

## Expression of the Prostate Specific Antigen Gene by Lung Tissue

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### ABSTRACT

We describe a female patient with lung adenocarcinoma whose tumor extract was highly positive for prostate specific antigen (PSA) immunoreactivity. PSA was present in its  $M_r$  33,000 free form. Using reverse transcription-PCR, we were able to amplify a 754-bp fragment that specifically hybridized to a PSA RNA probe on Southern blots. The PCR fragment was sequenced and found to represent PSA cDNA and not human glandular kallikrein cDNA. PSA immunoreactivity in the lung tissue was localized by immunohistochemistry to normal epithelial cells adjacent to the tumor which was completely negative for PSA. Tissue culture experiments suggested that beclomethasone, a glucocorticoid used to treat the patient, was able to up-regulate PSA gene expression. This is the first report that unequivocally demonstrates PSA expression in lung tissue. We speculate that PSA expression was mediated by the exogenously administered steroid beclomethasone.

### INTRODUCTION

There is now convincing evidence that the  $PSA^2$  gene is expressed in many human tissues (1). The highest levels of PSA are found in the prostate and in seminal plasma (2). Significant levels are also found in female breast discharge fluid, in breast milk (3), and in extracts of normal, hyperplastic, and cancerous breast tissues (4-10). PSA was also found in some ovarian tumor extracts (11), in endometrium (12), salivary glands (13), and in many other tumor types (14).

Recently, Bilgrami *et al.* (15) described a male patient with lung carcinoma who had high serum PSA levels (15). The

authors postulated that the PSA may have arisen from the lung cancer tissue. However, the tumor stained positive for PSA by polyclonal but not monoclonal antibodies. We have also reported the presence of immunoreactive PSA protein in some lung tumors (16).

Because PSA is a protein thought to be expressed only in the prostate, any extraprostatic source must be rigorously verified for the following reasons: (a) PSA is highly homologous to the *hGK* gene (*hGK-1* or *KLK-2*) and antibodies against PSA (now designated as *KLK-3*) cross-react with *KLK-2* (17, 18); (b) in males, PSA is present at high levels in serum, and male tissue extracts may contain contaminating amounts of PSA from serum; and (c) any tissue in males may contain PSA due to metastatic spread of a primary prostatic tumor.

In this report, we describe a female patient with a primary lung tumor whose lung tissue produced PSA as verified by immunofluorometric analysis, immunohistochemistry, reverse transcription-PCR, and DNA sequencing. We provide evidence with tissue culture studies that PSA production in this patient was induced by glucocorticoid treatment.

### PATIENT AND METHODS

**Patient.** The patient was an 87-year-old female who was initially admitted to the Emergency Department of St. Joseph's Health Center, Toronto, with acute cholecystitis. A chest X-ray at the time of admission revealed that the patient had fairly severe lung disease with chronic emphysema but also a nodule in the right lung suspicious for carcinoma. The patient underwent wedge resection. On pathological examination, the lung lesion proved to be a moderately differentiated adenocarcinoma with a maximum dimension of 1.5 cm. The cancer arose in an area of a scar with extensive deposition of anthracotic pigment. The tumor consisted of sheets and glands of large cells with eosinophilic cytoplasm and large hyperchromatic nuclei with central nucleoli. The pleural surface and resection margin were free of tumor.

Postoperatively, the patient did well initially; however, she soon developed abdominal pain and was totally unable to eat. With a diagnosis of cholecystitis, the patient underwent a video-assisted cholecystectomy. Postoperatively, she developed complications with abdominal bleeding and died 32 days after admission.

During the course of her disease, she was treated with the following drugs: Zantac (ranitidine hydrochloride;  $H_2$  receptor antagonist); diltiazem (antianginal agent; calcium channel blocker); Ventolin (salbutamol; bronchodilator,  $\beta$ -2 adrenergic stimulant); Lasix (furosemide, diuretic), Theo-Dur (theophylline, bronchodilator); Demerol (meperidine, narcotic, analgesic); morphine (narcotic, analgesic); and Becloforte (beclomethasone dipropionate, antiinflammatory corticosteroid).

Tumor tissue was snap-frozen in liquid nitrogen and subsequently stored at  $-80^\circ\text{C}$ . In parallel, tumor tissue and adjacent normal tissue excised during surgery were fixed in formalin and embedded in paraffin. Fresh frozen tissue was used to prepare

Received 1/2/97; revised 3/20/97; accepted 3/27/97.

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<sup>2</sup> The abbreviations used are: PSA, prostate specific antigen; HPLC, high-performance liquid chromatography; RT-PCR, reverse transcription-PCR; DIG, digoxigenin; hGK, human glandular kallikrein.

cytosolic extracts and for RNA extraction; the paraffin-embedded tissue was used for histopathology and immunohistochemistry.

**Preparation of Cytosolic Extracts.** Approximately 0.2 g of tissue from the lung adenocarcinoma was pulverized to a fine powder at  $-80^{\circ}\text{C}$ , and then the cells were lysed for 30 min on ice with 1 ml of lysis buffer [50 mM Tris buffer (pH 8.0), containing 150 mM NaCl, 5 mM EDTA, 10 g/liter NP40 surfactant, and 1 mM phenylmethylsulfonyl fluoride]. The lysate was centrifuged at  $15,000 \times g$  at  $4^{\circ}\text{C}$  for 30 min, after which the supernatant was immediately assayed for PSA immunoreactivity and total protein content.

**HPLC.** HPLC analysis was performed with a Hewlett Packard 1050 system. The mobile phase for the gel filtration chromatography was a 0.1 M sodium sulfate and 0.1 M sodium dihydrogen phosphate, pH 6.80. The size exclusion column used was a TSK-GEL G3000SW, 60 cm  $\times$  7.5 mm (TosoHaas, Montgomeryville, PA) and was calibrated with a molecular mass standard solution from Bio-Rad (Bio-Rad Laboratories, Hercules, CA). The flow rate was 0.5 ml/min, and HPLC was run isocratically. After injection of 500  $\mu\text{l}$  of centrifuged sample, fractions of 0.5 ml were collected and analyzed for PSA (see below).

**PSA Measurements.** PSA in the cytosolic lung extract was measured with a highly sensitive and specific immunofluorometric technique established previously and described in detail elsewhere (19). In brief, the PSA assay uses a mouse monoclonal anti-PSA capture antibody coated to polystyrene microtiter wells, a biotinylated monoclonal anti-PSA detection antibody, and alkaline phosphatase-labeled streptavidin. In this immunoassay, 100  $\mu\text{l}$  of sample are incubated with the coating antibody in the presence of 50  $\mu\text{l}$  of assay buffer containing the monoclonal biotinylated anti-PSA detection antibody. After 1 h incubation followed by washing six times, the alkaline phosphatase-labeled streptavidin conjugate is added for 15 min followed by another washing six times. The activity of alkaline phosphatase is then measured by adding the substrate 5'-fluorosallyl phosphate, incubating for 10 min, and then by adding a  $\text{Tb}^{3+}$ - and EDTA-containing developing solution. After 2 min, the fluorescence is measured in the time-resolved fluorometric mode with the CyberFluor-615 immunoanalyzer (CyberFluor, Inc., Toronto, Ontario, Canada). This assay has a detection limit of 1 ng/liter of PSA.

**Isolation of Total RNA.** For total RNA isolation, we used the Trizol reagent (Life Technologies, Inc., Gaithersburg, MD) following the instructions of the manufacturer. The integrity of the RNA was checked electrophoretically, and its amount and purity were established by spectrophotometry at 260 and 280 nm.

**RT-PCR.** We synthesized cDNA from the isolated total RNA with a first-strand cDNA synthesis kit and SuperScript II reverse transcriptase (Life Technologies, Inc.). In brief, 1–2  $\mu\text{g}$  of total RNA and 500 ng of oligo(dT)<sub>12–18</sub> primers were first denatured for 10 min at  $70^{\circ}\text{C}$ , chilled on ice for 1 min, and then incubated for 5 min at  $42^{\circ}\text{C}$  in a 19- $\mu\text{l}$  reaction mixture containing PCR buffer, 2.5 mM  $\text{MgCl}_2$ , 10 mM DTT, and 1 mM deoxynucleoside triphosphates. Then, 200 units (1  $\mu\text{l}$ ) of SuperScript II reverse transcriptase were added to the reaction mixture, which was then incubated for 50 min at  $42^{\circ}\text{C}$ , termi-

nated at  $70^{\circ}\text{C}$  for 15 min, and chilled on ice. Before proceeding to the amplification of the target cDNA, we treated the mixture with 1  $\mu\text{l}$  of RNase H for 20 min at  $37^{\circ}\text{C}$ . Negative control reactions for RT-PCR were performed by using all other reagents but the SuperScript II. We used two oligonucleotide primers to amplify a 754-bp region of PSA cDNA. The primers, originally described by Deguchi *et al.* (20), have the following sequences: PSA-A1, 5'-TGCGCAAGTTCACCCTCA-3'; and PSA-A2, 5'-CCCTCTCCTTACTTCATCC-3'.

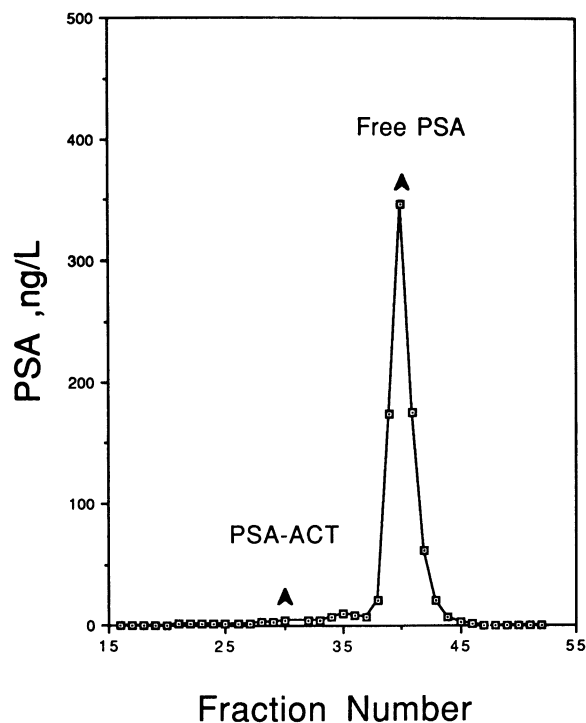
For actin cDNA amplification, we used the following primers published previously (21): 5'-ACAATGAGCTGCGTG-TGGCT-3' and 5'-TCTCCTTAATGTCACGCACG-3'. Actin primers amplify a 372-bp fragment.

PCR was performed in 0.2-ml thin-walled microAmp reaction tubes on a Perkin-Elmer (Palo Alto, CA) GeneAmp 2400 system. Total volume was 50  $\mu\text{l}$ . The reaction mixture contained PCR buffer [50 mM KCl, 10 mM Tris buffer (pH 8.3), 1.5 mM  $\text{MgCl}_2$ , and 10 mg/liter gelatin], 200  $\mu\text{M}$  deoxynucleoside triphosphatase, 1  $\mu\text{M}$  PCR primers, 2.5 units of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN), and 5  $\mu\text{l}$  of cDNA target (added last). The PCR for PSA and actin was performed with 1 cycle at  $94^{\circ}\text{C}$  for 5 min, 30 cycles with denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $56^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 30 s and 1 cycle at  $72^{\circ}\text{C}$  for 7 min. Portions (20  $\mu\text{l}$ ) of the PCR reaction products were electrophoresed on 2% agarose gels and visualized with ethidium bromide staining. Negative controls did not contain template DNA.

**Cloning of PCR Products.** We have generated a recombinant pCR 2.1 plasmid by cloning the 754-bp PCR product of PSA cDNA into the pCR 2.1 plasmid using the TA cloning kit (Invitrogen Corp., San Diego, CA). We followed the protocol recommended by the manufacturer. Large quantities of the plasmid were prepared by culturing transformed *Escherichia coli* cells in LB-ampicillin media and extracting with the Qiagen Midi plasmid purification kit (Qiagen, Inc., Chatsworth, CA).

**RNA Labeling by *in Vitro* Transcription.** The recombinant pCR 2.1 transcription vector contains promoters for SP6 and T7 RNA polymerases. After linearization of the vector with *Hind*III restriction enzyme, the T7 RNA polymerase was used to create "run-off" transcripts using the DIG RNA labeling kit (Boehringer Mannheim, Mannheim, Germany). DIG-UTP is used as a substrate and was incorporated into the transcript. The DIG-labeled RNA was used as a nonradioactive probe in Southern blots (see below).

**Hybridization.** Aliquots of PCR products (20  $\mu\text{l}$ ) were electrophoresed on 2% agarose minigels containing ethidium bromide. The gels were then Southern-transferred onto positively charged nylon membranes (Boehringer Mannheim) by overnight alkali capillary blotting with use of 0.4 M NaOH. The membranes were then baked for 15–30 min at  $120^{\circ}\text{C}$ . For hybridization, the membranes were placed in tubes with 20 ml of hybridization buffer per 100  $\text{cm}^2$  of membrane. We used a commercial hybridization solution (DIG Easy Hyb; Boehringer Mannheim). Prehybridization was at  $68^{\circ}\text{C}$  for 1 h. The solution was then replaced with 5 ml per 100  $\text{cm}^2$  of membrane of hybridization buffer containing 200 ng/ml of DIG-labeled PSA RNA probe. Hybridization was carried out for 12–16 h at  $42^{\circ}\text{C}$ . Filters were subsequently washed twice with  $2 \times$  SSC contain-

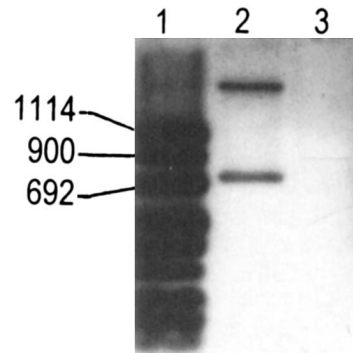


**Fig. 1** Molecular weight identification of PSA immunoreactivity in the lung tissue extract using HPLC with a gel filtration column. After injecting 500  $\mu$ l of tumor extract, HPLC fractions (0.5 ml) were analyzed with an assay that recognizes both free PSA ( $M_r$  33,000) and PSA- $\alpha_1$ -antichymotrypsin (PSA-ACT) complex ( $M_r$  100,000). All PSA immunoreactivity in the lung tumor extract elutes at a molecular weight of  $M_r$  33,000 (fraction  $40 \pm 2$ ) and represents free PSA. PSA- $\alpha_1$ -antichymotrypsin complexes (eluting at fraction  $32 \pm 2$ ) were not found.

ing 1 g/liter SDS at room temperature (5 min per wash) and twice with  $0.1 \times$  SSC containing 1 g/liter SDS at  $68^\circ\text{C}$  (15 min per wash). Detection was accomplished by using an antibody against digoxigenin, labeled with alkaline phosphatase and the chemiluminogenic substrate CDP-Star (Tropix, Bedford, MA). We followed the instructions of the manufacturer and captured the chemiluminescence on X-ray film using exposure times of 10–60 s.

**DNA Sequencing.** PCR products were directly sequenced using the Thermo Sequenase kit (Amersham International, Buckinghamshire, England). The protocol recommended by the manufacturer was used throughout. Sequencing primers, labeled at the 5'-end with Cy5 fluorescent dye, were as follows: PSA-S1, 5'-AAGGTGACCAAGTTCATG-3' (binds 19 nucleotides internally from PCR primer PSA-A1); and PSA-S2, 5'-CCATCCCATGCCAAAGGA-3' (binds 19 nucleotides internally from PCR primer PSA-A2). All sequencing reactions were loaded on the ALF Express automatic sequencer (Pharmacia Biotech, Inc., Uppsala, Sweden). Sequence comparisons were performed with the BLAST software.

**Immunohistochemistry.** Immunohistochemical stains for PSA protein were performed on 4- $\mu\text{m}$ -thick paraffin sections of selected blocks of the primary lung adenocarcinoma using a streptavidin-biotin technique and a polyclonal antiserum directed against human seminal plasma PSA protein (Biomeda,



**Fig. 2** RT-PCR of RNA extracted from the lung tissue of the patient under discussion. The PCR product was run on an agarose gel, Southern-blotted, and hybridized with a DIG-labeled RNA probe. Detection of hybrids was achieved with chemiluminescence. Lane 1, DIG-labeled molecular weight markers with length in base pairs shown on the left. Lane 2, RT-PCR product for the patient. The 754-bp PCR product is clearly identified along with a higher molecular weight product (genomic DNA). Lane 3, PCR negative control.

Foster City, CA). Sections were pretreated with microwave heating to facilitate antigen retrieval. Human prostate was used as a positive control, whereas nonimmune rabbit serum served as a negative control for assessment of nonspecific staining.

**Tissue Culture Experiments.** All compounds used in this study were obtained from Sigma Chemical Co. (St. Louis, MO), except for the beclomethasone dipropionate, which was obtained from Glaxo Wellcome, Inc., (Mississauga, Ontario, Canada). Stock solutions ( $10^{-2}$  or  $10^{-3}$  M) were prepared in absolute ethanol. Further dilutions were also prepared in the same solvent.

**Stimulation Experiments.** The BT-474 breast carcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). This cell line is positive for estrogen, progesterone, androgen, and glucocorticoid receptors. BT-474 cells were cultured in RPMI 1640 media (Life Technologies, Inc.) supplemented with glutamine (200 mM), bovine insulin (10 mg/liter), fetal bovine serum (10%), antibiotics (penicillin and streptomycin), and antimycotics (amphotericin B). The cells were cultured to near confluency in plastic culture flasks and then transferred to phenol red-free media containing 10% charcoal-stripped fetal bovine serum with antibiotics/antimycotics. Phenol red-free media were used because phenol red was found previously to have weak estrogenic activity, and charcoal-stripped fetal bovine serum is devoid of steroid hormones.

The BT-474 cells were aliquoted into 24-well tissue culture plates (Corning #25820) and cultured to confluency with a change in media at 3 days. Stimulations were carried out with confluent cells containing 2 ml of phenol red-free media with 10% charcoal-stripped FCS and antibiotics/antimycotics. Stimulation was initiated by adding 2  $\mu$ l of the testing compound dissolved in 100% ethanol and incubating for 5–8 days. Tissue culture supernatants ( $\sim 150$   $\mu$ l) were then removed for PSA analysis. Appropriate multiple positive and negative controls (only alcohol added) were included in each experiment. The final concentration of each steroid was used for data interpretation.

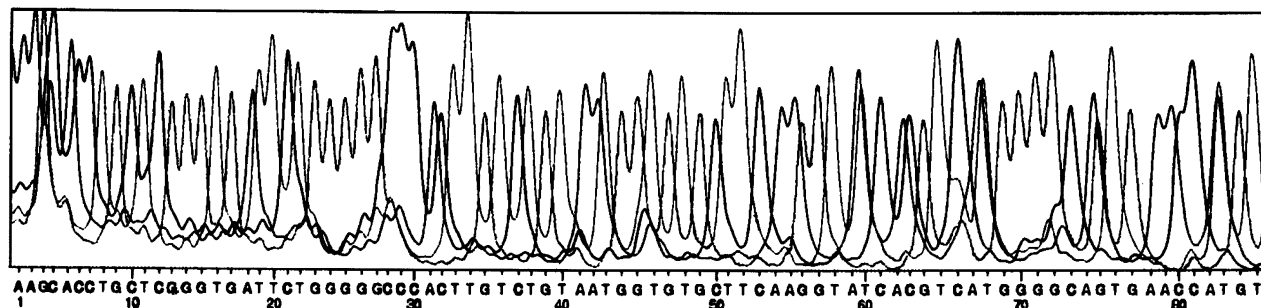


Fig. 3 Representative chromatogram of an 85-nucleotide region of the PSA cDNA sequence isolated from lung tissue. For details, see text.

1	AAGCACCTGC	<u>TCGGGTGATT</u>	CTGGGGGCC	ACTTGTCTGT	AATGGTGTGC
51	TTCAAGGTAT	CAGTCATGG	GGCAGTGAAC	CATGTGCCCT	GCCCGAAAGG
101	CCTTCCCTGT	ACACCAAGGT	GGTGCATTAC	CGGAAGTGG	TCAAGGACAC
151	CATCGTGGCC	AACCCCTG <u>AG</u>	CACCCCTATC	AAC <u>CC</u> CTAT	TGTAGTAAAC
201	TTGGAACCTT	GGAAATGACC	AGGCCAAGAC	TCAGGCCCTC	CCAGTTCTAC
251	TGACCTTGT	CCTTAGGTGT	GAGGTCCAGG	GTTGCTAGGA	AAAGAAATCA
301	GCAGACACAG	GTGTAGACCA	GAGTGTCTT	TAAATGGTGT	AATTTTGTCC
351	TCTCTGTGTC	CTGGGGAATA	CTGGCCATGC	CTGGAGACAT	ATCACTCAAT
401	TTCTCTGAGG	ACACAGATAG	GATGGGGTGT	CTGTGTTATT	TGTGGGGTAC
451	AGAGATGAAA	GAGGGGTGGG	ATCCACACTG	AGAGAGTGG	GAGTGACATG
501	TGCTGGACAC	TGTCCATGAA	GCACTGAGCA	GAAGCTGGAG	GCACAACGC

Fig. 4 Complete sequence of the 549-nucleotide region of the PSA cDNA sequence from the mRNA isolated from the lung tissue. **Bold** and underline indicate the end of exons 4 and 5, respectively. Underlined sequences represent a six-base repeat in which one nucleotide (double underlined) is frequently polymorphic (Table 1). Sequence variation was also seen at positions 234 and 184 (underlined).

## RESULTS

The tumor extract from the primary lung adenocarcinoma was measured for PSA protein; the PSA concentration obtained was 958 ng/liter and was considered highly positive for PSA immunoreactivity. In contrast, another 10 lung adenocarcinomas from women, when extracted, exhibited PSA concentrations <20 ng/liter. Contamination of the tumor extract by blood PSA is unlikely because the PSA concentration in the serum of this woman was 4 ng/liter preoperatively and 5 ng/liter postoperatively.

To establish the molecular weight of the immunoreactive species in the tumor extract, we performed HPLC with a gel filtration column (Fig. 1). Virtually 100% of the immunoreactive species elutes at fraction 40, corresponding to a molecular weight of approximately  $M_r$  33,000 (free PSA). No complex between PSA and  $\alpha_1$ -antichymotrypsin (molecular weight  $M_r$  ~100,000) was seen.

Total RNA extracted from the lung tissue was reverse-transcribed to cDNA and then amplified by PCR using primers specific for the PSA cDNA sequence. Southern blot hybridization of the PCR product with a PSA DIG-labeled RNA probe detected the PSA band of the expected size (754 bp) in the sample but not in the negative control (Fig. 2). This band was reamplified (to obtain enough material) and sequenced. Both strands were sequenced, and the data were compared with the published PSA and glandular kallikrein cDNA sequences.

Partial sequencing data are shown in Fig. 3. The entire sequence obtained is presented in Fig. 4. The sequence, spanning 549 nucleotides from exons 4 and 5 and the 3'-untranslated

region has >99% sequence similarity to the published sequence of PSA cDNA or genomic DNA (Table 1). However, there is only about 80% similarity with the human glandular kallikrein gene, in accordance with literature reports (22).

We found four positions where there is variation between our sequence and those reported in the literature. These differences, all present in the 3' untranslated region, may represent polymorphisms. At two positions (position 427 and 447) the variation appears in the same spot (underlined) of the sequence repeat TGGGGT (Fig. 4).

We localized the PSA immunoreactivity with immunohistochemistry (Fig. 5). Although the lung tumor was entirely negative for PSA, trapped nontumorous bronchioles at the edge of the tumor contained focal PSA immunoreactivity in epithelial lining cells.

Because the *PSA* gene is regulated by steroid hormones (5), we examined whether the only steroid administered to the patient during hospitalization (beclomethasone dipropionate) was able to stimulate PSA production in a tissue culture system. The breast carcinoma cell line BT-474 does not produce PSA when cultured in the absence of steroid hormones, but it does so when stimulated by androgens, progestins, and glucocorticoids.<sup>3</sup> Beclomethasone dipropionate was able to stimulate PSA production in this cell line in a dose-dependent manner at levels  $10^{-9}$ - $10^{-7}$  M (Fig. 6).

<sup>3</sup> Unpublished data.

Table 1 Comparison of DNA sequence of PCR product found in this study with sequences deposited in GenBank

GenBank accession	DNA	Position <sup>a</sup>			
		427	447	234	184
This study (lung tissue)	cDNA	G	G	G	T
X07730	cDNA	G	G	G	T
X05332	cDNA	G	G	A	C
X13944	Genomic	A	G	A	C
M21895	cDNA	G	T	A	T
M21896	cDNA	G	T	A	T
M24543	Genomic	G	T	A	T
M26663	cDNA	G	G	A	T
M27274	Genomic	A	G	A	T

<sup>a</sup> Refers to nucleotide number shown in Fig. 4. Nucleotides not shown are identical in all sequences.

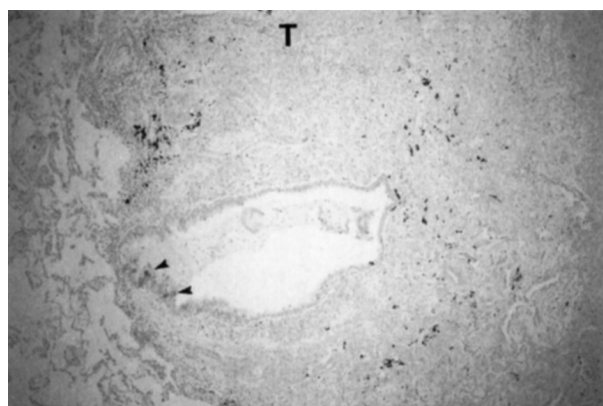


Fig. 5 Immunohistochemical localization of PSA in lung tissue with polyclonal antibody. A trapped nonneoplastic bronchiole at the edge of the tumor contains PSA immunoreactivity in epithelial lining cells. The cytoplasmic positivity is strong but restricted to scattered cells (arrowheads). Tumor cells (T) around the bronchiole are negative. Streptavidin-biotin technique,  $\times 250$ .

## DISCUSSION

PSA is a  $M_r$  33,000 serine protease produced by the prostate gland and secreted into the seminal plasma. It has been suggested that PSA is involved in semen liquefaction (22). More recently, others have proposed that PSA may cleave biologically important substrates like insulin-like growth factor binding protein 3 (23), parathyroid hormone related peptide (24), or unknown substrates that release peptides with smooth muscle contraction activity (25).

The search for new biological functions of PSA has recently been intensified after the realization that PSA is expressed in diverse tissues (1). Striking similarities between PSA expression and expression of the breast cancer susceptibility gene *BRCA-1* have been noted (26). Extraprostatically, PSA was found in a very high proportion of normal, benign, and cancerous breast tissues, in a small proportion of diverse malignancies (14), in the salivary glands (13), ovarian tumor extracts (11), and endometrium (12). The highest concentration of PSA, except for seminal plasma, was found in breast discharge fluid (up to 15 mg/liter) and in milk of lactating women (up to 0.3 mg/liter; Ref. 3).

It has been known for years that, in the prostate, the *PSA* gene is regulated by androgens through the androgen receptor

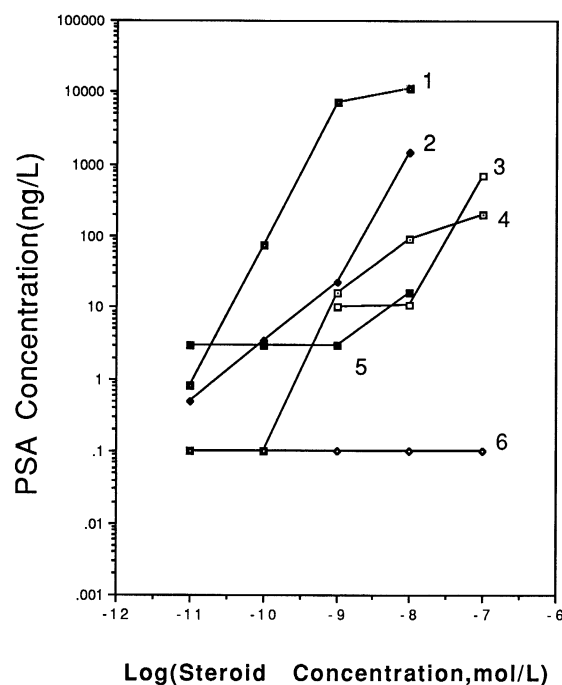


Fig. 6 Production of PSA by the breast carcinoma cell line BT-474 after stimulation by beclomethasone dipropionate and other steroids. PSA was measured in the tissue culture supernatant 5 days after stimulation of confluent cells with a single dose. 1, Norgestrel (progestin); 2, Beta-methasone (glucocorticoid); 3, dexamethasone (glucocorticoid); 4, beclomethasone (glucocorticoid); 5, prednisone (glucocorticoid); 6, estradiol (estrogen). Dose-dependent PSA production is seen at beclomethasone levels between  $10^{-9}$ - $10^{-7}$  M.

(22). Using a tissue culture system with breast carcinoma cell lines, we have demonstrated that androgens, progestins, and glucocorticoids can independently up-regulate the *PSA* gene (5). We have also provided evidence that this regulation occurs *in vivo* by describing a woman whose breast tissue PSA was very high due to stimulation by a progestin-containing oral contraceptive (8) and another woman whose ovarian tumor was producing PSA, likely due to stimulation by glucocorticoids (11).

Although two previous studies have suggested that PSA may be produced by lung tissue and tumors (15, 16), their data were based only on protein measurements. Because PSA has extensive sequence homology with the *hGK-1* gene and protein and PSA antibodies cross-react with hGK-1 (17, 18), the only certain way to verify the presence of PSA in lung is by either sequencing the purified protein (which is impractical due to the very low levels present) or by molecular analysis of the expressed PSA mRNA. We describe here a patient whose lung tissue extract was highly positive for PSA protein in its  $M_r$  33,000 form. In this patient, we were able to amplify PSA mRNA and sequence PSA cDNA to verify its identity as PSA cDNA and not hGK-1 cDNA. Kallikreins are widely distributed and expressed in various tissues including salivary glands, intestine, lung, brain, and nerve tissue (27). Immunohistochemical localization has shown that the PSA-positive tissue is in the normal lung epithelium adjacent to the tumor. This is in accord with data from breast tumors in which we noted more frequent staining for PSA of normal and hyperplastic

breast epithelium and well-differentiated tumors. Poorly differentiated and steroid hormone receptor-negative breast tumors are usually negative for PSA.<sup>3</sup>

This patient was unique because her lung tissue contained more than 50 times the PSA immunoreactivity of another 10 lung tumor extracts from women. We postulate that in this patient, the PSA was up-regulated by the steroid beclomethasone dipropionate because this drug was found to be able to up-regulate PSA production *in vitro* in a dose-dependent manner (Fig. 6). These data parallel observations in a patient with ovarian carcinoma (11) and a patient receiving oral contraceptives (8). Recently, in support of our hypothesis, Beattie *et al.* (28) reported the presence of high affinity receptors for androgen, estrogen, and glucocorticoids in lung tissues and carcinomas. Beer and Malkinson (29) reported similar data in mouse lung tumors. We conclude that PSA can be expressed in a variety of tissues bearing steroid hormone receptors and that its expression can be manipulated by exogenously administered hormones. The biological role of PSA in these tissues is still unknown. In further support of this work are data demonstrating that by using sensitive RT-PCR methods, at least 50% of normal and cancerous lung tissues can be found positive for PSA mRNA (30).

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