBP-3 in patients with prostate cancer (38). Killian *et al* have recently found that PSA has mitogenic activity, presumably due to the activation by PSA of latent transforming growth factor ß (TGF-ß) and through modulation of cell adhesion (39). Others have shown that PSA binds and inactivates protein C inhibitor (40). Our findings of PSA's presence in breast, colon, ovarian, parotid, kidney, lung and liver tumors, stimulated normal breast, amniotic fluid and breast milk and data presented by others for the 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. Given the new evidence that PSA may be a candidate growth factor or a cytokine or growth factor regulator, the biological role of PSA in normal tissues, tumors and during pregnancy may be much more complex than thought and raises numerous questions which will only be answered by further investigation.

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