Prostate-specific antigen in the breast

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Introduction

Prostate-specific antigen (PSA) is a serine protease and is a single chain glycoprotein of around 33 kDa molecular mass (see reviews by Peehl 1995, Duffy 1996, Lilja 1997). It was first described in seminal plasma (Hara et al. 1971) and was later isolated from the prostate (Wang et al. 1979). PSA is a product of the epithelial cells of the prostate and is secreted into the seminal fluid. It is believed that the main biological role of this protease in semen is to digest the protein semenogelin on ejaculation (Lilja 1985), thus liquefying the seminal fluid and allowing increased sperm motility. However, it should be noted that PSA is present in such high concentrations in semen that even after considerable dilution it could possibly have proteolytic effects on substrates in the female reproductive tract.

PSA is coded for by a 6 kilobase length of DNA on human chromosome 19 and has a high degree of homology with pancreatic/renal kallikrein (termed hK1) and a prostate specific glandular kallikrein (hK2). For this reason PSA is also given the alternative name of hK3. Genes for all three proteases are closely grouped together on chromosome 19 (see Kumar et al. 1996).

PSA exists in several forms, beginning as a proprotein with no proteolytic activity and being converted to an active protease by the removal of several N-terminal amino acids (Lovgren et al. 1997). It has recently been shown that the kallikrein hK2 can convert proPSA into its active form (Kumar et al. 1997, Lovgren et al. 1997, Takayama et al. 1997) and it seems very likely that hK2 is responsible for activation of PSA in the prostate.

Active PSA can interact with several protease inhibitors, the most abundant being alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin (AMG). These are covalent interactions which usually occur only with PSA which is enzymatically active (Leinonen et al. 1996). If PSA enters the circulation then a large proportion of it will immediately become complexed to AMG; however, because protease-AMG complexes are so rapidly cleared the major circulating complex will be PSA-ACT (Stenman 1997).

PSA is also found in a ‘nicked’ form (Stenman 1997) in which the protein has undergone one or more internal proteolytic cleavages, e.g. after residues 54, 57, 145 or 146 (Chen et al. 1997, Noldus et al. 1997) but retains the overall structure, held together by disulphide bridges. This form is enzymatically inactive and so does not usually interact with the protease inhibitors.

Many immunoassays have been established for PSA, some of which measure ‘total’ PSA, that is PSA whether or not it is bound to an inhibitor, while others measure ‘free PSA’, that is PSA not bound to an inhibitor which could comprise both enzymatically active PSA and the inactive ‘nicked’ form. Some assays are not equimolar, that is they detect both the free and the bound form but will report different levels depending on the proportion of PSA which is bound to inhibitors (reviewed by Semjonow et al. 1996). PSA bound to AMG may not be detected at all because the PSA molecule can be entirely masked from antibody detection by the large AMG molecule.

For some time it was believed that PSA was exclusively expressed in the prostate and that PSA in the circulation must be prostatic in origin. This has been the basis for the use of PSA as a tumour marker for prostate cancer and the detection of this cancer by measuring circulating PSA levels (Peehl 1995, Duffy 1996, Lilja 1997). In healthy men circulating PSA is below, and usually well below, 4 ng/ml (which is very low compared with the level in seminal fluid of 1 mg/ml) and is assumed to derive from the leakage of a small proportion of PSA from the prostate and seminal fluid into the general circulation. In men with prostate cancer circulating PSA is often above 10 ng/ml, probably due to secretion from the tumour cells and disruption of the prostate architecture which normally retains the PSA in the seminal fluid. One of the drawbacks of using circulating PSA as a screening test for prostate cancer is that elevated levels are also found in the much less serious condition of benign prostatic hyperplasia. It has been suggested that the two conditions could be distinguished by examining the proportion of the circulating PSA which is complexed to ACT compared with that which is free (Leinonen et al. 1993, Lilja et al. 1991). The free-to-total ratio is expected to be lower in patients with prostate cancer than in those with benign prostatic hyperplasia. This ratio is now believed to be useful in increasing the specificity of diagnosis (Bangma et al. 1997, Egawa et al. 1997), to be the earliest serum marker of prostate cancer (Pearson et al. 1996) and to predict the aggressiveness of subsequent tumours (Carter et al. 1997). Probably the free-to-total PSA ratio is higher in benign prostatic hyperplasia than in prostate cancer because more of the circulating PSA is in...
the nicked form which is unable to interact with ACT. Despite these complexities PSA remains the most important diagnostic and prognostic marker for human prostate cancer (Duffy 1996, Gao et al. 1997).

**Extraprostatic PSA**

With the availability of highly sensitive immunoassays it has become apparent that PSA is expressed in non-prostatic tissues. Since the levels expressed in the prostate are so much higher than in other tissues this has not proved an obstacle to the use of PSA as a prostate cancer marker. However, it has been suggested that highly sensitive techniques (such as the use of polymerase chain reaction (PCR) to detect circulating prostate cancer cells) might suffer from false positives due to this extra-prostatic expression (Eldgamal et al. 1996), but this view has been vigorously opposed (Horan 1996). This extraprostatic expression will be discussed first with regard to the wide variety of tissues expressing PSA and then more specifically with regard to PSA expression in the breast.

PSA has been reported to be present in low levels in several biological fluids apart from serum, for example in ascitic fluid (Mannello et al. 1997a), pleural effusions (Mannello et al. 1997b) and cerebrospinal fluid (Melegos et al. 1997a). Since levels were unaffected by the sex of the patient these observations cannot be explained by spillover derived from prostatic sources. Furthermore, PSA was consistently found in amniotic fluid (Yu & Diamandis 1995a), was largely in the free form and increased in concentration as gestational age increased. Amniotic fluid PSA levels were lower in cases where the fetuses were trisomic, anencephalic or had renal malformations, suggesting that amniotic PSA has potential as a diagnostic test (Melegos et al. 1996).

PSA expression has also been reported in a wide variety of tumours. It has been detected immuno-cytochemically in many primary and metastatic melanomas (Bodey et al. 1997a) and in lung tumours and normal lung tissue both by immunoassay and by reverse transcriptase (RT)-PCR which detects mRNA coding for the protein (Zarghami et al. 1997a). Similarly, RT-PCR has detected PSA mRNA at low levels in some pituitary tumours and normal tissue (Clements et al. 1996). There is also a case report of a patient with adenocarcinoma of the colon and highly elevated serum PSA levels which fell after surgery, presumably indicating PSA secretion by the tumour (Yamamoto & Miyake 1997). In addition there are reports of a cystic ovarian teratoma in which PSA was detected immuno-cytochemically (Tremblay et al. 1996) and a primary ovarian carcinoma in which PSA expression was confirmed by immunoassay, immuno-histochemistry, RT-PCR and sequencing of the PCR product (Yu et al. 1995a).

Many of these studies on extraprostatic PSA were inspired by reports that PSA could be detected in breast tumour cytosols (Diamandis et al. 1994, Yu et al. 1994a). Using a highly sensitive immunofluorimetric assay, chromatography and Western blotting, PSA was detected in 30% of breast tumours. Similar percentages have been found in other studies using immunoassay (Dibbelt et al. 1996) and immunocytochemistry (Bodey et al. 1997b). Although there is one report that PSA cannot be detected in formalin-fixed paraffin-embedded tissue from breast tumour or normal breast (Kenwright & Thornton 1997), this has not been the experience of other groups (Howarth et al. 1997).

PSA expression has now been shown in benign breast disease such as fibroadenoma and in normal breast tissue as well as in tumours by using techniques such as immunocytochemistry (Howarth et al. 1997) and immunoassay and RT-PCR (Yu et al. 1996). Because of these observations PSA levels have been measured in fluids associated with breast tissue. PSA was found in nipple aspirate fluid (Sauter et al. 1996) and in the milk of lactating women (Yu & Diamandis 1995b). This PSA in human milk was largely in the free form, uncomplexed with ACT, and declined in concentration in the two weeks post delivery.

PSA has also been detected in breast cyst fluid (BCF) which is aspirated from benign breast cysts (Filella et al. 1996a). A large proportion of this PSA is complexed with ACT (Diamandis et al. 1996, Mannello et al. 1997c), which is consistent with a previous report that BCF contains ACT (Mannello et al. 1994). BCF can be classified into Type 1 (high potassium levels, derived from apocrine cysts) and Type 2 (high sodium levels derived from cysts with flattened epithelia) and it has been reported that PSA levels are higher in the Type 1 BCF (Mannello et al. 1996, Parish et al. 1996), although another study found no significant difference (Lai et al. 1996).

Since PSA was originally identified in the prostate, it has long been assumed that women, lacking a prostate, would have no circulating PSA. However, with the advent of highly sensitive assays it has become clear that there are low but detectable levels of PSA in the circulation of women (Giai 1995, Filella 1996b). The source of this PSA has not yet been completely determined but it seems highly likely that it is derived from the normal breast (Diamandis 1995, 1996a, 1997). In women with detectable circulating PSA, levels varied with the menstrual cycle, peaking in the mid to late follicular phase (Zarghami et al. 1997b).

Circulating levels of PSA are not elevated in women with breast cancer (Giai 1995); however, PSA levels in the serum of some women with fibroadenomas or with breast cysts can attain the same levels as seen in men with...
prostate cancer, reaching 55 ng/ml in one case (Borchert et al. 1997a). This PSA is largely in the free form.

**Functions of extraprostatic PSA**

There is a well established function for proteolytically active PSA in seminal fluid, the cleavage of seminogelin resulting in liquefaction of semen and increased sperm motility. While it is possible that PSA has other functions in seminal fluid none have been categorically established. It is less clear what functions extraprostatic PSA might perform. Several suggestions have been made and are discussed below but it should be pointed out that most depend on the extraprostatic PSA having proteolytic activity. While PSA in seminal fluid is known to be proteolytically active, the situation is less clear for extraprostatic PSA. In environments such as the circulation, where there is a large excess of protease inhibitors such as ACT and AMG, it would be expected that almost all active PSA would become complexed and thus inactive. As discussed above, much of the extraprostatic PSA is in the free form and this could be either because it is in a tissue compartment in which it is not exposed to protease inhibitors and thus retains its proteolytic activity, or because the PSA is nicked and thus enzymatically inactive and unable to interact with the protease inhibitors. Hopefully, these two possibilities will be resolved eventually, for example by using peptide substmates to measure enzymatically active PSA (Denmeade et al. 1997).

One of the additional functions that PSA may perform in seminal fluid is the regulation of the interaction between insulin-like growth factor-I (IGF-I) and one of its binding proteins, IGFBP-3. PSA has been identified as the highly active protease in semen which proteolytically cleaves IGFBP-3 and thus drastically reduces its affinity for IGF-I (Cohen et al. 1992). The growth of prostate epithelial cells in culture is stimulated by IGF-I and this stimulation can be blocked by the addition of IGFBP-3; however, treatment of IGFBP-3 with PSA reversed this blockade (Cohen et al. 1994). These results have led to the suggestion that PSA, both in the prostate and extraprostatically, may be involved in local paracrine IGF pathways, releasing IGF-I from its binding protein and affecting cell growth (Diamandis & Yu 1995). Thus it may be involved in abnormal proliferation and even tumour growth.

It is also possible that PSA is involved in the breakdown of extracellular matrix. It has been reported that PSA is able to convert the precursor of urokinase type plasminogen activator into its active form (Yoshida et al. 1995), the result of which would be to begin the protease cascade which results in matrix metalloproteinase activation and extracellular matrix breakdown (Parish 1994). In tumours this breakdown is implicated in tumour growth and metastatic spread. However, recent studies using both recombinant proteases (Takayama et al. 1997) and proteases isolated from seminal plasma (Frenette et al. 1997) have demonstrated that PSA is unable to activate urokinase but that hK2 does activate it. Thus the previous results are likely to be due to contamination of the PSA preparation with hK2 which is another kallikrein and has a high degree of homology to PSA. However, there is another mechanism by which PSA could be involved in matrix breakdown, as PSA can directly degrade the extracellular matrix glycoproteins fibronectin and laminin (Webber et al. 1995). Using gel zymography and immunoneutralisation, this protease activity was shown to be due to PSA itself, rather than to a contaminant.

To explain the frequent occurrence of osteoblastic metastases in prostate cancer, another role for PSA has been suggested. Transforming growth factor (TGF)-β is mitogenic to osteoblasts and it has now been demonstrated that PSA is able to convert latent TGF-β to its active form (Killian et al. 1993). It is possible that PSA could affect cell growth by activating latent growth factors in bone and also in other tissues (Diamandis & Yu 1995).

Because PSA is present in prostate tumours and is now known to occur in many other tumours (see above), the roles proposed for PSA have largely centred on the stimulation of tumour growth or metastasis by release of growth factors from binding proteins, extracellular matrix breakdown or growth factor activation. However, the presence of PSA in breast tumour cytosol is a favourable prognostic factor (see below), which is incompatible with these explanations. Attempts have been made to suggest a role for PSA in the breast consistent with a favourable prognosis. It has been suggested that PSA might generate bioactive peptides from the BRCA1 gene product which might be growth inhibitory (Diamandis 1996b). It has also been observed that PSA added to the hormone-dependent human breast cancer cell line MCF-7 is growth inhibitory and stimulates conversion by the cells of oestradiol to the less potent oestrogen, oestrone (Lai et al. 1996). Since the cells are hormone-dependent this action of PSA could mediate its growth inhibitory effect on the cell line. In confirmation of this hypothesis PSA had no effect on the growth of the hormone-independent cell line MDA-MB-231.

Since it is not clear in many cases how much proteolytic activity extraprostatic PSA has, and since many suggested roles are not compatible with its prognostic indications in breast cancer, it must be concluded that the role of extraprostatic PSA has still not been determined.

**Regulation of extraprostatic PSA**

The presence of PSA in breast cancer cytosols is significantly associated with the expression of
progestosterone receptors in the same cytosols (Yu et al. 1994a, Levesque et al. 1995). There is also an association with oestrogen receptor expression but this is secondary to a known association of progesterone and oestrogen receptors in breast tumours (Yu et al. 1994a). The expression of PSA in the prostate is regulated by steroids, for example constitutive secretion of PSA from LNCaP prostate epithelial cells is stimulated by testosterone (Gau et al. 1997). This raises the question of whether the two tissues share a steroid-stimulated regulation of PSA expression.

When the steroid hormone receptor-positive cell lines T-47D and MCF-7 are treated with androgens, progestins or glucocorticosteroids they are stimulated to produce PSA in the culture medium (Yu et al. 1994b). In the T-47-D cells this stimulation is blocked by oestrogens. It is known that androgen, progestin and glucocorticoid receptors share a common hormone response element (HRE) which differs from that of the oestrogen receptor (Yu et al. 1994b). Presumably it is this common HRE which is associated with the PSA gene. The mRNA coding for PSA appeared within 2 h of progestin stimulation of T47-D cells and the PSA protein appeared after 4-8 h (Zarghami et al. 1997c). Only androgens and progestins are active at nanomolar concentrations.

There is also in vivo evidence that extraprostatic PSA, is under steroid hormonal control. When cytosols from normal breast tissue from a series of nine women undergoing breast reduction surgery were assayed for PSA, only one contained significant levels of PSA and this was from a woman who was receiving a progestin-containing oral contraceptive (Yu et al. 1995b). It is assumed that the steroid stimulated PSA expression in this patient. Androgen stimulation also promotes PSA expression in women - in a group of twenty female-to-male transsexuals receiving testosterone treatment, high urinary levels of PSA were detected (Breul et al. 1997). It was assumed that this urinary PSA was produced by the periurethral gland under androgen stimulation. Hirsute, hyperandrogenic women have significantly higher serum PSA levels than normal controls and these PSA levels correlate with 3α-androstanediol glucuronide (a specific metabolite of androgen action) levels (Melegos et al. 1997b). It seems likely that this PSA is derived from the breast (Diamandis 1995, 1996a, 1997). Total circulating PSA levels are not significantly different between breast cancer patients and controls (Giai et al. 1995). However, when the form of this PSA immunoreactivity was examined in a small group of women it was found that circulating PSA in healthy women is almost entirely in a complex with ACT, while that in cancer patients is almost entirely in the free form (Melegos & Diamandis 1996). In a larger study (Borchert et al. 1997b), it was confirmed that the major PSA form in normal or hirsute women is PSA-ACT while that in patients with malignant or benign breast disease is the free form. Presumably the free PSA in these cases is nicked so that it cannot interact with the great excess of protease inhibitor in the circulation. The authors suggest that this difference could form the basis of a diagnostic serological test for breast cancer which would be enormously exciting. However, it should be remembered that, because the PSA levels are close to the limits of detection of current assays, it was necessary to distinguish bound PSA from free by separation on gel filtration HPLC followed by immunoassay, which seems a procedure far too cumbersome for routine screening.

Conclusions

As its name suggests, expression of prostate-specific antigen was originally believed to be confined to the prostate and hence to men. With the advent of highly

Extraprostatic PSA in prognosis and diagnosis

Existing prognostic markers do not always provide all the information needed to select sub-groups of breast cancer patients for different postsurgical therapies. Therefore, there is great interest in possible new prognostic markers. In a recent study, breast tumour cytosol PSA levels were compared with other prognostic markers in a group of 174 patients with a median follow-up time of thirty-three months (Yu et al. 1995c). PSA-positive cytosols (those containing more than 0.03 ng PSA/mg protein) were associated with early disease stage, small tumours and oestrogen receptor-positive tumours. These PSA-positive tumours were also associated with a reduced risk of relapse or death, making PSA a favourable prognostic indicator for women with breast cancer. Furthermore, tumour cytosol PSA had a good prognostic value for relapse-free survival in patients with oestrogen receptor-negative tumours, leading the authors to suggest that high tumour PSA values might identify that group of patients which has receptor-negative tumours but which would respond to endocrine therapy (a group which is known to exist but for whom there is no current marker). They also suggest that the prognostic value of PSA does not arise from it performing any of the functions suggested above for extraprostatic PSA, but rather from it being a marker of the endogenous hormone balance between androgens, progesterone and oestrogen.

A diagnostic serological test for breast cancer would be of immense value. Circulating PSA levels in female patients were examined with high sensitivity assays to determine if they could form the basis of such a test, since this circulating PSA is assumed to derive from the breast (Diamandis 1995, 1996a, 1997). Total circulating PSA levels are not significantly different between breast cancer patients and controls (Giai et al. 1995). However, when the form of this PSA immunoreactivity was examined in a small group of women it was found that circulating PSA in healthy women is almost entirely in a complex with ACT, while that in cancer patients is almost entirely in the free form (Melegos & Diamandis 1996). In a larger study (Borchert et al. 1997b), it was confirmed that the major PSA form in normal or hirsute women is PSA-ACT while that in patients with malignant or benign breast disease is the free form. Presumably the free PSA in these cases is nicked so that it cannot interact with the great excess of protease inhibitor in the circulation. The authors suggest that this difference could form the basis of a diagnostic serological test for breast cancer which would be enormously exciting. However, it should be remembered that, because the PSA levels are close to the limits of detection of current assays, it was necessary to distinguish bound PSA from free by separation on gel filtration HPLC followed by immunoassay, which seems a procedure far too cumbersome for routine screening.
sensitive assays it has become clear that it has a more widespread distribution, being found in many tumours and normal tissues. In particular it is found in breast tumour cytosols, in benign breast diseases and in normal breast tissue and fluids. Several functions have been suggested for this extraprostatic PSA but none have yet been completely confirmed. PSA expression in the breast is probably stimulated both by androgens and progestins. Elevated levels of PSA in breast tumour cytosols have been shown to be a favourable prognostic indicator in breast cancer. Most excitingly it has been suggested that the presence of circulating free rather than bound PSA in women could be used as a diagnostic test for patients with a high risk of subsequent breast cancer.

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Parish: PSA in breast


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