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Report

Prostate specific antigen – a new constituent of breast cyst fluid

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Summary

We demonstrate for the first time that prostate specific antigen (PSA) is a component of breast cyst fluid. At the cutoff level of 0.01 or 0.03 μg/L of PSA, 64% or 43% of cyst fluids are positive for PSA, respectively. PSA in cyst fluid, as characterized by high performance liquid chromatography, exists in almost equal concentrations as free PSA, with a molecular weight of 33 KDa, and as PSA bound to α_1-antichymotrypsin, with a molecular weight of 100 KDa. PSA presence was also characterized in cyst fluid by Western blot analysis. These data suggest that PSA is a frequent component of breast cyst fluid. More studies are needed to establish the role of this serine protease in normal breast, gross breast cystic disease, and breast cancer.

Introduction

Gross cystic breast disease is a very common mammary disease, affecting about 7% of premenopausal women [1]. There is convincing evidence that women with gross cystic breast disease are at a 2–4-fold greater risk of developing breast cancer than normal females [2, 3]. The cyst fluid composition has been the subject of many studies in an attempt to understand the mechanisms of cystic disease initiation and progression and the possible linkage of cyst components to carcinogenesis. Cyst fluids contain a wide variety of substances including steroids [4, 5], tumor markers [6, 7], epidermal growth factor [8, 9], and many proteins including apolipoprotein D, cathepsin D, Zn-α_2-glycoprotein, and other proteolytic enzymes [10–18]. The cystic fluid proteins are believed to be secretory products of epithelial cells surrounding the cysts [10, 11].

We have recently found that about 30% of female breast tumors produce prostate specific antigen (PSA) and that PSA production is closely linked to the presence of steroid hormone receptors [19, 20]. PSA seems to be a favourable prognostic indicator in breast cancer [21, 22]. Moreover, PSA is present in the normal breast of women receiving oral contraceptives [23], is secreted in the milk of lactating women [24], and is present in amniotic fluid [25]. These data suggest that the breast epithelial cells have the ability to produce PSA under conditions of stimulation by steroid hormones, a situation which was confirmed by tissue culture studies [26]. In this study we examine if PSA is a component of breast cyst fluid in women with gross cystic breast disease.

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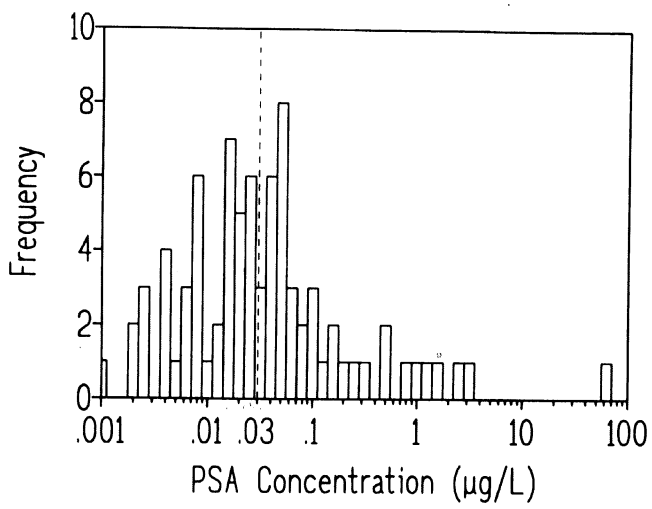

Fig. 1. Frequency distribution of PSA concentrations in 100 breast cyst fluids. The dotted line at 0.03 µg/L separates the cyst fluids in two groups. The cyst fluids with PSA > 0.03 µg/L comprise 43% of all samples.

Materials and methods

Samples

Cyst fluids were obtained, with informed consent, by needle aspiration from 100 women with gross cystic breast disease. The patients were all premenopausal. Cancer was excluded by clinical, echographic, mammographic, and cytological studies. Cyst fluids were centrifuged at 35,000 g for 1 h at 4° C and the supernatants stored at − 20° C until analysis.

Prostate specific antigen immunoassay

PSA was measured in all cyst fluids with a highly sensitive and specific assay described in detail elsewhere [27]. We have used two versions of our PSA immunoassay. One version quantifies both the 33 KDa free form of PSA and the 100 KDa form of PSA, which represents PSA bound to α_1-antichymotrypsin. The second version of our PSA immunoassay quantifies only the 100 KDa form of PSA. These assays have been described in detail elsewhere [27]. Our PSA assay has a biological detection limit of 0.01 µg/L.

High-performance liquid chromatography (HPLC)

We have used HPLC to separate free and α_1-antichymotrypsin-bound PSA of cyst fluid. The details of the method have been described elsewhere [27].

Western blot analysis

PSA in cyst fluid was characterized by Western blot analysis. We have used a polyclonal rabbit anti-PSA antibody and chemiluminescence detection. This method has been described in detail elsewhere [26].

Results

PSA concentration in cyst fluid

Table 1 shows numerical data of PSA concentration in 100 cyst fluids. The frequency distribution of values is shown in Fig. 1. The median value was 0.020 µg/L; the mean was 0.82 µg/L with a standard deviation of 6.60 µg/L. The highest PSA concentration observed was 82 µg/L. From all cyst fluids, 64% had a detectable PSA concentration (PSA > 0.01 µg/L) and 43% had PSA levels > 0.03 µg/L. The cyst fluid with a PSA concentration of 82 µg/L was also analysed by the IMx PSA assay (Abbott Diagnostics, Abbott Park, IL) and found to contain 66 µg/L of PSA.

Table 1. PSA concentration in 100 cyst fluids from patients with gross cystic breast disease

PSA, µg/L	Number of patients
< 0.010	36
0.010–0.030	21
0.031–0.050	12
0.051–0.10	13
0.11–0.50	9
0.51–1.00	3
1.01–2.00	3
> 2.00	3

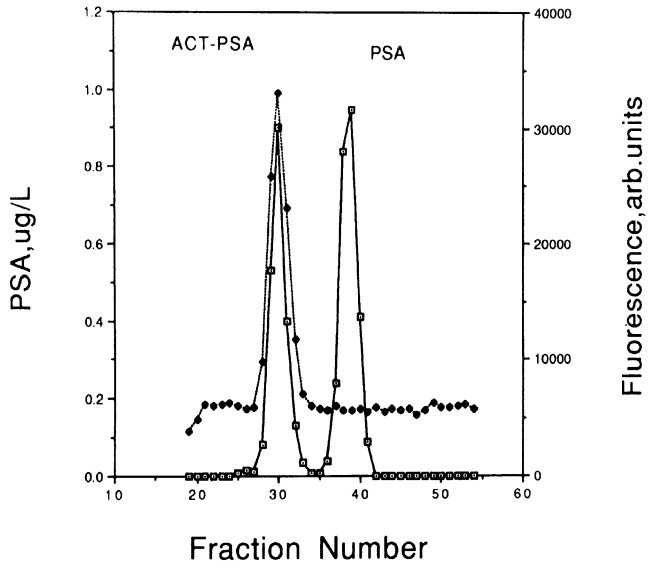

Fig. 2. Separation of PSA by high performance liquid chromatography and assay of the fractions with the time-resolved immunofluorometric procedure. The sample injected was a cyst fluid with PSA concentration of 82 µg/L (50 µL injected). Fractions of 0.5 µL were collected. The assay that measures both free PSA (PSA) and α_1-antichymotrypsin-bound PSA (ACT-PSA) (⊡) recognizes two peaks which correspond to molecular weights of 33 KDa (fraction 39) and 100 KDa (fraction 30). The assay that measures only the ACT-PSA complex (◆) recognizes only one peak which corresponds to a molecular weight of 100 KDa (fraction 30). The response of the latter assay is in arbitrary fluorescence units since no standard is available for the ACT-PSA complex. The HPLC gel filtration column was calibrated with molecular weight standards eluting at fraction 21 (thyroglobulin, 670 KDa), 28 (IgG, 158 KDa), 35 (ovalbumin, 44 KDa), 41 (myoglobin, 17 KDa), and 48 (cyanocobalamin, 1.4 KDa). Seminal PSA elutes at fraction 39 and a serum from a prostate cancer patient elutes as two PSA peaks at fraction 30 (major peak, ACT-PSA), and at fraction 39 (minor peak, free PSA) (data not shown).

HPLC

The cyst fluid with a PSA concentration of 82 µg/L was injected into the HPLC and separated on a gel filtration column. All fractions were then analysed with an assay that measures both free PSA (PSA) and α_1-antichymotrypsin bound PSA (ACT-PSA) and an assay that is specific only for the ACT-PSA complex [27]. The results are shown in Fig. 2. Cyst fluid contains PSA in two forms, free PSA ($\sim$ 33 KDa) and ACT-PSA ($\sim$ 100 KDa) at about the same proportions. The identity of ACT-PSA was confirmed with an assay that measures only ACT-PSA (Fig. 2).

Western blot analysis

One cyst fluid positive for PSA (82 µg/L) and one cyst fluid negative for PSA (< 0.01 µg/L) were separated on polyacrylamide gel electrophoresis along with a PSA-positive control (LNCaP cell line tissue culture supernatant, 120 µg/L) and molecular weight markers. After electroblotting to nitrocellulose membranes and probing with a polyclonal rabbit anti-PSA antibody, the bands were visualized with chemiluminescence captured on x-ray film [26]. The results are shown in Fig. 3.

PSA was detected in the cyst fluid that was positive for PSA but not in the cyst fluid that was negative. The identity of the other bands detected is not known; a strong immunoreactive band appearing at 25 KDa was also found in amniotic fluid [25] and normal breast extracts [23]. The nature of this band

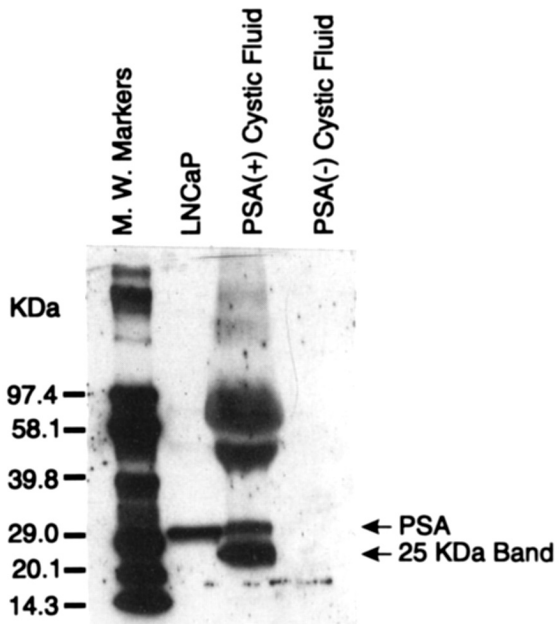

Fig. 3. Western blot analysis using a polyclonal anti-PSA antibody and chemiluminescence detection based on horseradish peroxidase. Lanes with molecular weight markers, an LNCaP prostate carcinoma cell line tissue culture supernatant (120 µg/L of PSA), a cyst fluid positive for PSA (82 µg/L), and a cyst fluid negative for PSA (PSA < 0.10 µg/L) are shown. PSA immunoreactivity is shown by an arrow in the two positive lanes. The identity of the 25 KDa band is unknown. The molecular weight markers were biotinylated and visualised with streptavidin-horseradish peroxidase.

is currently under investigation; it may represent a PSA fragment, non-glycosylated PSA, a PSA fragment that is released from a binding protein during the Western blot, a PSA form synthesized by alternative mRNA splicing [28], or it may represent a cross-reacting substance.

Discussion

PSA was until recently considered a highly specific biochemical marker of prostatic epithelial cells and is currently used as a valuable marker for diagnosis and monitoring of prostatic adenocarcinoma [29]. We have recently provided convincing biochemical and molecular evidence [30] showing that PSA is a ubiquitous protein expressed by 30% of breast tumors [19, 20], normal breast [23], and in a smaller percentage of many other tumors including those of the lung, ovary, liver, kidney, adrenals, colon, and parotid [31, 32]. PSA is present in milk of lactating women [24] and in amniotic fluid [25]. Others have recently found PSA in human endometrial tissue [33]. *In vitro* experiments with breast cancer cell lines have shown that PSA production is mediated by steroid hormone receptors and that the steroid hormones able to upregulate the PSA gene are androgens, progestins, mineralocorticoids, and glucocorticoids, but not estrogens [26]. PSA is a good prognostic indicator in breast cancer [21, 22]. These new findings have recently been reviewed [34].

We hypothesized that breast cyst fluid may also contain PSA. Breast cyst fluids contain many different substances including growth factors, proteinases and their inhibitors, proteins of unknown function, steroid hormones, electrolytes, etc. Many of these substances are produced by the epithelial cells surrounding the cysts. The regulation of a number of these proteins is under the control of steroid hormones and their receptors.

We present data showing that 64% of all cyst fluids contain PSA at levels higher or equal to 0.010 μg/L, which is the detection limit of the assay used. At a level greater than 0.030 μg/L, which is easily measurable by the technique used and previously utilized as a cutoff point for breast tumor extracts [20], 44% of cyst fluids were positive (Table 1). Some fluids had relatively high levels of PSA (Fig. 1) and the highest level identified was 82 μg/L. PSA in cyst fluid was found in both its free, 33 KDa form and in its 100 KDa form; the latter represents PSA bound to α_1-antichymotrypsin (Fig. 2). Western blot analysis confirmed the presence of the 33 KDa form of PSA and identified another 25 KDa protein of unknown structure. The identity of this band, which was also seen in normal breast extracts and amniotic fluids but not in breast tumor extracts, is currently under investigation.

The physiological role of PSA in breast cyst fluid is unknown at present. Based on numerous data presented earlier by our group, we speculate that PSA is the product of breast epithelial cells surrounding the cyst. We further speculate that the PSA gene expression by these cells is under the control of steroid hormone receptors and their cognate steroid hormones. Our tissue culture experiments have shown that PSA gene expression in breast cancer cells is upregulated by androgen, progestin, mineralocorticoids, and glucocorticoids, and downregulated by estrogen [26]. Other data support the same mechanism *in vivo* [23]. We believe that the same regulatory mechanisms may be operating in breast cyst epithelial cells but proof must await further experimentation.

Recent data support the view that PSA may act as a growth factor or cytokine regulator [34]. PSA can enzymatically digest insulin growth factor binding protein-III (IGFBP-3). This activity may regulate insulin growth factor-1 (IGF-1) concentration since digestion of IGFBP-3 by PSA releases biologically active IGF-1. Other groups have reported mitogenic activity of PSA, presumably due to activation by PSA of latent transforming growth factor-β and through modulation of cell adhesion. PSA can also inactivate protein C inhibitor.

These new findings converge to the conclusion that PSA may have an important biological role in the female breast. However, extraprostatic substrates for PSA are currently unknown. It would be interesting to further study the mechanism of appearance of this prostatic marker in breast cyst fluid, establish its inhibitors and substrates, and define its biological role in normal breast, breast cyst disease, and breast cancer.

In the meantime, PSA appears to be one of the promising favourable prognostic indicators in breast cancer, and it may find application for patient prognosis and selection of therapy.

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