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Report

Detection of prostate-specific antigen immunoreactivity in breast tumors

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Abstract

Prostate-specific antigen (PSA) is a glycoprotein produced by the epithelial cells of the prostate. PSA is currently used in clinical practice to facilitate diagnosis and monitoring of prostate carcinoma. The prostate is an organ that possesses androgen, estrogen, and progesterone receptors, and in this respect is similar to the breast. We postulated that breast tumors might also have the ability to produce PSA. We performed these studies on a collection of 525 tumor specimens collected for routine biochemical determination of estrogen and progesterone receptors. Using a highly sensitive immunofluorometric procedure, we measured the p53 tumor suppressor gene product and PSA. Twenty nine percent of the breast tumor extracts contained detectable levels of PSA immunoreactivity (> 0.05 μg/L). The immunoreactive PSA content was associated with estrogen and/or progesterone receptor-positive tumors ($P < 0.002$). No association was found between PSA immunoreactivity and levels of the p53 tumor suppressor gene product ($P = 0.37$). High performance liquid chromatography and Western blot analysis revealed that the PSA immunoreactivity in the tumor had a molecular weight of 30 kDa, similar to that of seminal PSA. Immunoreactive PSA-positive tumors were associated with younger women ($P = 0.012$) and earlier disease stage ($P = 0.064$). We postulate that PSA immunoreactivity may be an additional marker of steroid hormone receptor-ligand action.

Introduction

Prostate cancer is a leading cause of mortality and morbidity among men [1, 2]. Prostate tumors have been shown to contain receptors for androgens, estrogens, and progestins. Prostate tissue and cancer is responsive to steroid hormones, and therapy which takes advantage of this is routinely used [3, 4]. One of the hallmarks of prostate cancer is the elevation in serum concentrations of a 30–33-kDa glycoprotein, prostate specific antigen (PSA) [5]. PSA is secreted by the epithelial cells of prostate tissue and is widely used as a tumor marker for monitoring prostate carcinoma. We recently developed a new method for measuring PSA, based on time-resolved fluorometry (TR-FIA) [6, 7] which is at least 10-fold more sensitive than other commercially available and widely used immunometric techniques. This method has been thoroughly evaluated and can easily and precisely quantitate PSA levels as low as 0.05 μg/L.

Breast cancer is a leading cause of mortality and morbidity among women [8–11]. Breast cancer has been shown to contain androgen, estrogen, and

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progesterone receptors, and normal breast and cancer tissue has been shown to be responsive to steroid hormones. Therapy which makes use of these hormones is routinely used [12, 13]. One of the priorities of breast cancer research is to define new biochemical markers which could be used to further define the prognostic ability of available markers and to facilitate treatment strategies on the basis of the probable likelihood of response.

In this paper, we have examined if breast tumors have the ability to produce PSA and, if PSA is produced, whether it has any correlation with estrogen and/or progesterone receptors, the p53 tumor suppressor gene product, patient age, or disease stage.

Methods

Patients – breast tumors

The primary breast tumors used in this study were collected from female patients at hospitals collaborating in the Ontario Provincial CEA/Receptor Program. The primary breast tumor tissue was immediately stored in liquid nitrogen after surgical resection, transported to the laboratory, and subsequently stored at – 70° C until extraction was performed (~ 1–2 weeks). Approximately 0.5 g of tumor tissue was weighed out, fragmented with a hammer if necessary, and pulverized in a Thermovac tissue pulverizer at liquid nitrogen temperature. The resulting powder was transferred into 50-mL plastic tubes along with 10 mL of extraction buffer (0.01 mol/L Tris, 1.5 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L sodium molybdate, pH adjusted to 7.40 with 5 mol/L HCl). The suspended tissue powder was solubilized on ice with a single 5 s burst of a Polytron homogenizer. The particulate material was pelleted by centrifugation at 105,000 g for one hour. The intermediate layer (cytosol extract) was collected without disturbing the lipid or particulate layers. Protein concentration of the cytosol extract was determined by the Lowry method. The remainder of the extract was stored at – 70° C until analysis (up to three weeks). Studies have shown that p53 protein and PSA in cytosol extracts are stable for at least four months in these conditions.

Measurements

For quantitative analysis of estrogen and progesterone receptors (ER, PR) we have used the Abbott enzyme immunoassay kits (Abbott Laboratories, North Chicago, IL 60064). The kits were used according to the manufacturer's instructions. Analysis of PSA and p53 was performed as described elsewhere [7, 14]. Briefly, the methods are based on the following principles. The PSA assay [7] uses a mouse monoclonal anti-PSA capture antibody coated to polystyrene microtiter wells, a biotinylated polyclonal rabbit detection antibody, and alkaline phosphatase-labeled streptavidin (SA-ALP). In the assay, 50 µL of sample is first incubated with the coating antibody in the presence of 50 µL of assay buffer. After 3 h incubation and washing × 6, the biotinylated polyclonal anti-PSA antibody is added and incubated for 1 h. After washing × 6, the SA-ALP conjugate is added for 15 min, followed by another washing × 6. The activity of ALP is then measured by adding the substrate 5-fluorosalicylphosphate, incubating for 10 min, and then adding a Tb^{3+}-EDTA solution to form a ternary fluorescent complex between the released 5-fluorosalicylate, Tb^{3+}, and EDTA. The fluorescence is measured in the time-resolved fluorometric mode [6].

For the p53 assay [14] we used goat anti-mouse immunoglobulin coated to polystyrene microtiter wells, a mouse monoclonal anti-p53 capture antibody (mutant specific, PAb 240), a rabbit polyclonal anti-p53 antibody (CM-1, wild-type and mutant specific), and alkaline phosphatase-labeled goat anti-rabbit immunoglobulin (GARIg-ALP). In the assay, 50 µL of sample is incubated along with 100 µL of mouse PAb 240 antibody, for 3 h, followed by washing × 6. The rabbit polyclonal CM-1 antibody is then added for 2 h followed by washing × 6. The GARIg-ALP conjugate is then added for 1 h, followed by washing × 6. The activity of ALP is then measured as described for the PSA assay.

PSA was also measured in selected tumor extracts with commercially available kits: (a) the Hy-

britech Tandem™-R PSA kit (Hybritech Inc, San Diego, CA 92126), and (b) the IRMA-Count™ PSA kit (Diagnostic Products Corp., Los Angeles, CA 90045). High performance liquid chromatography was performed with a Shimadzu system (Shimadzu Corp., Kyoto, Japan), isocratically, using a mobile phase of 0.1 mol/L Na_2SO_4-0.1 mol/L NaH_2PO_4, pH 6.80. Flow rate was 0.5 mL/min. The column used was a Bio-Sil SEC-400, 600 mm × 7.5 mm (BioRad Labs, Richmond, CA). The column was calibrated with a molecular weight standard solution from BioRad. Fractions of 0.5 mL each were collected after injecting 150 µL sample.

All necessary reagents and equipment for Western blot analysis were purchased from Novex, San Diego, CA 92121. The manufacturer's protocols were followed throughout. Samples for Western blot analysis were electrophoresed under reducing conditions on 8–16% gradient polyacrylamide mini gels and proteins were transferred to polyvinylidene difluoride (PVDF) membranes by electroelution. Membranes were blocked for 1 h at 45° C in 50 mL of a 2% blocking solution, commercially available from Boehringer Mannheim Canada, Laval PQ. The primary anti-PSA polyclonal rabbit antibody (2 mg/mL, from Medix Biotech, Foster City, CA) was used at a dilution of 1 : 500 in blocking solution, for 1 h, with gentle shaking at room temperature (10 mL per membrane). After washing 3 times, 15 min each, with 100 mL of washing solution (50 mmol/L Tris buffer, containing 150 mmol NaCl and 0.5 g Tween 20 per liter), the membranes were immersed in 10 mL of a 1 : 2000-fold diluted goat anti-rabbit antibody (1 mg/mL, from Jackson Immunoresearch, West Grove, PA) conjugated to alkaline phosphatase, in 2% blocking solution, for 1 h at room temperature. Ten minutes before the end of the incubation we added 0.5 µL of a 1 mg/mL streptavidin-alkaline phosphatase conjugate (also from Jackson) to visualize the biotinylated molecular weight markers. After a further washing × 3 as above, the membrane bands were visualized with a BCIP/NBT solution from Boehringer Mannheim. Purified PSA from seminal plasma (from Scripps Laboratories, San Diego, CA) and diluted seminal plasma were used as controls. Breast tumor extracts (10 µL) were used without any further treatment.

Statistical analysis

The chi-square (χ^2) test was used to determine the statistical significance of differences in distributions and all chi-square values and the corresponding P values were calculated by the statistical software SAS (SAS Institute Inc., Cary, NC, USA).

Results

The arbitrarily selected cutoff value of 0.05 µg/L for PSA immunoreactivity was based on the PSA assay sensitivity. PSA values > 0.05 µg/L can be easily and precisely quantified by using the developed assay [7]. We analyzed PSA in 525 breast tumor extracts and the results are shown in Table 1. From these tumor extracts, 374 (71.2%) had immunoreactive PSA (IR-PSA) levels < 0.05 µg/L and were considered negative for PSA. One hundred and fifty-one (28.8%) of the tumor extracts had IR-PSA levels ≥ 0.05 µg/L, 96 (18.3%) had IR-PSA levels ≥ 0.1 µg/L, and 49 (9.3%) had IR-PSA levels ≥ 0.3 µg/L. Twenty-five samples with a IR-PSA concentration of ≥ 0.3 µg/L, which is potentially measurable by commercial kits, were also analyzed by the Hybritech Tandem™-R PSA kit and by the IRMA-Count™ PSA kit. The results are shown in Fig. 1. There is good correlation between the results of our method and the commercial kits for all samples tested. To further exclude the possibility of non-specific effects, we repeated our assay with the 25 highly positive samples (IR-PSA ≥ 0.3 µg/L) under the following conditions: (a) the assay was run in the absence of capture mouse monoclonal anti-PSA antibody; (b) the assay was run by using an irrelevant capture mouse monoclonal antibody (against alpha-fetoprotein); (c) the assay was run after substitution of the polyclonal rabbit detection antibody

Table 1. Analysis of PSA in breast tumor extracts

	PSA, Immunoreactivity, µg/L			
	< 0.050	≥ 0.050	≥ 0.10	≥ 0.30
Number (total = 525)	374	151	96	49
% of samples	71.2%	28.8%	18.3%	9.3%

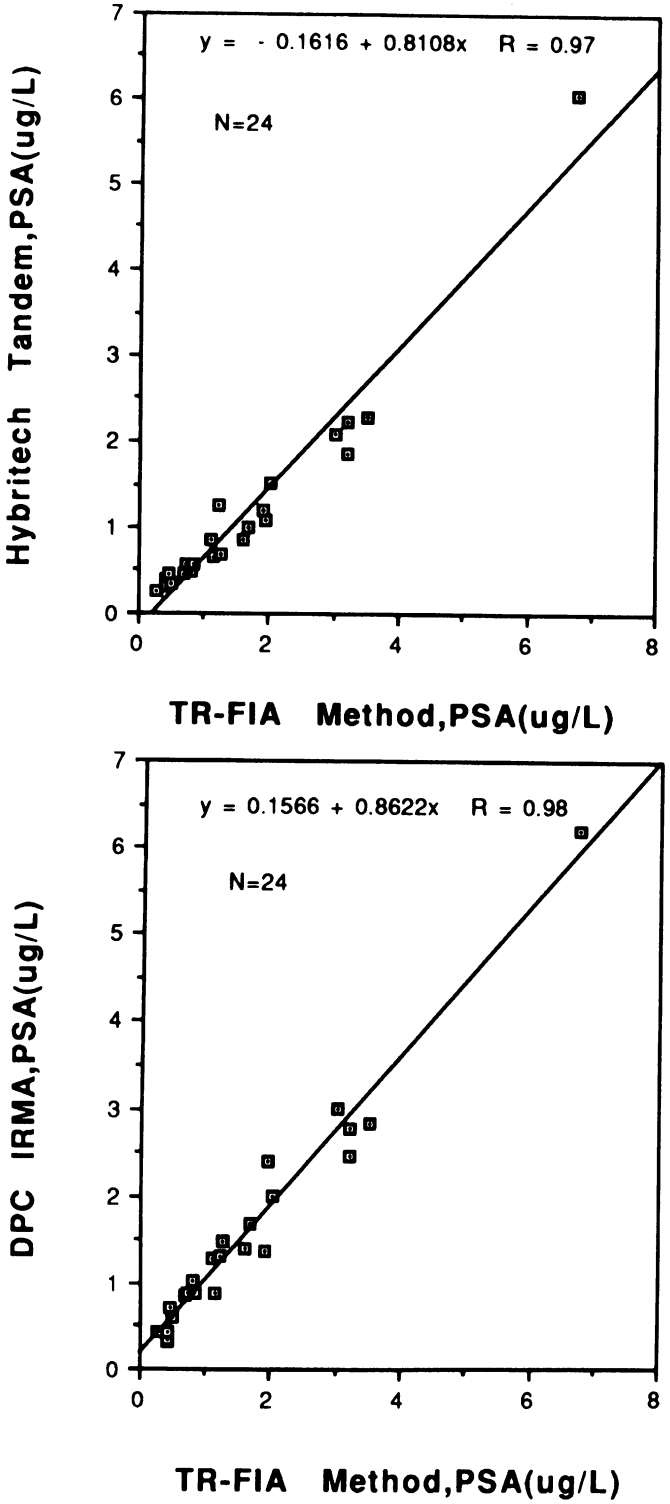

Fig. 1. Analysis of PSA in breast tumor extracts by a TR-FIA method [7] and two immunoradiometric techniques which are currently commercially available. Upper panel: Comparison of TR-FIA with the Hybritech Tandem™ PSA kit for 24 breast tumor extracts with PSA > 0.3 µg/L. Lower panel: Comparison of TR-FIA with the DPC IRMA-Count™ PSA kit for the same extracts. The equation represents linear regression analysis and R is the correlation coefficient. One tumor extract sample, not included in the graph, had a PSA value of 61.4 µg/L by TR-FIA, 39.4 µg/L by Hybritech and 51.8 µg/L by the DPC kit. These data confirm the presence of PSA in the 25 breast tumor extracts by three different immunological techniques.

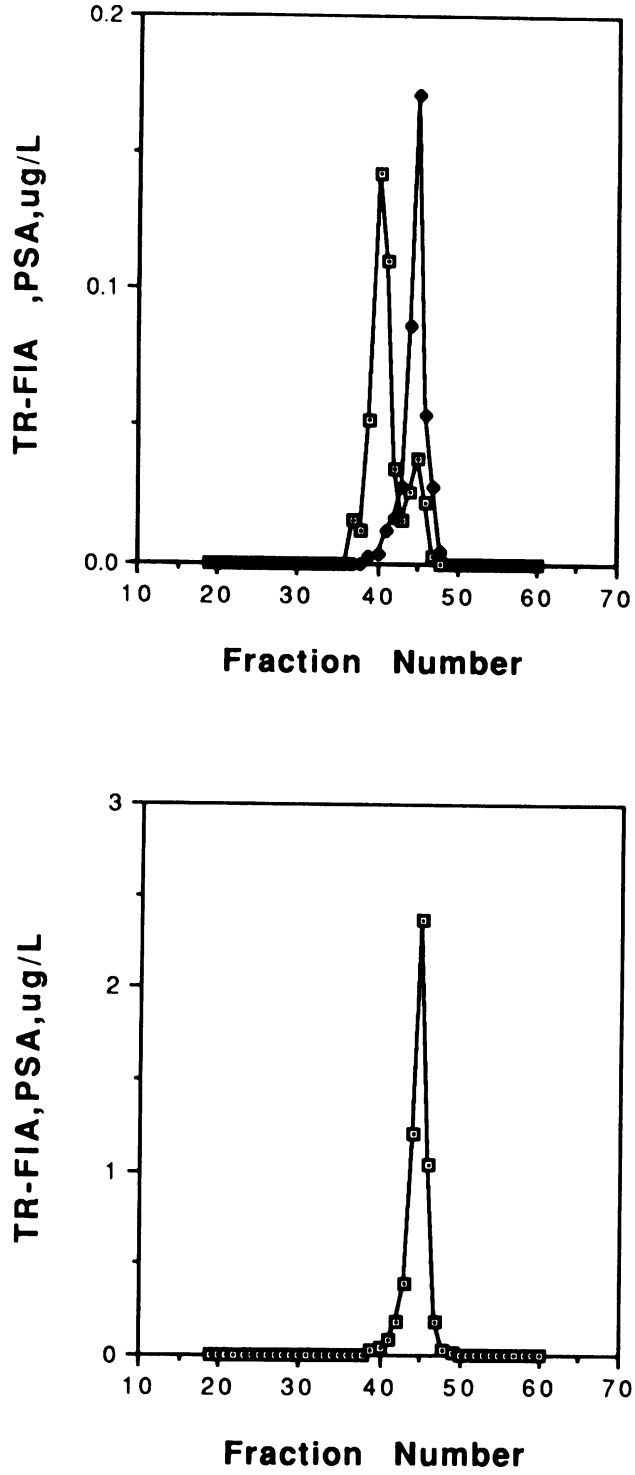

Fig. 2. Analysis of PSA by TR-FIA in high performance liquid chromotographic (HPLC) fractions. The column was calibrated with a molecular weight standard solution containing thyroglobulin (670 kDa), IgG (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and cyanocobalamin (1.4 kDa). Upper panel: Breast tumor extract with PSA of 6.7 µg/L by TR-FIA (◆) and a male serum sample with 4.27 µg/L of PSA by TR-FIA (⊡). Lower panel: Breast tumor extract with PSA of 61.4 µg/L by TR-FIA. The peak at fraction 45 corresponds to a molecular weight of approximately 30 kDa and represents free PSA. The peak at fraction 40 corresponds to a molecular weight of approximately 100 kDa and represents PSA bound to α_1-antichymotrypsin [7, 15, 16].

with biotinylated rabbit IgG. In all cases, we obtained fluorescence signals similar to those of the zero standard, verifying that non-specific effects were absent.

We have further investigated the PSA immunoreactivity in two breast tumor extracts by using high performance liquid chromatography (HPLC). One male serum sample with a PSA concentration of 4.27 µg/L by TR-FIA and one negative breast tumor extract were used as positive and negative controls. Analysis of PSA was performed in the HPLC fractions and the results are shown in Fig. 2. The PSA-negative breast tumor extract, run between the positive samples, gave undetectable readings in all fractions, in all cases. The PSA immunoreactivity in the two breast tumor extracts elutes as a single peak at fraction 45 and corresponds to a molecular weight of approximately 30 kDa. The PSA immunoreactivity in the male serum sample elutes in two peaks at fractions 40 and 45 and corresponds to molecular weights of approximately 100 kDa and 30 kDa, respectively. These two peaks correspond to PSA bound to α_1-antichymotrypsin and to free PSA, respectively [7, 15, 16]. These findings suggest that the PSA immunoreactivity in the breast tumor extracts is present in a 30 kDa form which has the same molecular weight as seminal PSA. Western blot analysis of four breast tumor extracts (2 PSA-positive and 2 PSA-negative by the immunological assay) has shown positive bands only in the two PSA-positive samples (Fig. 3). These bands are positioned in the same spot as seminal PSA and purified PSA from seminal plasma.

In order to exclude the possibility of contamination of the initial extracts, we have reextracted the initial breast tumor tissue that remains stored at – 70° C in six PSA-positive and six PSA-negative tumors. Rerun of the fresh extracts with the TR-FIA assay confirmed the original results in all cases. We have also obtained 94 breast tumor extracts from another steroid hormone receptor laboratory serving different hospitals in Toronto and obtained

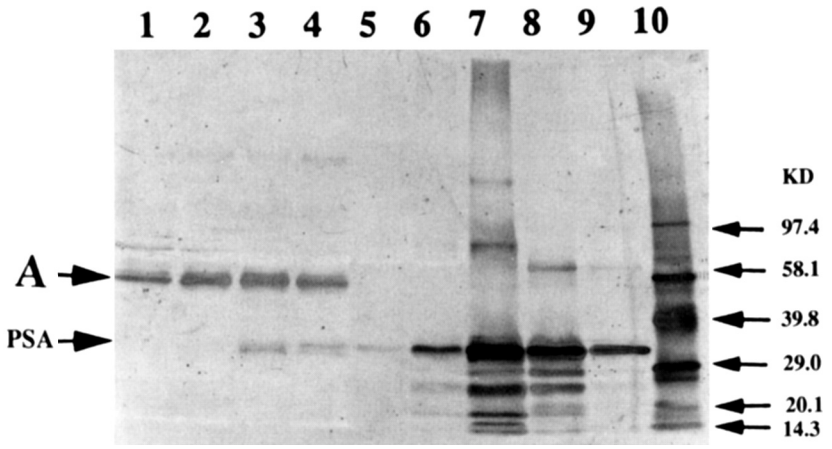

Fig. 3. Western blot analysis of breast tumor extracts (lanes 1–4) seminal plasma (lanes 5–7), a purified PSA preparation from seminal plasma (lanes 8, 9), and biotinylated molecular weight markers (lane 10). The major band at the molecular weight of ~ 33 kDa is the monomeric form of PSA. PSA immunoreactivity is present in the two PSA-positive breast tumor extracts (lanes 3, 4, about 60 and 20 µg/L, respectively) but not in the two PSA-negative breast tumor extracts (lanes 1, 2) as analyzed by the immunofluorometric assay. Lanes 5, 6, and 7 contain seminal plasma diluted 10,000, 1000, and 100 fold, respectively. Lanes 8 and 9 contain purified seminal plasma PSA, 20 ng and 2 ng, respectively. The nature of the band at a molecular weight of ~ 58 kDa, present in all tumor extracts (denoted by arrow A, left) is not known. The molecular weight markers used were: phosphorylase B (97.4 kDa); catalase (58.1 kDa); alcohol dehydrogenase (39.8 kDa); carbonic anhydrase (29.0 kDa); trypsin inhibitor (20.1 kDa); lysozyme (14.3 kDa).

positivity rates similar to those of Table 1. Recovery experiments done by spiking tumor extracts with seminal plasma PSA gave values averaging 83%. Dilution experiments were performed by diluting a breast tumor extract with a high PSA concentration (20.4 μg/L) with either a 6% (w/v) bovine serum albumin solution or a PSA-negative breast tumor extract. The obtained values, at dilutions ranging from 2 to 32-fold, were very close to those predicted by the PSA-value in the undiluted specimen (mean ± 1 standard deviation = 100 ± 5%). We have also sent a batch of 16 breast tumor extracts (four with IR-PSA < 0.05 μg/L and twelve with IR-PSA > 1 μg/L) to two different laboratories performing routine PSA assays by the Hybritech and DPC methods. In both cases, their values were very similar to the ones obtained by our method. These data further support our suggestion that our findings are not due to any non-specific effects and that contamination is extremely unlikely.

For most of the tumor extract samples analyzed for PSA we had data available for both estrogen (ER) and progesterone (PR) receptor concentrations. We have also analyzed 474 samples for the presence of the p53 tumor suppressor gene product, using a method previously described [14]. Tumors were then classified as being positive or negative for ER, PR, p53, and IR-PSA using the following negativity cutoff levels: < 10 fmol/mg of total protein for ER and PR [14], < 3 U/L for p53 (based on an arbitrary p53 standard established in our laboratory), and < 0.05 μg/L for PSA. The data are summarized in Table 2.

Clearly, there is a very strong association between the presence of estrogen and/or progesterone receptors and the presence of PSA immunoreactivity in the tumors ($P < 0.002$). PSA immunoreactivity is independently associated with either ER or PR because tumors which are ER(+) only or PR(+) only still have higher percentage of positivity for PSA immunoreactivity in comparison to tumors which are negative for both receptors. Additionally, the highest percentage of PSA-positive tumors is associated with tumors that are positive for both the ER and PR (Table 2). There is no association between the presence of PSA and the presence of the p53 tumor suppressor gene product ($P = 0.37$). It has recently been shown that the latter is strongly associated with estrogen and/or progesterone receptor-negative tumors [14], an association also shown in Table 2 for the samples of this study ($P < 0.001$).

No linear correlation was seen between the values of IR-PSA and ER or PR. For all samples of this study (N = 525) we obtained the following Pearson correlation coefficients: $r = -0.023$, not significantly different from zero (NS), $P = 0.60$ for ER, and $r = -0.015$, (NS), $P = 0.71$ for PR. When only the IR-PSA-positive tumors were used for correlation (N = 151) the following Pearson correlation coefficients were obtained: $r = -0.015$, (NS), $P = 0.85$ for ER, and $r = -0.068$, (NS), $P = 0.40$ for PR.

We also found that PSA was more likely to be positive in patients ≤ 55 years ($P = 0.012$, Table 3).

Tumor stage information was available in 203 patients. We analyzed the distribution of IR-PSA-pos-

Table 2. Relationship between estrogen and progesterone receptors, PSA immunoreactivity, and p53 levels in breast tumor extracts[1]

Samples (N = 525)	PSA (+) (%)	PSA (−) (%)	P Value
ER (+) 393	127 (32.3)	266 (67.7)	
ER (−) 132	24 (18.2)	108 (81.8)	0.002
PR (+) 321	111 (34.6)	210 (65.4)	
PR (−) 204	40 (19.6)	164 (80.4)	< 0.001
ER (+) or PR (+) 407	131 (32.2)	276 (67.8)	
ER (−) and PR (−) 118	20 (16.9)	98 (83.1)	0.001
ER (+) and PR (+) 307	107 (34.8)	200 (65.2)	
ER (+) and PR (−) 86	20 (23.3)	66 (76.7)	
ER (− and PR (+) 14	4 (28.6)	10 (71.4)	
ER (−) and PR (−) 118	20 (16.9)	98 (83.1)	0.002
Samples (N = 558)	**p53 (+) (%)**	**p53 (−) (%)**	**P Value**
ER (+) 416	64 (15.4)	352 (84.6)	< 0.001
ER (−) 142	50 (35.2)	92 (64.8)	
PR (+) 338	47 (13.9)	291 (86.1)	< 0.001
PR (−) 220	67 (30.4)	153 (69.6)	
ER (+) or PR (+) 428	68 (15.9)	360 (84.1)	< 0.001
ER (−) and PR (−) 130	46 (35.4)	84 (64.6)	
Samples (N = 474)	**p53 (+) (%)**	**p53 (−) (%)**	**P Value**
PSA (+) 90	20 (22.2)	70 (77.8)	0.37
PSA (−) 384	103 (26.8)	281 (73.2)	

[1] For negativity cutoff levels see text. Values in brackets are percentages.

itive tumors by stage, and the results are shown in Table 4. Clearly, there is a trend for the IR-PSA-positive tumors to be preferentially associated with earlier disease stage, but the preference did not reach statistical significance, perhaps because the number of samples per group was relatively small.

Some breast tumors had very high IR-PSA immunoreactivity. Highest values were obtained for five tumors in which PSA levels were > 20 μg/L in the extracts and between 200–1000 ng of IR-PSA per g of breast tumor tissue.

Discussion

Our data strongly suggests that breast tumors produce PSA, an antigen that was originally thought to be highly specific for the prostate. Previous immunohistochemical studies found no PSA immunoreactivity in breast or other tumors [17], or found occasional PSA immunoreactivity with polyclonal but not monoclonal antibodies, suggesting cross-reactivity effects [18].

The immunoreactive PSA species identified in breast tumors exhibits important similarities with seminal PSA. They both react with monoclonal and polyclonal antibodies incorporated in FDA-approved PSA methods, and exhibit the same molecular weight as assessed by high performance liquid chromatography and Western blot analysis. However, final identification of the PSA immunoreactivity in breast tumors by protein sequencing, after purification in sufficient quantities, must await further experimentation.

Table 3. Distribution of PSA-positive, estrogen receptor-positive, and progesterone receptor-positive tumors in various age groups

Age	% of positive tumors[1]		
	PSA (+)	ER (+)	PR (+)
< 35 (N = 18)	33.3 (6/18)	50.0 (9/18)	44.4 (8/18)
35–44 (N = 66)	36.4 (24/66)	71.2 (47/66)	62.1 (41/66)
45–54 (N = 104)	38.5 (40/104)	64.4 (67/104)	58.7 (61/104)
> 55 (N = 336)	23.8 (80/336)	80.1 (269/336)	62.5 (210/336)
P Value [2]	0.012	0.001	0.45

[1] In brackets are numbers of positive tumors per total number of tumors in each group. N = number of patients per group.

[2] P value for comparing the distribution of positive tumors for each parameter, in the various age groups.

The percentage of tumors containing immunoreactive IR-PSA is significant in our study (approximately 30%) and is similar to the percentage of tumors with amplification of the HER-2 oncogene [19]. The PSA immunoreactivity in the tumor has a molecular weight of approximately 30 kDa which is identical to that of the free PSA molecule. We speculate that the appearance of PSA in breast tumors is due to PSA gene derepression by steroid hormone receptors bound to either estrogens, progestins, or androgens. This notion is supported by our finding that most tumors producing IR-PSA contain receptors for estrogen and/or progesterone. Only 20 of the 151 IR-PSA-positive tumors were negative for estrogen and progesterone receptors. From these 20 tumors, 15 had detectable estrogen and/or progesterone receptors but their concentration was below the cutoff point of 10 fmol/mg of protein, used in this study. Only five IR-PSA-positive tumors had undetectable estrogen or progesterone receptors (receptors < 1 fmol/mg of protein). In these five tumors the PSA immunoreactivity was < 0.34 μg/L.

Previous studies have shown an association of estrogen, progesterone, and androgen receptors [20]. We do not have the facility to measure the androgen receptors on the specimens utilized in this study. It may well be that an even higher correlation in the presence of receptors and PSA immunoreactivity would have been reserved had it been possible to include the androgen receptors in the studies undertaken. Our observation that the IR-PSA in breast tumors is associated with the presence of estrogen and/or progesterone receptors, while there is no correlation between the *levels* of IR-PSA and receptors, prompts us to speculate that the receptors may facilitate but are not sufficient for IR-PSA production. We postulate that one or more as yet unidentified ligands act in a permissive environment, regulated by steroid hormone receptors, that in turn, regulates PSA gene derepression [21–24]. These events only occurred in a third of the steroid hormone receptor positive tumors. It is not clear whether the ligand necessary for this activity is absent in the remaining cells, or the receptors are de-

fective as previously suggested [25], or the ligand-receptor complexes are formed but are ineffective.

Our suggestion of the mode of PSA gene derepression in breast cancer is further supported by our finding that IR-PSA production is associated with younger patient age (P = 0.012, Table 3). In patients over the age of 55, only 24% of tumors produce IR-PSA despite the fact that 80% of the tumors were steroid hormone receptor-positive. In patients under the age of 35, 33% of tumors produced IR-PSA despite the fact that only 50% of these tumors were steroid hormone receptor-positive. To further demonstrate the effect of age on IR-PSA production we calculated the percentages of tumors that produce IR-PSA from the total number of estrogen or progesterone receptor-positive tumors. These are 67% (6/9) and 75% (6/8), respectively, for the age group < 35 years, and 29.7% (80/269) and 38.1% (30/210), respectively, for the age group > 55 years (data from Table 3). Although more work is needed to clarify this point, it is tempting to speculate that the higher IR-PSA positivity rate among younger patients is related to production of the putative ligands by the functioning ovaries. The relationships between age, receptor status, and IR-PSA levels are further developed in Table 3.

The higher IR-PSA positivity rates among tumors which are positive for both estrogen and progesterone receptors and the intermediate positivity rates among tumors which are positive for only estrogen or only progesterone receptors, in comparison to the tumors which are negative for both receptors (Table 2), suggests that either receptor can potentiate the production of IR-PSA.

Table 4. Association of PSA-positive tumors with disease stage

Disease stage	% with PSA-positive tumors[1]
0	42.9 (6/14)
1	30.7 (35/114)
2	22.0 (13/59)
3	12.5 (2/16)
	P = 0.18
0–1	32.0 (41/128)
2–3	20.0 (15/75)
	P = 0.06

[1] In brackets are numbers of positive tumors per total number of tumors in each group.

Although disease stage was available only for 203 patients, the association between IR-PSA positivity and disease stage is illuminating (Table 4). There is a trend for IR-PSA-positive tumors to be preferentially associated with lower disease stage.

Our findings may have practical implications. It will be of interest to examine which ligands are involved in steroid hormone receptor binding and PSA gene regulation in breast cancer. Breast tumors producing IR-PSA constitute a sizable group (29% of patients) who may be examined in retrospective or prospective studies to establish if they have a different prognosis or favorable response to treatment. Although preliminary, our data suggests that IR-PSA may be a favourable prognostic indicator because it is associated more strongly with tumors that are positive for both receptors and with lower disease stage. The suggested mode of PSA production based on the findings that the overwhelming majority of IR-PSA-positive tumors have detectable receptors (146/151 or 97%) and that younger patients are more positive than older patients, prompts us to speculate that the PSA-positive tumors are a subgroup that possess 'effective' receptors and ligands, capable of gene regulation, as exemplified by IR-PSA production. It will be of interest to examine if this is the group of patients most likely to respond to steroid hormone therapy. This notion was recently suggested for the steroid hormone receptor-inducible pS_2-BCEI protein, another potential prognostic indicator in breast cancer [26, 27].

PSA is an active kallikrein-like serine protease, and the breast tumor PSA immunoreactivity may be in an enzymatically active form. Like some other proteases, PSA might facilitate cancer cell migration and invasion by digesting the basement membrane, extracellular matrix, and connective tissue. This mechanism has been proposed for cathepsin D, which is another steroid hormone receptor-inducible breast cancer prognostic indicator [28, 29]. However, we have no indication that PSA immunoreactivity is associated with more aggressive breast tumors.

We conclude that our finding of immunoreactive PSA production by 29% of breast tumors underscores the similarities between breast and prostate

tissues and holds promise of being used in breast cancer for prognosis, selection of therapy, or devising new therapeutic interventions. Apart from its potential clinical usefulness in breast cancer, PSA immunoreactivity could also be used in more basic studies as a biochemical marker of gene regulation by steroid hormone receptors. Clearly, more studies are needed to further investigate the importance and implications of our findings.

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