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Review

Prostate-specific antigen (PSA/hK3): a further player in the field of breast cancer diagnostics?

Ferdinando Mannello and Giancarlo Gazzanelli

Libera Università Studi, Urbino, Italy

Correspondence: Dr F Mannello, Istituto di Istologia e Analisi di Laboratorio, Facoltà Scienze MFN, Libera Università Studi, Via Zeppi, 61029 Urbino (PU), Italy. Tel: +39 722 320168; Fax: +39 722 322370; e-mail: mannello@uniurb.it

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Abstract

Since its identification, much information has been obtained about prostate-specific antigen (PSA, or human glandular kallikrein 3 [hK3]), a kallikrein-like serine protease that is the most valuable tumour marker for the screening, diagnosis and management of human prostate carcinoma. Recently, it has become widely accepted that PSA is also present in many nonprostatic sources, casting doubts about the specificity of its tissue expression. Here we summarize the findings on the biomolecular expression of PSA in breast secretions, cells and tissues of healthy and diseased females. Although several studies have strongly suggested that the molecular forms of PSA seem to represent a potential tool for the risk assessment of breast cancer, recent reports have yielded conflicting results. Although several studies have suggested new biological function(s) for PSA in breast physiopathology, more studies are needed to enlist PSA unequivocally as an additional weapon in the anticancer armoury in breast cancer diagnostics.

Keywords: breast, breast cyst fluid, cancer, nipple aspirate fluid, prostate-specific antigen

Introduction

> *Omnia, quae nunc vetustissima creduntur, nova fuere* [All, which we consider now as most ancient, was new once]
>
> Tacitus, *Annals*, Book XI, 24

As with everything else in science, biological research involves the continuous updating of physiological phenomena, sometimes subverting the certainty 'written in stone'. That is also true of prostate-specific antigen (PSA), an antigen discovered in the 1970s and introduced to urological practice about 15 years ago [1]. Although it is widely used as the most sensitive marker available so far for screening, diagnosis and monitoring human prostate cancer progression as well as response to therapy, discoveries over the past decade have unequivocally indicated that the original antigen PSA is no longer prostate-specific, shedding light on the multifunctional behaviour of this 'novel' serine protease [2]. The main characteristics of this kallikrein, which belongs to the family of serine proteases, have previously been described in detail [3] and are therefore mentioned only briefly here. The glandular kallikrein gene family is composed of three genes, localized on chromosome 19q13.3–q13.4; the KLK-3 gene locus encodes the extracellular serine protease PSA, which has also been named human glandular kallikrein 3 (hK3). In the prostate, PSA expression is localized to the differentiated, secretory columnar cells of the glandular epithelium. Biochemically, it is a 33 kDa single-chain glycoprotein with chymotrypsin-like activity that requires post-translational processing for its full proteolytic activity.

Physiologically, PSA has a role in the dissolution of the gel structure of freshly ejaculated semen, through specific

BC = breast cancer; GCBD = gross cystic breast disease; NAF = nipple aspirate fluid; PSA = prostate-specific antigen.

Table 1

Main reports of biomolecular expression of prostate-specific antigen related to the human female breast

Source	References
Tissue from healthy breast	[16–18]
Tissue with benign breast disease	[5,16]
Tissue with breast cancer	[6–8,13,14–16,21]
Association with steroid-hormone receptors	[6,8,11,13–15,17]
Use as a prognostic indicator	[7,14,16,20–23,45]
Gene and mRNA	[9–12,15]
Breast cancer cell lines	[11,24,25,34]
Milk	[26,27]
Colostrum	[28]
Breast cyst fluid	[30,32–36]
Nipple aspirate fluid	[27,31,38–40]
Serum of healthy and diseased females	[16,19,20,22,35,41,43,44]

proteolysis of both high molecular mass semenogelin and fibronectin. In seminal fluid about two thirds of PSA is enzymically active, whereas the remainder is inactive and does not bind to protease inhibitors. Low levels of PSA are normally released into the blood and its predominant molecular form is that complexed with α_1-antichymotrypsin, whereas about 30% of serum PSA occurs in a noncomplexed free form. Several thousand reports on this indisputable urological marker have already been published, identifying PSA as the most useful biomarker for detecting prostate cancer at an early stage, for evaluating disease progression and for assessing therapeutic response, as well as for identifying tumour recurrence [1]. Only recently several publications have shown that PSA is expressed, at lower concentrations than in prostate or in seminal fluid, in a variety of human tumour types, healthy tissues, biological fluids and cell lines [4]. Here we summarize the biomolecular findings and the conflicting results on PSA expression in female breast tissues and fluids (Table 1), focusing our attention on its potential usefulness for clinical applications in breast diseases.

PSA synthesis and secretion in normal and diseased breast tissues

In 1989, PSA expression was first discovered [5] through immunohistochemical reactions in some apocrine foci of female fibrocystic breast tissue as well as in breast cancer (BC), casting doubt on the specificity of this kallikrein only for prostatic epithelium. Starting from 1994, Diamandis *et al* [6] identified PSA immunoreactivity in at least 30% of BC cytosolic extracts. Immunohistochemical studies revealed that the intense PSA immunoreactivity identified in BC was well correlated with that detected by immunoassays [7]; western blotting and chromatographic analyses demonstrated that breast tumour PSA had the same molecular mass as seminal or prostatic PSA [6,8]. Molecular analysis revealed that the messenger RNA of breast tumour PSA was identical in sequence to prostatic PSA [9]; DNA sequencing confirmed that no mutations were present in the coding region of the PSA gene in BC, whereas frequent multiple mutations were revealed in 5′-flanking regions [10].

Although the molecular and physiological mechanisms behind PSA gene regulation by steroid hormones have not yet been fully explained, the expression of PSA gene in human female breast tissue is under the control of steroid hormones, in particular androgens and progestins but not oestrogens [11], through the activation of steroid-responsive elements in the PSA promoter/enhancer region [12]. Not surprisingly, PSA positivity in breast tumours was not univocally associated with oestrogen and progesterone receptor positivity [13,14]: in fact, most PSA-producing BCs are positive for steroid hormone receptor, but not all tumours that are positive for steroid hormone receptor produce PSA [15].

PSA production in healthy mammary tissue and in benign breast disease tissues has also been debated; in fact, several authors have demonstrated that PSA is expressed at low levels in about 30% of normal breast tissues [16] and that this production is under a specific hormonal control (for example PSA production through the action of progesterone and androgens but not oestrogens) [17]. Although the protein levels of PSA in female breast tissue are generally quite low (analysed both by commercially available enzyme immunoassays and by in-house immunofluorometric immunoassay [4]), the highest expression of PSA in breast tissue at both the protein and the mRNA levels is seen in benign diseases, and the lowest expression is seen in advanced stage cancerous tissue, suggesting that PSA expression in malignant breast tissues is generally lower than in benign hyperplastic foci or healthy tissue [16]. In contrast, recent evidence has reported a lack of expression in normal breast tissues [18], casting doubt on the breast tissue as the main source of PSA in human females.

PSA as a potential prognostic factor

The fact that not all breast tumours and BC cell lines produce PSA prompted studies on the use of PSA as a prognostic indicator in BC. Some authors found that PSA positivity in BC was significantly associated with smaller tumours, progesterone and/or androgen receptor positivity (and also with diploid tumours, early disease stage, younger patient age and lower risk of relapse), describing PSA as a valuable tool for the prediction of a 'favourable' BC prognosis and response to endocrine therapy [19,20].

In contrast, other studies have suggested a lack of value of PSA immunoreactivity in BC patients as a general prognostic marker for BC [7,14]. Moreover, induction of serum PSA is also associated with an 'unfavourable' prognosis in BC patients with tumours that are positive for oestrogen receptor [21,22] or in those receiving adjuvant treatment [23], contradicting the reports of 'favourable' outcome in patients bearing BC whose tumours showed PSA positivity and who received no drug therapy [20], and apparently in contrast with the induction of PSA synthesis observed *in vitro* [11,24]. This controversy has no obvious explanation at present and more studies are needed to understand the function of PSA in BC and to identify PSA unequivocally as a potential tool for the prediction of BC prognosis.

PSA production *in vitro*

Similarly to what has been shown *in vivo*, several BC cell lines that are positive for steroid hormone receptor can be induced (for example by mineralocorticoids, glucocorticoids, androgens and progestins but not by oestrogens) to produce PSA [11,24], even if not all of these cell lines can constitutively produce PSA [25]. The following BC cell lines positive and negative for steroid hormone receptor were used to evaluate the induction of PSA synthesis and secretion: BT-20, BT-474, MCF-7, MDA-MB-231, MDA-MB-435, MFM-223, T-47D and ZR-75-1 [11,24,25]. The lack of PSA expression in some receptor-positive BC cell lines (as has also been observed in certain receptor-positive breast tumours) might be due to different mechanisms affecting the expression level of PSA (for example either the absence or dysfunction of the necessary components for PSA transcription/translation or a mutation/polymorphism in the promoter/enhancer region of the gene encoding PSA) [12]. However, the production of PSA mediated by steroid hormones and their receptors suggests that PSA might be a new marker for steroid hormone action in female BC cells, even if the behaviour of this proteolytic enzyme *in vitro* is not always correlated with the results obtained *in vivo* (such as treatment with tamoxifen) [20,21,23,24].

PSA expression in breast secretions

Milk and colostrum

PSA immunoreactivity in breast milk of normal lactating women was first identified in 1995 [26]: PSA concentrations were quite variable, were not correlated with the age of the mother or the sex of the newborn, and tended to decrease with increasing time after delivery, perhaps because of the decrease in stimulating steroids after removal of the placenta. PSA was found in milk predominantly in its free form (about 75%) [26,27], whereas in colostrum around half of the molecules were complexed with protease inhibitors [28]. Surprisingly, only few results are available on the expression levels of PSA in milk during lactogenesis [26], and none have yet been published on the concentration of serum PSA during lactation, whereas these biological aspects have been widely studied during pregnancy [4,29]. Recent papers have demonstrated, through an ultrastructural analysis of breast duct cells found in milk and also in nipple aspirate fluids (NAFs) and breast cyst fluids, that these apocrine cells are able to synthesize and secrete PSA [27,30,31], suggesting that these biosynthetically active cells might be the source of PSA in milk and other breast extracellular fluids.

Although these results seem to suggest a possible synthesis and secretion of PSA during lactation, the biological function and physiological significance of different molecular forms of PSA in milk are still unknown (for example, is free PSA an enzymically active fraction involved in the hydrolysis of milk proteins or is it able to act as growth factor regulator?).

Breast cyst fluids

Starting from 1996 [32,33], it has been demonstrated that most breast cyst fluids aspirated by needle from women affected by gross cystic breast disease (GCBD) might accumulate appreciable amounts of immunoreactive PSA intracystically (up to 0.5 mg/l). Since then, over the course of several studies, it has been shown that the intracystic accumulation of PSA was associated more with metabolically active secretive/apocrine type I cysts than with transudative/flattened type II cysts and that the ratio of free PSA to complexed PSA differs between the two cyst types [34–36]. These findings suggest PSA as a further biomarker for the subclassification of the GCBD, providing a new and valuable tool for the discrimination of gross cysts at higher risk of subsequent BC [35,36]. Moreover, it has been shown that intracystic carcinoma of the female breast can produce and accumulate large amounts of PSA in extracellular fluid from breast cyst fluid, probably under the control of the progesterone receptors [8]. A recent study demonstrated that the intracystic PSA accumulated in breast cyst fluid is a secretory product of the apocrine cells lining type I cysts [30], suggesting the involvement of this serine protease in the mechanism of GCBD formation and in its predisposition to preneoplastic transformation [37].

NAFs

Several studies have demonstrated that NAFs contain immunoreactive PSA at very high levels (up to 10 mg/l) [27,38], suggesting PSA as a potentially useful biomarker in risk assessment for BC. In fact, the concentrations of PSA in NAFs were higher in premenopausal than in postmenopausal women, in association with the higher levels of circulating steroid hormones [39] (inversely associated with risk for BC [40]); higher levels of PSA were found in NAF from women with no risk factors for BC. Recently it has been demonstrated that breast epithelial cells found in NAF can synthesize and secrete PSA protein, suggesting that the ductal lobular unit of the breast is a possible

source of PSA that accumulates in very large amounts in extracellular fluids of NAF [31].

Molecular forms of PSA in serum from women affected by breast diseases

The recent use of ultrasensitive PSA immunoassays has enabled the detection of PSA in at least 50% of normal female serum [41], even if at concentrations of about 500 times lower than in male serum. This finding suggests as possible sources both the mammary ductal system [16,30] and the endometrium [29,42], two of the major hormonally responsive tissues in females. Not surprisingly, an association between serum PSA concentrations and circulating steroid hormone levels has been demonstrated [43].

Although serum PSA levels are elevated in most endocrine-dependent disorders [2,4], the presence of circulating serum PSA in both healthy females and in patients affected by breast diseases remains under dispute. In fact, several studies suggested that some benign breast diseases (for example GCBD and fibroadenoma) and breast tumours might be the sources of higher levels of PSA in serum [19,20,44] as the result of an altered hormonal balance [2], triggering the aberrant expression of hormone-dependent genes (such as PSA). Of particular importance were the findings that the two major molecular forms of PSA are differently accumulated in the serum of the healthy and the diseased female breast: in the vast majority of sera of healthy women and patients affected by benign breast diseases, PSA seems to be mostly complexed to α_1-antichymotrypsin [20], and in a significant proportion of females with BC the predominant serological isoform is the free form of PSA [44].

In contrast, recent studies have shown that the concentration of serum PSA in BC is not always directly correlated with that in breast tumours and that PSA expression in serum does not distinguish healthy women and/or women with benign breast diseases from patients with BC [7,14,18,22]. However, more evidence is needed to evaluate whether free PSA might have potential clinical applicability as a circulating marker for breast tumours, because free PSA occurs at very low concentrations in serum, is incapable of binding to proteinase inhibitors, and does not always permit a distinction between healthy and benign diseased patients [14,22]. Moreover, given that the gene and mRNA for PSA are not modified in breast tumours, it will be of greater importance to provide further information on the nature of circulating free PSA in BC patients (for example post-translationally modified PSA and/or free PSA circulating as nicked/zymogenic protein).

Conclusions

Although potentially exciting, the proposed use of PSA as a further biomarker for BC risk assessment must be viewed with caution; it is needed to understand the possible 'new' physiopathological function(s) of this 'old' antigen; in fact, this kallikrein-like protease is thought to participate in pathways that are involved in prostate tumour initiation and/or progression, modulated through its hormone dependence. A multitude of unanswered questions exist regarding the functional role(s) of extraprostatic PSA, in particular in human female breast tissues. Multidisciplinary results add support to the notion that PSA is a widespread kallikrein-like serine protease and focus attention on the novel expression of PSA by human breast tissue and fluids, in which PSA might act both as a growth factor modulator and as a translational/post-transcriptional protein regulator. In fact, PSA has the following properties: it has no kininogenase activity; it hydrolyses insulin chains, extracellular matrix laminin and fibronectin, and interleukin-2; it enzymically digests insulin-like growth factor binding proteins, and probably also single-chain urokinase-type plasminogen activator; it activates latent transforming growth factor; it inactivates protein C inhibitors; it stimulates the conversion of oestradiol to oestrone; it releases angiostatin-like fragments by digestion of plasminogen; and it regulates the hormonal bioactivity of parathyroid hormone-related protein [42,45].

The proteolytic activity of PSA on these different biological substrates, all detected in breast tissues and fluids, could explain in part the novel potential functions of PSA in female breast, not only as a sensitive molecular marker implicated in responsiveness to hormone but also as an initiator of the protease cascade, an important biological mechanism for tissue remodelling in the breast [8,16]. Moreover, PSA might represent a potential good therapeutic target, but both overexpression and underexpression of the gene encoding PSA should be examined because the enzymic activity of this serine protease could be either beneficial or deleterious. In fact, it remains uncertain whether mammary PSA is enzymically active (and therefore could be involved in the promotion of tumour growth and metastasis through the proteolytic degradation of peculiar proteins) or inactive (expressed as zymogenic, nicked or tumour-specific post-translationally modified enzyme, and then capable of having a detrimental role in cancer progression or even of having a functional role in the inhibition of tumour progression through apoptotic pathways) [46].

As with physiological phenomena, novel discoveries on PSA have obscured and complicated the understanding of this kallikrein-like serine protease, giving rise to the notion that PSA can no longer be regarded as a tissue-specific or tumour-specific marker only for prostatic tissue but as a ubiquitous molecule that can be synthesized and secreted by cells bearing specific steroid hormone receptors under conditions of steroidal modulation or stimulation. As always happens with biomedical novelties, the potential for clinical

application of PSA as a tumour-specific biochemical marker in breast diseases is under debate: the molecular forms of PSA are invaluable markers in human male prostatic diseases, but much literature is accumulating at an alarming rate on extraprostatic PSA, and in particular on the new function(s) of PSA both as a prognostic tool for BC risk assessment and a biomarker in the prediction of response to the treatment of breast diseases [19]. Given the dire need for tumour markers, only further studies can establish the utility of PSA in the detection and treatment of breast diseases and provide irrefutable evidence for its diagnostic and/or prognostic use as a new weapon against human breast cancer.

> *Nec scire fas est omnia* [We are not permitted to know everything]
>
> Horace, *Odes*, Book IV, 4

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