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Editorial: New Biological Functions of Prostate-Specific Antigen?

Prostate-specific antigen (PSA) was first discovered in seminal plasma 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 mol wt of about 33 kilodaltons (kDa) (4). The PSA gene was cloned and sequenced, and was found to have extensive homology with the genes encoding proteases of the kallikrein family. Kallikreins are serine proteases that 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 (5).

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. PSA is present in semen at concentrations of about 10^6 μg/L. In the seminal fluid, PSA is involved directly in liquefaction of the seminal coagulum that is formed at ejaculation by cleaving a seminal vesicle protein. PSA has chymotrypsin-like and trypsin-like enzymatic activity (4).

PSA is present in normal benign hyperplastic, malignant prostatic tissue, and metastatic prostatic carcinoma. In the serum of normal men, PSA levels are below 4 μg/L. Serum PSA is present in three molecular forms: 1) as free PSA, with a mol wt of about 33 kDa; 2) as PSA bound to the proteinase inhibitor α_1-antichymotrypsin (ACT), with a mol wt of about 100 kDa; and 3) as PSA bound to α_2-macroglobulin, with a mol wt of about 800 kDa (6). Current commercial PSA immunoassays can measure only the free PSA and the PSA-ACT complex; the PSA-α_2-macroglobulin complex is not recognized by anti-PSA antibodies. In serum, the ratio of PSA/PSA-ACT is about 1:4. PSA is one of the most useful tumor markers because of its tissue specificity. It is thus widely used for screening, diagnosis, and monitoring patients with prostate cancer. 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.

Over the last few years, a number of reports have challenged the absolute tissue specificity of PSA. Papotti *et al.* (7) 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 was found positive by either antibody (7). More recently, McLachlin and Srigley (8) reported two cases of mature cystic teratomas of the ovary which contained prostatic tissue that stained positive for PSA. Pummer *et al.* (9) 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, as a more specific monoclonal antibody-based assay was free of such interference. More recently, Van Krieken (10) presented evidence that PSA is rarely produced by salivary gland neoplasms.

A number of other groups have demonstrated the presence of PSA in the periurethral and perianal glands (11–13). This finding led Wernert *et al.* (14) to propose that the "female prostate" is the periurethral glands, as 67% of them stained positive for PSA and had a histological appearance similar to that of the prostate gland before puberty. 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 unknown. In a study of 1061 sera from healthy and hospitalized women, we showed that 1.5% of them had serum PSA levels of 0.10 μg/L or more (15). We found that these PSA-positive sera were associated with women over the age of 50 yr.

The isolated reports that 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 recently reported that PSA is frequently present in female breast tumor cytosolic extracts (16, 17). At the cut-off level of 0.015 ng PSA/mg total protein, which is easily measurable by these ultrasensitive PSA assays, 50% of the breast tumors are positive for PSA (17). At the cut-off level of 0.030 ng/mg, the positivity rate is 30%. Some female breast tumors contained PSA levels of 50 ng/mg or more. The mol wt of PSA in female breast tumors was identical to that of seminal PSA and free serum PSA (~33 kDa). This form of PSA is presumably enzymatically active, but such activity was not tested in breast tumor extracts. Molecular characterization of breast tumor PSA messenger ribonucleic acid (mRNA) with reverse transcription-polymerase chain reaction and nucleic acid sequencing techniques has shown that this mRNA is identical in sequence to PSA mRNA from prostatic tissue (18). Association analysis between PSA levels and levels of progesterone and estrogen receptors (ERs) in female breast cancer for 1275 tumors clearly showed that PSA was associated with the presence of the progesterone receptor (PR), but not the ER. Survival analysis demonstrated that patients with breast tumors producing PSA live longer and relapse less frequently than patients with tumors that do not produce PSA (19) (Yu H., M. Giai, E. P. Diamandis, unpublished data) Thus, PSA is a new favorable prognostic indicator in female

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breast cancer. A tissue culture system that was developed with female breast cancer cell lines showed that PSA production in these cell lines was mediated through the action of the PR, androgen (AR), mineralocorticoid (MR) and glucocorticoid (GR) receptors, but not the ER (21). 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 (22).

Subsequent to our studies on breast cancer, Clements and Mukhtar (23) recently reported in this journal that PSA is present in normal endometrial tissue. The 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 ubiquitous enzyme, and that breast cancer and endometrial cells are not the only tissues that can produce it. For example, we found that normal female breast can produce easily measurable amounts of PSA in subjects who receive progestin-containing oral contraceptives (24). Moreover, postpregnancy, the normal breast produces PSA and secretes it into the milk of lactating women (25). Some milk contains more than 300 μg/L PSA, whereas other milk contains traces only. PSA was also found by us in breast cystic fluid and amniotic fluid (26). In amniotic fluid as well as in serum from pregnant women, the PSA concentration increases with gestation from weeks 11–21 and plateaus thereafter (26). Cases of fetuses with various congenital abnormalities associated with abnormal PSA levels in amniotic fluid have been reported by us (Ref. 25 and unpublished data).

A more recent survey of various tumors revealed that at least some ovarian, liver, kidney, adrenal, colon, parotid, and lung tumors produce PSA (27). As many of these tissues are known to contain steroid hormone receptors, these findings have led us to speculate that any tissue that contains steroid hormone receptors has the ability to produce PSA provided that the cognate steroid hormones are also available. The stimulating hormones could be exogenously administered or endogenously released by the adrenals or ovaries. As mentioned above, AR, GR, MR, and PR have the ability to regulate PSA gene expression through the same hormone response element present in DNA (21, 22). At this point, we would like to emphasize that although many tissues could produce PSA, the amounts produced are much lower compared to the amounts produced by prostatic cells (*e.g.* by a factor of at least 10^4).

What is the physiological role and importance of our findings and those of Clements and Mukhtar? At present, only proposals can be made. PSA is a serine protease, and in all tissues and fluids examined, the predominant form is the noncomplexed, 33-kDa, free PSA monomer that is the enzymatically active form of this enzyme; complexes with proteinase inhibitors also exist, but at much lower concentrations. It would be reasonable to propose that PSA must enzymatically act upon one or more substrates and modify 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 the 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%) (28). In addition, PSA can enzymatically digest insulin growth factor-binding protein-III (IGFBP-3). This activity is thought to regulate the insulin growth factor-I (IGF-1) concentration, because digestion of IGFBP-3 by PSA releases biologically active IGF-I (29). Findings by other groups further support the hypothesis that PSA is a regulator of IGFBP-2 and IGFBP-3 in patients with prostate cancer (30). Killian *et al.* (31) recently found that PSA has mitogenic activity, presumably due to activation by PSA of latent transforming growth factor-β and through modulation of cell adhesion (31). Others have shown that PSA binds and inactivates protein-C inhibitor (32). Our finding of PSA in breast, colon, ovarian, parotid, kidney, lung, and liver tumors; stimulated normal breast; amniotic fluid; and breast milk as well as the data presented by Clements and Mukhtar for normal endometrium (23) 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 that 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 and tumors and during pregnancy may be much more complex than thought and raises numerous questions that will only be answered by further investigation. At this point, PSA shows promise of being routinely used as a favorable prognostic indicator in female breast cancer.

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