PRODUCTION OF PROSTATE-SPECIFIC ANTIGEN (PSA) BY A BREAST CANCER CELL LINE, SK-Br-3

E. KAMALI SARVESTANI,*
A. GHADERI, AND B. GHARESI-FARD

From the Department of Immunology,
Shiraz Medical School,
Shiraz University of Medical Sciences, Shiraz, I.R. Iran.

ABSTRACT

PSA is a 33-KDa serine protease that is produced predominantly by prostate epithelium. However, it has been shown that about 30-40% of female breast tumors produce PSA and its production is associated with the presence of estrogen and progesterone receptors. We have now developed a new tissue culture system to study PSA production in breast cancer and its association with prognostic factors such as progesterone receptor and c-erbB-2. For this purpose we investigated the ability of PSA production in five different cell lines, including two breast cancer cell lines, SK-Br-3 and MDA-MB-453. The PSA in tissue culture supernatant and cytoplasm of the Sk-Br-3 cell line was detected by western blotting and immunoperoxidase, respectively. Furthermore, we found lower expression of c-erbB-2 in Sk-Br-3 than non-PSA producer breast cancer cell line, MDA-MB-453. Progesterone receptor was expressed by both PSA-positive and -negative cell lines and only the intensity of staining and the number of positive cells in Sk-Br-3 population was higher than MDA-MB-453. According to our findings PSA can be considered as a good prognostic factor in breast cancer and we suggest that these two cell lines are a good *in vitro* model to study the relationship of different breast cancer prognostic factors and their regulations.

Keywords: PSA, c-erbB-2, Progesterone receptor, Breast cancer, SK-Br-3.

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INTRODUCTION

Prostate-specific antigen (PSA) is a 33-KDa single-chain glycoprotein produced by prostate epithelium[1] and is a major protein in seminal plasma with chymotrypsin-like substrate specificity.[2] PSA has been widely used as a marker for diagnosis and monitoring of prostate cancer.

Originally, the main physiological function of PSA was thought to be liquefaction of seminal coagulum.[3] However, PSA has been found to catalyze the hydrolysis of other substrates such as insulin-like growth-factor binding protein-3 (IGFBP-3).[4] IGFBP-3 is one of the serum binding proteins for insulin-like growth factor II (IGF-II), which is a growth factor for a variety of cells. Thus, PSA mediated hydrolysis of IGFBF-3 may modulate the levels of IGF and consequently the rates of cellular proliferation. PSA was shown to catalyze degradation of the extracellular matrix proteins, fibronectin and laminin,[5] and thereby is implicated in the invasion of tissues by tumor cells.

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*Correspondence: E. Kamali Sarvestani, Dept of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. P.O. Box: 71345-1798 Tel/Fax: (00)98-711-2304069, E-mail: immunol2@sums.ac.ir
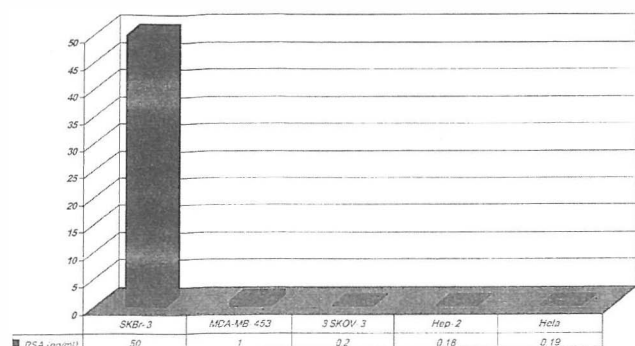
Fig. 1. Cell lines were cultured in the same condition and supernatants were collected three days after final refreshing of the medium. The presence of PSA was checked by a commercial ELISA kit. Average of three separate experiments are shown.

Recently using an ultrasensitive PSA assay, it has been reported that 30% of female breast tumor cytosolic extracts contain PSA immunoreactivity, accounting for greater than 0.03 ng/mg of total protein.[6,7] Also one study has shown that women whose breast tumors are positive for PSA have a better clinical prognosis.[8] Therefore, availability of an *in vitro* model to study PSA role(s) and it's relationship to other prognostic factors in breast cancer is desired. In this respect, we investigated the ability of five different tumor cell lines for PSA production. Among them a breast carcinoma cell line, SK-Br-3, was able to produce PSA. Furthermore, comparison of SK-Br-3 with a non-PSA producing breast cancer cell line provided evidence that PSA production is inversely correlated with the expression of a poor prognostic factor, c-erbB-2. We proposed SK-Br-3 as a good *in vitro* model for study of the role of PSA in breast tumor biology and it's relationship to other prognostic factors.

MATERIAL AND METHODS

Cell cultures

Five human cell lines obtained from Ludwig institute for cancer research, London, included two breast cancer cell lines, SK-Br-3 and MDA-MB-453, and Hep-2 (liver), Hela (cervix) and SKOV-3 (ovary) as three non-breast cancer control cell lines. Cells were propagated in Nunc 24-well culture dishes with Dulbecco's modified medium supplemented with 10% FCS at 37°C and 5% CO_2 (all reagents were obtained from Sigma Chemical Co.). To test the presence of PSA in cell cultures supernatants, after reaching approximately 80% confluency, cultures were continued for over five days and supernatants were collected.

PSA Immunoassay

The presence of PSA in culture supernatants was determined by an Immunoenzymatic assay using the Can-Ag PSA Kit (Can-Ag Diagnostic, Sweden).

Western blot analysis

For Western blotting, cells were grown in low FCS (3%) containing medium and the collected supernatants were concentrated 20-fold by ammonium sulfate. After extensive dialysis in normal saline, samples were electrophoresed under reducing conditions on 10% polyacrylamide gel and separated protein bands were transferred to nitrocellulose membranes. Saturation was performed for 1 h at 37°C in blocking solution (20 g/L BSA), pH 7.2, containing 0.05 % Tween 20.

After that, saturated strips were incubated with the supernatant of an anti-PSA monoclonal antibody (produced in our laboratory) at room temperature for 2h. After several washes in PBS buffer containing 0.05 % Tween 20, membranes were incubated for 1h at room temperature with 1/1000 dilution of a horseradish peroxidase-conjugated rabbit anti-mouse antibody (Sigma Chemical Co.). The antibody binding was revealed by exposure to 100 nmol/L tris HCl, pH 9.5, containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB). Molecular mass markers and purified PSA from semen, as described previously,[9] were used as calibrator and positive control, respectively.

Immunoperoxidase analysis

After trypsinization, drops of the cell suspension in culture medium were added directly on to the sterilized slides. Cells were spread properly after about 48 h of culture. Slides were then rinsed in PBS and fixed in a freshly prepared and chilled mixture of 50% acetone-50% methanol with gentle agitation for 2 minutes. The slides were incubated for 30 minutes with normal rabbit serum to inhibit non-specific binding of second antibody. This was followed by washing and incubation with the supernatant of an anti-PSA producing hybridoma for 1h. Later, the slides were washed and incubated with 1/50 HRP-conjugated anti-mouse antibody for 1h. Following an extensive wash with PBS, slides were stained with 0.05% DAB in 100 nmol/L tris HCl, pH 9.5, and counterstained with hematoxylin, dehydrated and finally mounted.

C-erbB-2 expression in cell lines were investigated in the same manner except for primary and secondary antibodies which were ICR-12 (gift from Dr. Modjtahedi) and sheep anti-rat peroxidase conjugated antibody (Amershaam).

The progesterone receptor level was determined by LSAB2 (Dako, Denmark) Kit, as recommended by the manufacturer. Briefly after quenching endogeneous per-
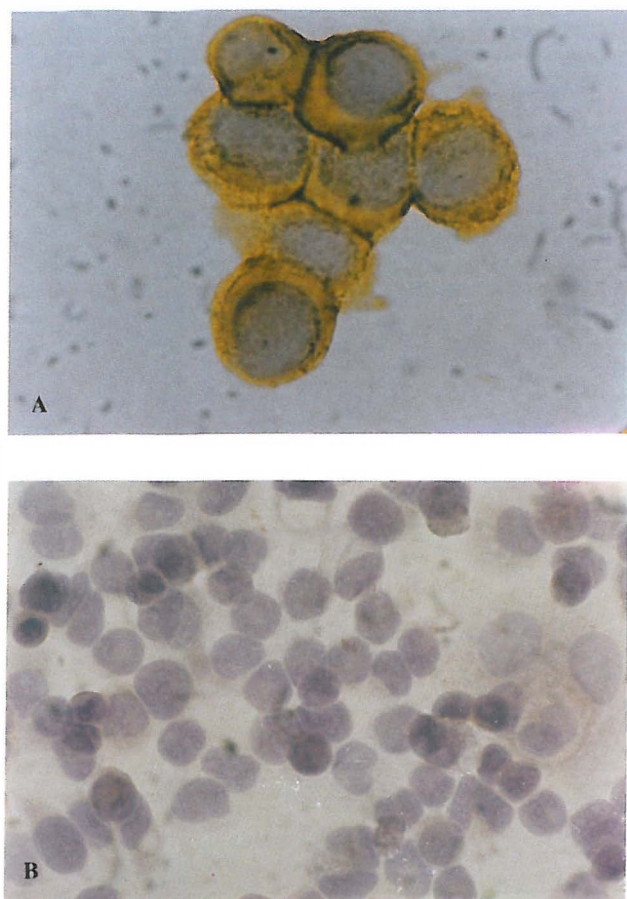
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Fig. 2. Immunohistochemical analysis of PSA production in two different breast cancer cell lines. **A)** The presence of an immunoreactive intracytoplasmic molecule(s) in Sk-Br-3 was shown using an anti-PSA monoclonal antibody (original magnification 1000×). **B)** This reactivity was not detected in MDA-MB-453 (original magnification 400×).
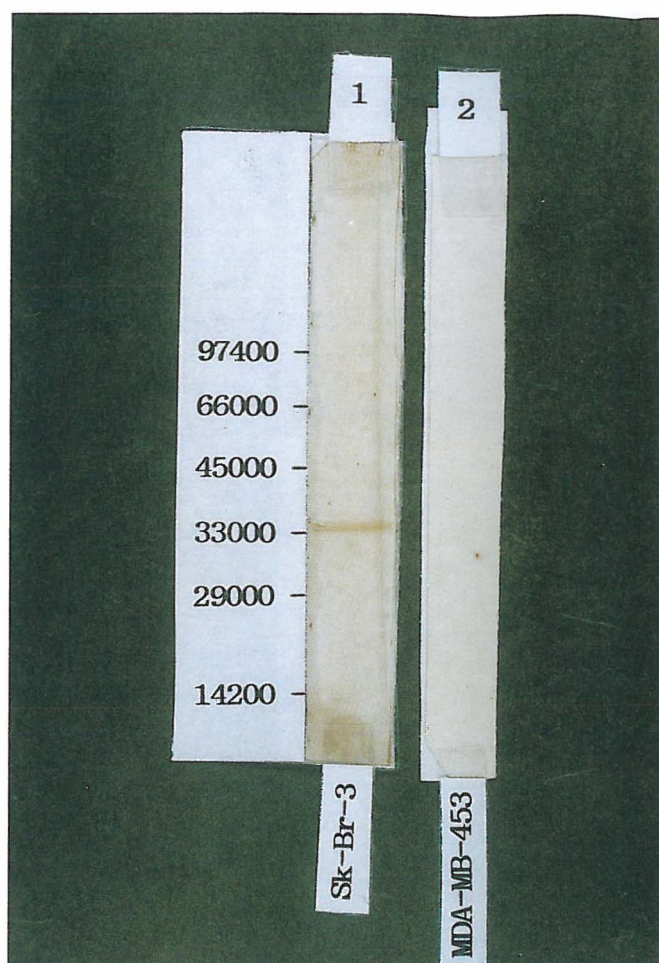

Fig. 3. Western blot analysis of Sk-Br-3 and MDA-MB-453 supernatants using an anti-PSA monoclonal antibody. Results showed that a 33 KDa immunoreactive protein band is present only in SK-Br-3 supernatant.

oxidase by immersing the sections in PBS containing 3% H_2O_2, the specimens were incubated with an appropriately characterized and diluted anti-ER mouse primary antibody. Biotinylated anti-mouse antibody was used as secondary antibody. Peroxidase labelled streptavidin was used for detection of primary and secondary antibody linking. Final reactions were visualized by using DAB as chromogen, the sections were counter-stained in hematoxylin and mounted with Entellan.

RESULTS

The possibility of PSA production in five different cell lines was investigated by three different methods. The presence of an immunoreactive molecule(s) in the culture media of SK-Br-3 was detected using a PSA commercial kit (Figure 1). The reactivity of anti-PSA monoclonal antibody with intracytoplasmic molecule(s) in SK-Br-3 cell line was also shown by an immunoperoxidase

technique (Figure 2). Finally concentrated media of SK-Br-3 and MDA-MB-453 were checked in Western blotting. Results indicated the recognition of a 33 KDa molecule by anti-PSA monoclonal antibody in the culture media of SK-Br-3, but not in MDA-MB-453 (Figure 3). The expression of two breast cancer prognostic factors, c-erbB-2 and progesterone receptor, were also investigated using immunoperoxidase method. Our results showed lower expression of c-erbB-2 in PSA producer breast cancer cell line, SK-Br-3 (Figure 4). Interestingly the expression of progesterone receptor was not significantly different, and only the number of positive cells and the intensity of staining in the SK-Br-3 population was slightly higher than MDA-MB-453 (Figure 5).

DISCUSSION

Until recently, PSA was thought to be a prostatic tissue-specific protein that is not expressed in any other
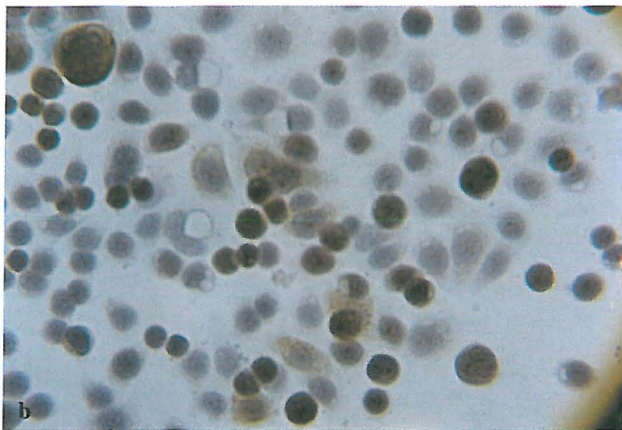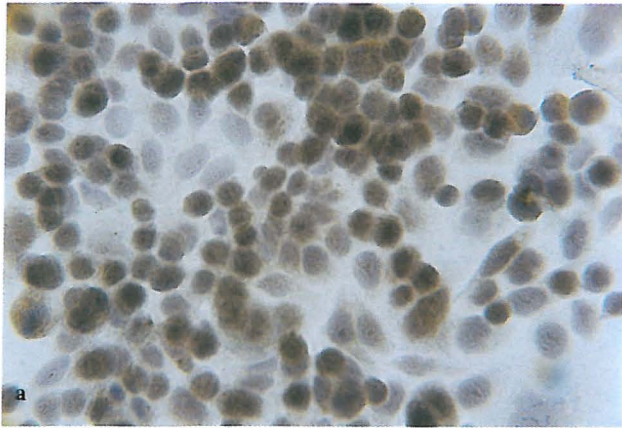
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Fig. 4. Immunohistochemical staining of c-erbB-2 in **a)** SK-Br-3 and **b)** MDA-MB-43 cell lines. Results indicate the lower expression of c-erbB-2 in SK-Br-3 (original magnification 400×).

Fig. 5. Immunohistochemical staining of progesterone receptor in **a)** SK-Br-3 **b)** MDA-MB-453 cell lines. The intensity of staining and the number of positive cells are higher in SK-Br-3 than MDA-MB-453 (original magnification 400×).

tissue in men or women. It was recently shown that PSA production occurs in the female breast and some other tissues in both men and women.[10] Normal and benign breast tissues produce PSA,[11] but many malignant breast tumors lose their ability to produce PSA. Therefore PSA appears to be a prognostic indicator of breast cancer.[12]

In this study we developed a tissue culture system to study PSA production and its probable function in breast cancer. Using this system, we analyzed the relation between PSA production and other breast cancer prognostic factors, such as progesterone receptor and c-erbB-2.

c-erbB-2 is a member of a class of molecules called growth factor receptors, and has a profound significance on metastatic breast cancer. Following discovery of c-erbB-2 in 1987, it was determined that 25% to 30% of women with breast cancer have amplification of this gene,[13] and amplification results in overexpression in 95% of cases. Currently this molecule appears to be an independent prognostic factor in node-positive disease and may be predictive in node-negative disease.[14] The c-erbB-2 amplification induces cells toward more aggressive behavior and markedly boosts the metastatic poten-
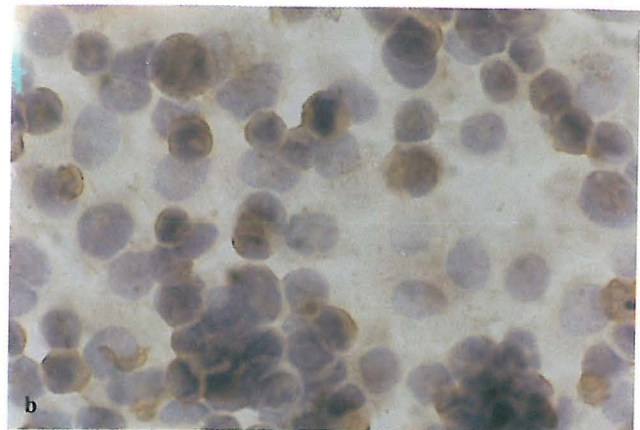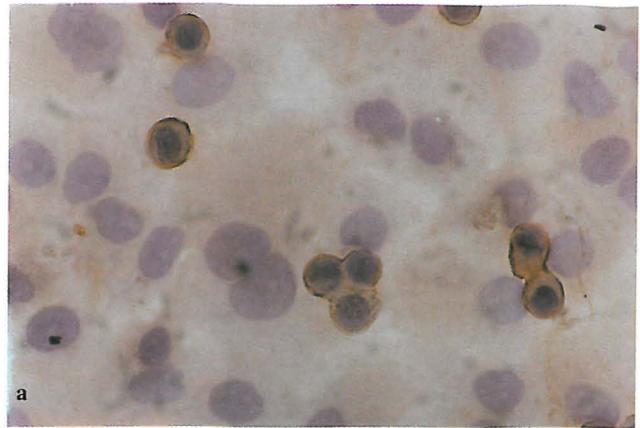
tial of the tumors.[15] Furthermore, more than half of the women who are c-erbB-2 positive are estrogen/progesterone-negative and women who are c-erbB-2-positive and estrogen/progesterone-positive may not respond to hormone therapy.[16]

On the other hand Yu and his colleagues have reported that about 30-40% of female breast tumors produce PSA and there is a close association between PSA and progesterone receptor presence in breast tumors.[17] It has been also demonstrated that the steroid hormone receptor-positive breast carcinoma cell line T47-D can be stimulated by progestins to produce PSA.[19] Thus, it can be postulated that steroid hormones regulate the PSA gene in the female breast. Furthermore, steroid hormones and their receptors may be necessary but not sufficient for PSA production. Zarghami and his colleagues reported that the steroid hormone receptor-positive cell lines SAOS (osteosarcoma) and BG-1 (ovarian carcinoma), were not able to produce PSA.[18]

The relationship between three breast cancer prog-

nostic indicators (PSA, c-erbB-2 and steroid receptors) have not been investigated in a same study, although availability of a proper model for such an investigation would be remarkable. We have now developed a tissue culture system to study the relationship of PSA production and two other prognostic factors in breast cancer. By comparing SK-Br-3 with another non-PSA producer breast cancer cell line, MDA-MB-453, we showed an inverse correlation between PSA production and an unfavorable prognostic factor, c-erbB-2. This finding is in accordance with the study of Yu and his colleagues that many patients with malignant breast tumors lose their ability to produce PSA and therefore PSA appears to be a good indicator in breast cancer.[11]

Furthermore, in this model we demonstraed that there is no relationship between PSA and progesterone receptor expression. In fact, both PSA producing and nonproducing cell lines have the ability for progesterone receptor expression. This finding is also in accordance with the previous finding of Zarghami that steroid receptors may be necessary but not sufficient for PSA production.[18] In our model two breast cancer cell lines have progesterone receptor, but only the SK-Br-3 can produce PSA. This is probably due to the presence of another factor(s) that exists only in SK-Br-3 cell line and the elucidation of their nature requires further investigation.

With respect to the relationship of c-erbB-2 and steroid receptor expression, our findings also confirm the previous reports.[16] In fact the intensity of staining of the progesterone receptor in SK-Br-3 cells was higher than MDA-MB-453 cells, but the former express lower levels of c-erbB-2. Therefore, as anticipated, SK-Br-3 with higher levels of progesterone receptor expresses lower levels of the poor prognostic factor, c-erbB-2.

In conclusion, we introduce a new *in vitro* model for the study of breast cancer prognostic factors. Our finding can be used as an *in vitro* model to study the role and involvement of different breast cancer prognostic factors and the regulation of their genes in breast cancer.

ACKNOWLEDGEMENT

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