

Expression of the Prostate-specific Antigen Gene by a Primary Ovarian Carcinoma¹

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Abstract

We describe a patient with primary ovarian carcinoma that developed after liver transplantation whose tumor was highly positive for prostate-specific antigen (PSA). PSA in tumor tissue was characterized by two immunoassays, HPLC, immunohistochemistry, reverse transcription-PCR, Southern blotting, and DNA sequencing. PSA in the ovarian tumor was present as free, M_r 33,000 protein (>90%) and as PSA bound to α_1 -antichymotrypsin (M_r 100,000; <10%). Immunohistochemistry localized PSA in the cytoplasm of epithelial cells of the tumor. Two separate reverse transcription-PCRs for PSA amplified the expected products which hybridized specifically to a PSA cDNA probe on Southern blots. Sequencing of the PCR products, representing the whole coding sequence of the PSA gene, revealed identity with the sequence of PSA cDNA from prostate tissue. These data suggest that the PSA produced by the ovarian tumor was identical in molecular weight and sequence to prostatic PSA. Based on data of tissue culture experiments with breast carcinoma cell lines, we speculate that the PSA gene in the tumor of this patient was up-regulated by the therapeutically administered glucocorticoids after liver transplantation.

Introduction

PSA³ is a M_r 33,000 serine protease present at high concentrations in seminal plasma (1, 2). Since its identification in 1979, PSA was believed to be secreted exclusively by the epithelial cells of the prostate gland. More recently, it has been shown that PSA could also be found in the periurethral and perianal glands (3-5) and very rarely in tumors of the skin (6), salivary glands (7), and in ovarian teratomas (8). We found that PSA is present in 30% of female breast tumors and that its production in these tumors is associated with the presence of steroid hormone receptors (9, 10). PSA-positive breast tumors have a better prognosis than PSA-negative breast tumors (11). PSA production by breast cancer cell lines can be induced by glucocorticoids, androgens, and progestins (12). Normal breast can be stimulated by oral contraceptives to produce PSA (13). PSA is also found in the milk of lactating women (14) and in amniotic fluid (15).

Based on a study of 98 primary ovarian tumors, we have recently concluded that PSA is found in minute amounts in only 5% of such tumors (16). The highest PSA content observed in primary ovarian tumor extracts was 0.40 ng PSA/mg of total protein. We here report a patient who developed a primary ovarian tumor after liver transplantation and who was receiving glucocorticoids for many years prior to the diagnosis of the ovarian neoplasm. This primary ovarian

tumor contained high levels of PSA (5.6 ng/mg). Molecular analysis confirmed that this PSA was identical to PSA produced by the prostate gland. We speculate that the high level expression of the PSA gene in this patient was regulated by the exogenously administered glucocorticoids. The physiological role of PSA in ovarian tissue and tumors and its association with cancer initiation and progression in this patient remains to be determined.

Materials and Methods

Patient. The patient was a 52-year-old female who underwent liver transplantation 7 years ago for Budd-Chiari syndrome. Postoperatively, her course was complicated by a gastric lymphoma which was managed by partial gastric resection. The patient had severe viral encephalitis 4 years ago, which resolved entirely. Three years ago, the patient was diagnosed by ultrasound to have a left adnexal mass comprised of solid and cystic components. The mass was monitored by frequent clinical examinations and ultrasound; continued growth led to surgical resection 2 years after detection. Her medications before surgery but after the liver transplantation were cyclosporin A (200 mg daily), prednisone (7.5 mg daily), and coumadin (3 mg daily). The patient underwent hysterectomy and bilateral salpingo-oophorectomy. Pathological examination revealed a complex neoplasm in the left ovary with focal areas of adenofibroma and benign cystadenoma and, in addition, extensive areas of invasive, moderately differentiated endometrioid adenocarcinoma with prominent stromal luteinization. The stroma was not malignant. The left and right fallopian tube as well as the right ovary were within normal limits.

Tumor tissue was snap-frozen in liquid nitrogen and subsequently stored at -80°C . In parallel, tumor tissue and normal tissue excised during surgery was formalin-fixed and paraffin-embedded. Fresh frozen tissue was used to prepare 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 ovarian tumor was pulverized to a fine powder at -80°C , and the cells were lysed for 30 min on ice with 1 ml of lysis buffer [50 mmol/liter Tris buffer (pH 8.0) containing 150 mmol/liter NaCl, 5 mmol/liter EDTA, 10g/liter NP40 surfactant, and 1 mmol/liter phenylmethanesulfonyl fluoride]. The lysate was centrifuged at $15,000 \times g$ at 4°C for 30 min, after which the supernatant was immediately assayed for PSA protein.

Total protein concentration of the tumor extract was determined by the bicinchoninic acid method commercially available by Pierce Chemical Co. (Rockford, IL). The PSA content of the tumor was expressed as ng of PSA/mg of total protein to compensate for the amount of tissue extracted.

PSA Measurements. PSA in the tumor extract was analyzed with a highly sensitive and specific immunofluorometric procedure described in detail elsewhere (17). The tumor extract was also analyzed by the IM_x^R automated PSA assay (Abbott Laboratories, Abbott Park, IL). An assay that can specifically quantify the PSA-ACT was also used; this assay has been described in detail elsewhere (17). All measurements were performed in duplicate.

HPLC. HPLC was performed as described previously (17).

RNA Extraction and cDNA Synthesis. RNA extraction and cDNA synthesis were performed as described previously (18).

Oligonucleotide Primers. The PSA oligonucleotide sequences used as primers were: PR4, 5'TAAGAAACACTCTGGTCT3'; PR5, 5'AGCCCCAAGCTTACCACCT3'; PR6, 5'CACAATCCGAGACAGGAT3'; PR7, 5'GCCCACTTGCTGTAATG3'; and PR8, 5'CAGGGCACATGGTTCCTG3'. PR2 and PR3 sequences have been published previously (19). Amplification

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³ The abbreviations used are: PSA, prostate-specific antigen; PSA-ACT, PSA- α_1 -antichymotrypsin complex; bp, base pair; TR-IFA, time-resolved immunofluorometric assay.

yielded a 571-bp and a 636-bp PSA cDNA fragments with PR5 (from exon 1) and PR2 (from exon 4) and with PR3 (from exon 3) and PR4 (from the 3' untranslated region) oligonucleotide primers, respectively. The primers used for amplification of human β -actin sequences were described previously and generate a 154-bp cDNA product (18).

PCR Procedure. One μ l of cDNA was added to 39 μ l of PCR mix containing 1X PCR buffer (Boehringer Mannheim, Indianapolis, IN), 0.4 μ mol/liter of each primer, 250 μ mol/liter of deoxynucleoside triphosphates, and 1.25 units of Taq DNA polymerase (Boehringer Mannheim). PCR was performed with primers PR5 and PR2 and with primers PR3 and PR4 for 30 cycles according to the following program: 94°C for 1 min (2 min for the first cycle), 56°C for 1 min, and 72°C for 2 min (5 min for the last extension). Actin gene fragments were amplified from 1 μ l of the cDNA preparation under the same conditions used for PSA PCR. Twenty μ l of each PCR reaction were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining. One μ l of the PCR reaction was used as a template for a second round amplification in order to provide sufficient material for subsequent analysis. Finally, the fragments were purified from agarose gel by ion exchange columns (Qiagen, Dusseldorf, Germany) according to the instructions of the manufacturer.

Sequencing. An automated 373A DNA sequencer (Applied Biosystems, Foster City, CA) and dye terminator kits from the same manufacturer were used for direct sequencing of the PSA cDNA fragments by the dideoxynucleotide chain termination method using fluorescent labels (20). The coding and noncoding strands of each fragment were sequenced with primers generating overlapping sequence data. DNA sequence analysis was performed with PSA oligonucleotide primers PR2, PR3, PR5, and PR6 for the 571-bp fragment and with PR2, PR3, PR4, PR7, and PR8 for the 636-bp fragment. Sequences were reassembled and analyzed using the SAP program (21).

Immunohistochemistry. Immunohistochemical stains for PSA protein were performed on 4- μ m-thick paraffin sections of selected blocks of the ovarian tumor using a streptavidin-biotin technique and a polyclonal antiserum directed against human seminal plasma PSA (201 M, diluted 1:2 from original prediluted preparation; Biomedica, Foster City, CA). Sections were pretreated with microwave heating to facilitate antigen retrieval (22). Human prostate was used as a positive control, while nonimmune rabbit serum served as a negative control for assessment of nonspecific staining.

Results

Tissue extracts from the ovarian tumor were analyzed for PSA by the TR-IFA procedure and by a commercially available automated PSA assay (Abbott Laboratories). The latter assay, IM_x^R , is used widely for serum PSA monitoring of prostate cancer patients. The concentration of PSA in two separate ovarian tumor extracts was 37.1 and 28.0 μ g/liter by TR-IFA and 27.8 and 22.7 μ g/liter by the IM_x^R assay, respectively. Both methods detected very high levels of immunoreactive PSA; the TR-IFA method gave approximately 19–25% higher readings. In order to verify the molecular weight of immunoreactive PSA, we have separated the ovarian tumor extracts on a gel filtration column using HPLC and then analyzed all fractions with an assay that measures both free and α_1 -antichymotrypsin-bound PSA (17) and an assay that measures only the α_1 -antichymotrypsin-bound PSA (17). The results are shown in Fig. 1. Free PSA elutes at fraction 39 corresponded to a molecular weight of \sim 33,000. The PSA-ACT complex elutes at fraction 30, corresponding to a molecular weight of \sim 100,000. The identity of the PSA-ACT complex was verified with an assay that measures the PSA-ACT complex but not free PSA (Fig. 1). Fig. 1 reveals that free PSA constitutes more than 90% of the total immunoreactive PSA of the ovarian tumor extract.

The identity of the immunoreactive species was further confirmed with molecular studies. Total RNA extracted from cancer tissue was reverse transcribed to cDNA and then amplified by PCR using primers specific to the PSA cDNA sequence. Two overlapping regions, from nucleotides 1 to 571 and from nucleotides 354 to 990 of the PSA cDNA, were amplified by using two sets of primers derived from exons 1 and 4 and from exon 3 and the 3'-untranslated region, respectively. Ethidium bromide-stained agarose gel electrophoresis demonstrated the presence of the expected 571 and 636 bp cDNA fragments, indicating the presence of a PSA transcript in this tumor (Fig. 2). Southern blot hybridization of the PCR products with a PSA

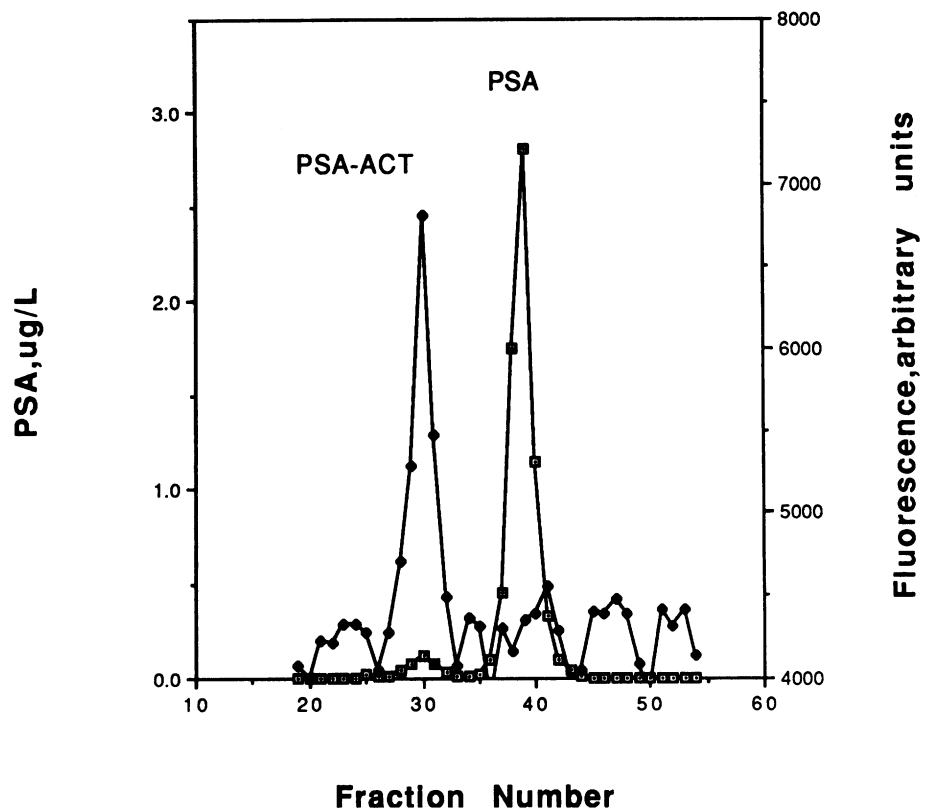


Fig. 1. HPLC with a gel filtration column. Details of the method are given by Yu and Diamandis (17). Each HPLC fraction (0.5 ml) was analyzed with an assay that measures both free PSA and PSA-ACT (\square) or an assay that measures only PSA-ACT (\blacklozenge). The response of the latter assay is in arbitrary fluorescence units since no PSA-ACT standard is available. Purified seminal plasma PSA elutes at fraction 39, corresponding to a molecular weight of 33,000, and PSA-ACT elutes at fraction 30, corresponding to a molecular weight of 100,000 (data not shown). The ovarian tumor extract contains two immunoreactive peaks at fraction 30 and 39, respectively. Only the former peak is recognized by the PSA-ACT assay. Over 90% of the total PSA immunoreactivity is due to free PSA. The HPLC column was calibrated with molecular weight standards eluting at fraction 21 (M_r 660,000), 28 (M_r 160,000), 37 (M_r 44,000), 42 (M_r 17,000), and 49 (M_r 1,400).

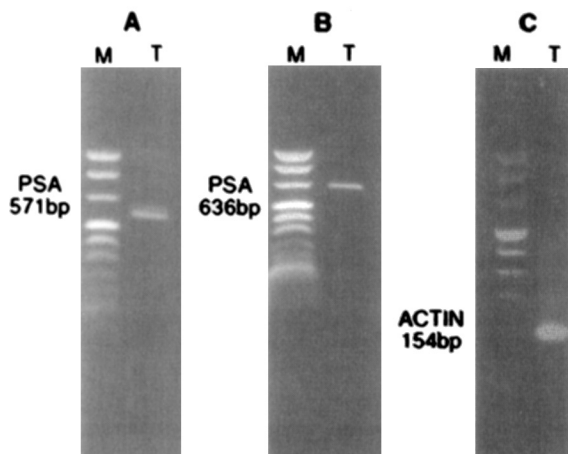


Fig. 2. Reverse transcription-PCR of RNA extracted from the ovarian tumor (*T*). In (A), the PCR was performed with primers PR5 and PR2, generating a 571-bp fragment. In (B), the PCR was performed with primers PR3 and PR4, generating a 636-bp fragment. In (C), the PCR was performed with β -actin primers, generating a 154-bp fragment. *M*, molecular weight standards. The PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide.

cDNA probe further confirmed the identity of these fragments (data not shown).

In order to determine whether the mRNA sequence was identical to the PSA cDNA sequence derived from prostate and breast tissues, the PCR products were purified, and the DNA sequence was determined. Both strands of each PCR product were sequenced. The obtained sequence, corresponding to the entire coding region and part of the 3' untranslated region of the PSA gene showed 100% identity to the PSA cDNA sequence derived from prostate tissue (Genbank accession no. U17040).

Immunohistochemistry revealed focal positivity for PSA in scattered epithelial cells of the endometrioid adenocarcinoma (Fig. 3). The stromal elements were negative, as were the adenofibroma and benign cystadenoma. Immunoreactivity was seen predominantly in basal and parabasal cells of the thick epithelial lining. Many positive cells exhibited faint diffuse cytoplasmic positivity, several contained intense juxtannuclear reactivity, and a few were diffusely and strongly stained. Positivity was most intense within lumina in areas containing positive lining cells (data not shown). The specificity of the results was confirmed in negative control slides using nonimmune rabbit serum.

Discussion

Although expressed at much lower levels in comparison to the prostate gland, PSA was found in 30–40% of female breast tumors and at a lower frequency in tumors of the lung, colon, ovary, liver, kidney, adrenal, parotid, skin, and salivary glands (23). In addition, PSA was found in normal endometrial tissue (24), in normal breast tissue of women receiving oral contraceptives (13), in milk of lactating women (14), and in amniotic fluid (15). Based on these data, we have speculated that PSA could be produced by any tissue that possesses steroid hormone receptors provided that the tissue is stimulated by steroid hormones. Working with a tissue culture system and steroid hormone receptor-positive breast carcinoma cell lines, we have recently demonstrated that glucocorticosteroids, mineralocorticosteroids, androgens, and progestins but not estrogens could mediate PSA gene expression (12).

In a previous study of 98 primary ovarian carcinomas from patients not receiving any medication, we have concluded that expression of

PSA is a relatively rare event and that the levels of PSA in the PSA-positive ovarian tumors are very small in comparison to the levels of PSA in breast tumors (16). We have also reported two cases of ovarian metastasis from a primary breast carcinoma. One of the two tumors was PSA-positive, and the patient responded very well to tamoxifen treatment, despite the tumor being steroid hormone receptor negative. This patient is still alive 6 years after surgery. The other tumor was PSA-negative, and the patient did not respond to tamoxifen treatment, despite the tumor being steroid hormone receptor positive. This patient died 1 year after diagnosis (16). We have further provided evidence that PSA-positive breast tumors respond better to endocrine treatment and patients live longer and relapse less frequently than PSA-negative breast cancer patients (11). These data collectively suggest that PSA is a favorable prognostic marker associated with better patient survival and an increased likelihood of response to endocrine treatment.

In this study, we describe a patient who developed a primary endometrioid ovarian carcinoma after liver transplantation. Primary breast carcinoma in this patient was excluded by physical examination and mammography. The tumor of this patient contained large amounts of PSA. The PSA was present in its free, M_r 33,000 form. Molecular analysis has confirmed that the immunoreactive PSA was identical to the PSA present in seminal plasma. After sequencing the entire coding sequence of PSA cDNA from this tumor, we were unable to find any mutations. PSA was localized to epithelial cells in the adenocarcinoma. The pattern of juxtannuclear globular positivity and luminal reactivity is consistent with packaging of PSA in the Golgi apparatus and active secretion. We speculate that the high-level expression of PSA in this patient is due to the administration of glucocorticoids (prednisone) for many years after liver transplantation. Our proposal is based on data recently published with breast carcinoma cell lines which were shown to express PSA after stimulation by glucocorticosteroids (12).

This is the first report describing high-level expression of PSA in a primary ovarian carcinoma. If our notion that the high-level expression of PSA in this tumor is associated with the stimulatory effect of the exogenously administered glucocorticoid, we could further speculate that other ovarian tumors or other tumors or tissues bearing steroid hormone receptors could also be exogenously stimulated to produce PSA. This was recently reported by our group for normal breast tissue stimulated by oral contraceptives (13).

The patient described in this report is still alive with no signs of

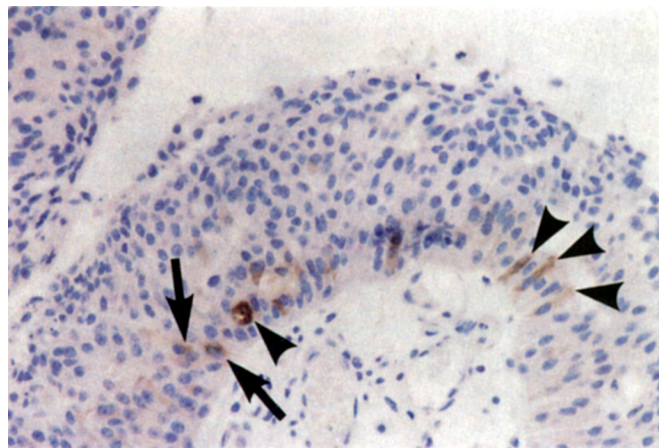


Fig. 3. Immunohistochemistry of the ovarian tumor with a polyclonal PSA antibody. PSA is localized in basal and parabasal cells of the thick epithelial lining. Elongated cells exhibit diffuse cytoplasmic positivity of variable intensity (arrowheads); some cells have an intense juxtannuclear globular pattern of staining consistent with Golgi localization (arrows). (Streptavidin-biotin technique; \times 170).

tumor relapse 3 years after diagnosis and more than one year after surgery. This finding further supports our previous proposal that PSA-producing tumors are associated with more favorable patient prognosis (11, 16). It would be important to delineate in the future if the favorable prognosis of patients with PSA-producing tumors is related to the enzymatic serine protease action of PSA or is an associated event. If PSA does play a direct role in tumor biology, then exogenously administered agents which regulate PSA gene expression may have some therapeutic potential. Alternatively, the exogenously stimulated expression of PSA in women may be used diagnostically for tumor localization by radioimaging.⁴

Recent literature reports by our group and other investigators suggest that PSA may play a role as a normal breast tissue regulator (14), a fetal growth regulator (15), an insulin-like growth factor (IGF-I) regulator (25), a cytokine adhesion molecule regulator (26), or a local uterine function regulator (24). Although the data are currently circumstantial, it seems that PSA can now be regarded as a ubiquitous molecule with extraprostatic functions related to its serine protease enzymatic activity. A substrate for this enzyme in extraprostatic tissue has not as yet been identified.

We have presented evidence that PSA gene expression could be manipulated with exogenously administered compounds. This may, in the future, have therapeutic or diagnostic applications. It will be of interest to clarify if the good prognosis of tumors positive for PSA is related to the PSA enzymatic activity. Clearly, PSA should no longer be regarded as a prostate-specific molecule but as a marker of steroid hormone action in many tumor types.

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⁴ Unpublished data.