

Immunohistochemical analysis of prostate-specific antigen in female breast cancer

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Summary

Purpose: Prostate-specific antigen (PSA), a glycoprotein initially thought to be produced only by the epithelial cells of the prostate, has recently been found in various tissues and tumors. It has been suggested that the expression of PSA in breast cancer is a good prognostic indicator and correlated with favorable prognosis. However, in recent years opposite results have been reported. In this study, we investigated the immunoreactivity of PSA in female breast cancer to find out any relationship between PSA and prognostic parameters.

Patients and methods: Sections of formalin-fixed, paraffin-embedded samples from 109 invasive ductal carcinomas were immunostained for PSA. The staining results were

analyzed in relation to age, tumor size, histologic grade, axillary lymph node status and steroid receptors.

Results: PSA immunoreactivity was seen in only 11 (10.1%) out of 109 cases. All PSA positive cases were also estrogen (ER) and progesterone receptor (PR) positive. We found a statistically significant correlation between PSA and the expression of steroid receptors, while no correlation was detected with the other factors.

Conclusion: The detection of PSA, using immunohistochemistry, does not seem to be a reliable prognostic criterion for female breast cancer patients or a marker of tumor origin.

Key words: breast cancer, immunohistochemistry, prognostic factors, prostate-specific antigen

Introduction

PSA is a valuable tumor marker used for diagnosis and overall management of prostate cancer [1]. Until recently PSA was seen as a highly specific biochemical marker of prostatic epithelial cells, and in practice its detection by immunohistochemistry has been widely used in determining the prostatic origin of metastatic cancer [2]. However, in recent years, numerous studies have demonstrated the production

of PSA in various non-prostatic normal tissues such as the parotid gland, kidney, pancreas, breast and in their malignant counterparts as well [3-8].

PSA is a serine protease controlled by one of the three members in the human glandular kallikrein gene family. The other two are tissue kallikrein and human glandular kallikrein-1 [9]. There are about 60% and 80% sequence homology between PSA and these two serine proteases, respectively [10-12]. The high levels of homology may explain the positive immunohistochemical reactions for PSA in non-prostatic tissues.

The presence of PSA has been shown in normal, hyperplastic and neoplastic breast tissues [6]. A number of reports concerning PSA positivity in breast cancer tissues proposed its utility as a prognostic marker for breast cancer [13-15]. It has been suggested that the production of PSA in breast tumors is regulated by steroid receptors [16] and associated with favorable prognosis [13, 14]. However, recent studies using breast cancer patients' serum, tumor cytosolic extracts and paraffin-embedded breast cancer

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tissues, have not revealed any prognostic significance of PSA in breast cancer [17-19].

In this study, we investigated the immunoreactivity of PSA in female breast cancer tissues to find out if there was any relationship between PSA and some well-known prognostic parameters of breast cancer.

Patients and methods

One hundred and nine cases of invasive ductal carcinoma of the breast from female patients were enrolled in this study. The histologic grading was done according to the modified Bloom-Richardson method [20]. The status of ER and PR, determined by immunohistochemistry, was obtained from pathology reports. We prepared 3 µm-thick sections from formalin-fixed, paraffin-embedded tissue samples and performed immunohistochemical staining using monoclonal PSA antibody. The sections were mounted on poly-L-lysine-coated slides, deparaffinized in xylene, rehydrated in graded series of ethyl alcohol and blocked with 3% hydrogen peroxide for 15 min. For antigen retrieval, they were immersed in citrate-phosphate buffer, and microwaved at 100° C for 15 min. The sections were cooled to room temperature and then incubated with monoclonal antibody against PSA (Dako, USA; prediluted). After washing with phosphate buffer saline, the secondary antibodies were applied. AEC (3-amino ethylcarbazole) was used as chromogen. The slides were counterstained with hematoxylin. Normal prostatic tissue was used for positive control. The immunohistochemical examination was performed by two pathologists independently without any information about the patients. The intensity of positive immunostaining in the cytoplasm of the tumor cells was graded into 3 categories (weak, moderate, strong). The expression of PSA was compared with clinical and histological features including age, tumor size, axillary lymph node status, histologic grade, and ER and PR status. The strength of the associations was appraised by using chi-square and Fischer's exact tests. A p-value less than 0.05 was considered statistically significant.

Results

The age range of the patients was 30-72 years (median 56 years). Thirty-six out of the 109 cases were less than 50 years old. The size of the tumors varied from 1.2 to 3.5 cm in diameter (median 2.4

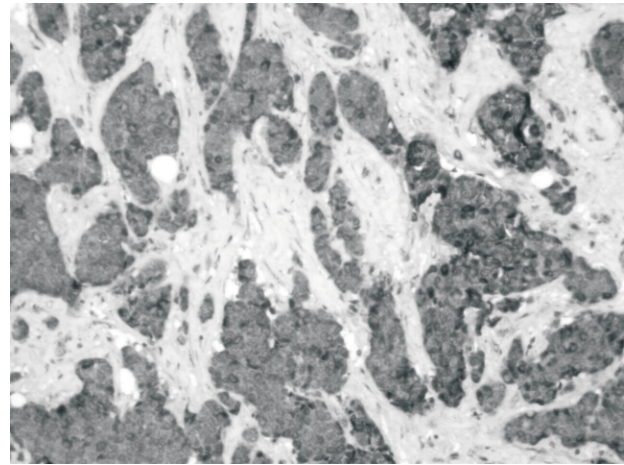


Figure 1. Strong cytoplasmic immunostaining for PSA in tumor cells ($\times 200$).

cm). Histologically, all of the tumors were invasive ductal carcinomas. Nineteen of the tumors were grade I, 60 grade II, and 30 grade III. In 62 cases axillary lymph node metastases were present. According to the pathology reports, 63 cases were positive for ER and 61 were positive for PR. Positive tumor staining with anti-PSA antibody was seen in 11 (10.1%) cases. In these cases, the percentage of positively stained cells was greater than 10%. The intensity of the immunoreactivity was weak in 3 cases, moderate in 6 cases and strong in 2 cases (Figure 1). All these PSA positive cases were also ER and PR-positive. The relation between PSA expression and clinicopathological parameters are shown in Table 1. In another 3

Table 1. Associations between PSA status and other variables

Variable	No. of patients		p-value
	PSA positive	PSA negative	
Age (years)			
< 50	4	32	1.00
≥ 50	7	66	
Tumor size (cm)			
< 2	3	35	0.744
≥ 2	8	63	
Histologic grade			
I	3	16	0.148
II	3	57	
III	5	25	
Nodal status			
negative	3	44	0.345
positive	8	54	
ER status			
negative	0	46	0.002
positive	11	52	
PR status			
negative	0	48	0.002
positive	11	50	

cases, normal breast tissue surrounding cancerous tissue showed positive staining, while the tumor itself was PSA-negative.

A statistically significant association with PSA and the expression of steroid receptors was found, whereas the other parameters showed no association with PSA positivity.

Discussion

It is now accepted that PSA is not a tissue-specific protein, so the term "prostate-specific antigen" is a misnomer. Recently, PSA has been found in various female hormonally regulated tissues, principally the breast and its secretions [6, 21]. The production of PSA in breast tissue is regulated by androgens and progestins at the level of transcription. The effect of ER in this event is indirect to the known association between ER and PR [16]. An experimental study showed that tumors stimulated with a progestin produce significantly more PSA than nonstimulated tumors [22]. The association between steroid hormone receptors and PSA production was also demonstrated *in vivo* in breast tissue. Breast cytosolic extracts from women receiving progestin-containing oral contraceptives had considerably more PSA immunoreactivity [23]. These findings confirmed the hormonal dependency of PSA production in breast cancer. Therefore, the majority of PSA-producing breast tumors are steroid hormone receptor-positive; however, not all steroid hormone receptor-positive tumors produce PSA. Yu et al. [24] revealed that PSA immunoreactivity in ER/PR positive tumors was 35%, while it was 14% in ER/PR negative tumors. In our study, all of PSA positive tumors were also found to be ER and PR positive.

The physiological role of PSA in breast tumors remains undetermined, but there is a hypothesis suggesting that PSA may be involved in a regulatory pathway of insulin-like growth factors (IGFs) with strong mitogenic effects. PSA is an insulin-like growth factor binding protein-3 (IGFB-3) protease. Degradation of IGFB-3 results in an increase in serum IGFs, which are known mitogens, and decrease in IGFB-3, a potential apoptosis mediator. It has been also demonstrated that PSA degrades the extracellular matrix, facilitates local invasion and stimulate cell detachment, suggesting a role for PSA in tumor progression or metastasis [21]. On the contrary, Fortier et al. [25] suggested that PSA may function in tumors as an endogenous antiangiogenic protein. Such a function and the association of PSA with steroid receptors in

some cases might explain, at least in part, its favorable value reported in breast cancer.

In breast cancers, the percentage of PSA positivity detected in both cytosolic extracts and paraffin-embedded tissues ranges from 9 to 70% [14, 19, 24, 26]. In our study, PSA positivity was detected in only 11 (10.1%) of 109 cases by using immunohistochemical staining in paraffin-embedded tissues. These different values may result from the different methods used. Howarth et al. [27] did not find any clear correlation between immunohistochemical results of PSA staining on paraffin sections and immunofluorometric PSA analysis in breast tumor cytosols, although there was a trend towards association of high immunofluorometric PSA levels with positive immunohistochemical PSA staining. The different PSA expressions, obtained from immunohistochemical staining on paraffin-embedded breast tumor sections, may result from the use polyclonal or monoclonal antibodies. Miller et al. [26] used monoclonal antibody as in our study, and observed PSA positivity in only 9% of breast cancers. This percentage is rather close to ours (10.1%), but lower than in the studies using polyclonal antibodies [28].

In numerous studies, PSA positivity in breast tumors has been found to be significantly associated with smaller tumors, steroid hormone receptor positivity, lower stage, younger age, differentiated tumor type, low tumor grade, low S-phase fraction, and longer overall survival [13,14,24,27]. But some authors could not detect any association between PSA immunoreactivity and prognostic parameters [19,29]. We only showed a statistically significant correlation between PSA expression and the ER/PR positivity, whereas, age, tumor size, histologic grade and axillary lymph node status were not found to be associated with PSA positivity.

Several authors have demonstrated that the patients with PSA positive breast tumors have better prognosis compared to those with PSA negative ones [13,14,30]. In addition, some studies have suggested that PSA is an independent favorable prognostic parameter for breast cancer [13,14]. In contrast with these findings, some authors using patients' serum, tumor cytosolic extracts or paraffin-embedded tissues reported no prognostic significance of PSA [17-19]. Moreover, PSA was found as an independent variable of poor prognosis by Foekens et al. [18], which is the largest relevant study so far.

In conclusion, due to the large variations of PSA expression and the contradictory results concerning its prognostic significance, this marker cannot be used reliably in clinical practice for female breast cancer

patients at the moment. In addition, it is important to keep in mind that there are a variety of normal tissues and neoplasms, which may express PSA and therefore, a definitive diagnosis of metastasis from a prostatic adenocarcinoma cannot be based only on immunohistochemical labelling of PSA.

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