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Ectopic Production of Prostate Specific Antigen by a Breast Tumor Metastatic to the Ovary

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We have recently reported that about 30% of breast tumors produce prostate specific antigen (PSA). We examined here, 99 primary ovarian cancer tumors and found relatively low levels of PSA in only three tumors. One patient with metastatic ovarian cancer from a primary breast tumor, produced relatively high levels of PSA and responded well to antiestrogen treatment although she was steroid receptor-negative. Another patient with metastatic ovarian cancer from a primary breast tumor did not produce PSA and did not respond to treatment although she was steroid hormone receptor-positive. This data describe for the first time, ectopic PSA production by a breast tumor at the ovarian metastatic site and further support the view that PSA is a favourable prognostic indicator in breast cancer.

INTRODUCTION

Prostate specific antigen (PSA) is a 34 kD glycoprotein produced by the epithelial cells of the prostate and is used as a tumor marker for the diagnosis and monitoring of prostatic carcinoma (1). Recently, we found that about 30% of primary breast tumors produce PSA and that the presence of PSA is associated with steroid hormone receptor-positive tumors, earlier disease stage, and younger patient age (2). Based on these data we have postulated that the PSA presence in breast tumors is a new favourable prognostic indicator and that PSA positive tumors belong to a subgroup of patients who respond to endocrine treatment.

In this paper, we have analyzed for PSA 101 ovarian tumor cytosolic extracts and found no PSA in 95 tumors, and relatively low concentrations of PSA ($0.0076 < PSA < 0.40$ ng/mg of total protein) in 5 tumors. However, one tumor (Patient A) that metastasized to the ovary from breast produced relatively high levels of PSA (tumor extract PSA concentration of 1.40 ng/mg of total protein). Another ovarian tumor (Patient B) also metastatic from breast, did not produce PSA. Patient A was steroid hormone receptor-negative but responded to tamoxifen therapy and is disease-free 18 months post-abdominal surgery. Patient B died one year post-abdominal surgery.

This is the first report of PSA production by a breast tumor at the site of its metastasis (ovary). Because PSA production seems to be a highly frequent event in breast cancer, finding PSA in a metastatic tumor of unknown origin in female patients may strongly suggest that the primary tumor is in the breast. Our findings also suggest that PSA production by primary ovarian tumors is a relatively rare phenomenon. The response of the metastatic breast tumor to tamoxifen in the absence of measurable receptors supports our current view that PSA production by breast tumors is a favourable biochemical prognostic marker which may define a subgroup of patients who are responsive to endocrine treatment.

MATERIALS AND METHODS

Ovarian tumors were collected after surgical resection and stored at –80°C until use. Cytosol extracts of these tumors were prepared as follows: Approximately 0.5 g of tumor tissue is weighed out, smashed with a hammer if necessary, and pulverized in a Thermovac tissue pulverizer with liquid N_2. The resulting powder is transferred into 50-mL plastic tubes along with 10 mL of extraction buffer (0.01 mol/L Tris, 1.5 mmol/L ethylenediaminetetraacetic acid, pH adjusted to 7.40 with 5 mol/L HCl). If the tumor tissue is less than 0.5 g, the volume of the buffer used is reduced proportionally. The tissue powder is homogenized on ice with a single 5s burst of a Polytron homogenizer at setting 6. The particulate material is pelleted by 1 hour centrifugation at 105,000 g. The intermediate layer (cytosol extract) is collected without disturbing

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the lipid or particulate layers. Protein concentration of the resulting cytosol extract is determined by the bicinchoninic acid method, commercially available by Pierce Clinical Co., Rockford, IL. Extracts were analyzed within 24 hours.

Enzyme immunoassay kits (Abbott Laboratories, North Chicago, IL 60064) were used to measure the concentrations of estrogen and progesterone receptors in the tumor cytosols, following the methods described in the package inserts. Specimens were assigned positive receptor status if they exceeded 10 fmol/mg of protein.

The PSA assay (3) 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 hours incubation and washing × 6, the biotinylated polyclonal anti-PSA antibody is added and incubated for 1 hour. After washing × 6, the SA-ALP conjugate is added for 15 minutes, followed by another washing × 6. The activity of ALP is then measured by adding the substrate 5-fluorosalicylphosphate, incubating for 10 minutes and then adding a solution containing Tb^{3+} and EDTA. The fluorescence is measured in the time-resolved fluorometric mode.

Patients

We have studied 101 consecutive patients of whom 98 had primary ovarian cancer. The other three patients are described in detail below. All patients were diagnosed and treated at the Department of Gynecologic Oncology, University of Turin. Patient A (#100) was a 54 year old post menopausal female patient with primary breast cancer (maximum tumor diameter 1.5 cm) who underwent surgery in 1986 (quadrantectomy + axillary node dissection) followed by radiation therapy on the remaining breast and axilla, and by chemotherapy (CMF regimen) for 8 cycles. The breast tumor was an invasive ductal carcinoma, grade G3, negative for both estrogen and progesterone receptors (cutoff values < 10 fmol/mg of protein). Lymph node invasion was negative (00 in 19 nodes examined). The patient was given Tamoxifen for 20 months and she was in complete remission until April 1992. On May 1992, she was operated for an ovarian mass. She underwent total abdominal hysterectomy plus bilateral salpingoophorectomy, omentectomy, appendectomy, and pelvic lymph node dissection. Random biopsies were performed for an apparently ovarian cancer. All histologic specimens were negative except for her left ovary which had a metastatic tumor spread from the breast primary (histologically confirmed). The secondary ovarian tumor was used to prepare the cytosolic extract for PSA analysis. The patient is until now in complete remission. Patient B (#16) was also a primary breast cancer patient, positive for both estrogen and progesterone receptors who presented with widespread abdominal metastases including ovarian sites and was operated upon but died one year post surgery. Ovarian metastatic tumor was obtained for PSA analysis. Patient C (#99) was a primary breast tumor patient, negative for both receptors who also developed primary ovarian cancer. Ovarian tumor was used for PSA analysis. The primary breast tumors in these patients were not available.

RESULTS

From the 98 primary ovarian cancer patients (all but patients A, B, C), 93 were negative for PSA in the tumor cytosolic extracts (PSA < 0.007 ng/mg of total protein). Patients B and C were also negative. From the remaining tumors, three contained only traces of PSA (0.048, 0.034, and 0.0076 ng/mg of total protein). The remaining two tumors had PSA levels of 0.40 and 0.084 ng/mg of total protein. The cytosolic extract concentration of PSA in patient A was 1.40 ng/mg of total protein. This value was confirmed with another PSA assay which is commercially available (Abbott IMx Assay, Abbott Laboratories, Abbott Park, Chicago, IL).

DISCUSSION

Our results with the primary ovarian cancer tumors suggest that PSA production by ovarian tumors is a relatively rare event in comparison to breast tumors. We have previously found that PSA production by breast tumors is linked to the presence of steroid hormone receptors which interact with ligands and derepress the PSA gene. Steroid hormone receptors are not considered important disease mediators in ovarian cancer and are not useful for therapeutic manipulations (4). In the two cases where PSA production by primary ovarian tumors was above the cutoff level of 0.05 ng/mg, the concentrations found in the cytosolic extracts were relatively low in comparison to PSA-positive breast tumors (2). Only Patient A produced relatively high levels of PSA (1.40 ng/mg of total protein). This patient had a histologically confirmed metastatic ovarian tumor from a breast primary. Although the patient was negative for both ER and PR receptors, she responded to endocrine treatment very well and she is in complete remission. We have recently shown by using an in vitro system that PSA production in breast cancer cell lines is induced by androgens and progestins and inhibited by estrogens. We speculate that Patient A possesses very low concentrations of steroid hormone receptors which can nevertheless mediate the PSA gene derepression after association with appropriate steroid ligands. Patient B also had an ovarian tumor metastatic from breast but the tumor was PSA-negative. This patient did not respond to therapy and died one year after abdominal surgery. Although based on only two ovarian cancer patients, our data support our previous view that PSA may be a new favourable prognostic indicator in breast cancer (2).

Our report describes for the first time the production of PSA by a breast tumor at the site of its metastasis which suggests that the metastatic tumor cells in the ovary retain their ability to produce PSA with mechanisms similar to those in the primary tumor. Because PSA presence in breast tumors is a relatively frequent event, PSA detection in a tumor of unknown origin may suggest that the primary tumor originated from the breast. Our data also suggest that the metastatic tumor may respond to endocrine treatment in a manner similar to that of the primary tumor.

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REFERENCES

1. Osterling JE: Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J Urol* 145:907–923, 1991.
2. Diamandis EP, Yu H, Sutherland DJA: Detection of prostate specific antigen immunoreactivity in breast tumors. Breast Cancer Res Treat (in press).
3. Yu H, Diamandis EP: Ultrasensitive time-resolved immunofluorometric assay of prostate-specific antigen in serum and preliminary clinical studies. *Clin Chem* 39:2,2108–2112, 1993.
4. Young RC, Fuks Z, Hoskins WJ: Cancer of the ovary. In *Cancer, Principles and Practice of Oncology.* VT DeVita, S Hellman, SA Rosenberg, eds. J.B. Lippincott, Philadelphia, 1993, p 1,226–1,263.