

Immunohistochemical localization of prostate-specific antigen in benign and malignant breast conditions

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Abstract

We studied 24 selected breast tumors and 3 lymph nodes metastasis from patients with breast carcinoma. The biopsies were formalin-fixed, paraffin-embedded and sections were stained with haematoxylin-eosin. Additional sections from each case were immunostained for prostate specific antigen (PSA), using the EnVision technique. PSA was detected in 7% of normal breast tissues, in 54.5% of benign tumors and 46.5% of malignant tumors. The lesions with apocrine metaplasia were intense and constantly positive, the cystic dilated ducts and the areas with mastopathy were negative. Intense staining for PSA has been found in well-differentiated tumors, while the undifferentiated tumors were usually PSA-negative. The PSA-positivity in 2 of the 3 lymph nodes metastasis indicates that PSA immunoreactivity alone is not an individual prognostic indicator, but it correlates with the hormonal status of the female body. We discuss the results in terms of clinical implications of PSA immunoreactivity detection in mammary gland and other extra-prostatic sources.

Keywords: breast tumors, fibroadenoma, apocrine metaplasia, prostate-specific antigen (PSA).

Introduction

Prostate-specific antigen (PSA) is a glycoprotein initially isolated in 1979. PSA or kalikrein 3 (KLK3), is a 33-kDa serine protease found at high concentrations in seminal plasma and prostate epithelial cells and at low concentrations in healthy male serum. It is responsible for liquefying the clot formed immediately following ejaculation. Androgenic steroids that bind the androgen receptor and up-regulate transcription of the PSA gene regulate PSA production. Prostate-Specific Antigen is a highly specific tumor marker for prostate adenocarcinoma. Moreover, the determination of PSA in male serum is currently used as a biochemical marker for the early diagnosis and monitoring of prostate adenocarcinoma, even in screening actions [1, 2].

The immunohistochemical detection of PSA is very useful in the pathological typing of prostate carcinoma, differential diagnosis for urinary bladder neck tumors, and diagnosis of metastasis of unknown primary [3, 4].

Until recently, PSA was thought to be a prostatic tissue-specific protein that is not expressed in any other tissues in men or women. For these reasons, the studies that demonstrated non-prostatic sources of prostate-specific antigen were surprising. Further investigations demonstrated the presence of PSA immunoreactivity for many others normal or tumor tissues. PSA was found not only by immunochemistry but also by measurement of mRNA levels, with extremely sensitivity. PSA was demonstrated in salivary glands, pancreas, breast (healthy breast tissues and breast tumors, breast cystic disease), various breast secretions (nipple aspirate fluid, milk of lactating women), periurethral (Skene's) gland, endometrial tissue, amniotic fluid, bronchoalveolar washing, ascitic fluid, pleural effusions, cerebrospinal fluid, etc. Very low levels of circulating PSA are detectable in female sera [5–8]. PSA was also detected

in a large variety of tumors (ovarian tumors, thyroid neoplasm, bile duct neoplasm, lung neoplasm, bladder neoplasm, sweat gland neoplasm, paraurethral gland neoplasm, salivary gland neoplasm, pancreas neoplasm, kidney, colon, liver neoplasm), but the report of immunoreactivity was occasional and unspecific. For the female breast tumors already exists a great number of studies, that reveals PSA in 30–40% of breast tumors and the immunoexpression of PSA correlates with the hormonal status [9, 10].

There are still multiple unknown regarding the presence and the role of PSA in normal and tumor breast tissues. Therefore, there is no doubtlessness regarding the value of PSA as a prognostic indicator in breast cancer and there is not yet a sure correlation between PSA immunoexpression and the tumor grade. Therefore, the function of this tumor marker in breast tissues is not known. On the other hand, the existence of extraprostatic sources for PSA raises some delicate problems regarding its value in the diagnosis of prostate cancer. So, after radical prostatectomy, the postoperative PSA level should be reconsidered. It is not necessary to be zero, the measurement of serum PSA can be under the value of 1 ng/ml, in absence of the residual tumor. There are acknowledged cases with the serum PSA value between 4 and 10 ng/ml, in there, the repeated biopsies and the continuous surveillance does not reveal the malignancy. For all these reasons, we investigated the expression of PSA in the normal and malignant tissues. In this first phase, we report our results considering the benign and malignant breast tumors.

Material and methods

We studied 24 cases of breast tumors, 11 benign lesions and 13 breast cancers. Surgically obtained

biopsies were formalin-fixed and paraffin-embedded, according to the routine procedure. The histological grading and typing of the tumor were done on haematoxylin-eosin stained samples.

Additional sections from every case were immunohistochemically stained for PSA, using the EnVision working system, while it is actually the most sensitive and specific and without artifacts noticed sometimes in LSAB2 and EPOS systems.

The steps of the technique were: blocking of endogenous peroxidase, washing with Tris, incubation with monoclonal anti PSA antibody (rabbit anti-human PSA, DAKO, Denmark) – 30 minutes, washing with Tris, incubation with EnVision-peroxidase system 30 minutes, washing with Tris, visualization with diaminobenzidine (DAB). The outer positive control of the reaction was represented of sections from 7 cases of prostate adenocarcinoma and 3 cases of benign prostate hyperplasia. The results were estimated as negative (-), weak positive (+1), moderate positive (+2) and strong positive (+3). The evaluation of the immunoreactivity considered the dominant lesion, the additional lesions and the normal breast tissue that was present on sections in some cases.

Results and discussions

The results of the PSA immunoreactivity were estimated in accord with the pathological diagnosis of the breast tumor and the intensity of the final reaction product from the prostate lesions. On the sections from the 24 cases, normal breast tissue was identified in 9 cases, 8 from these being operated for benign lesions. The benign lesions that were identified were: fibroadenoma, apocrine metaplasia, cystic dilatations, adenosis and mastopathy.

The malignant breast tumors were represented by: 10 carcinomas with different differentiation grade and 3 lymph node metastasis derived from breast cancer. The outer positive control of PSA immunoreactivity revealed the presence of the final product of the reaction, intensively stained in brown (+3), either the cases of benign hyperplasia or the carcinoma cases.

The PSA immunoreactivity was positive in the secretory cells of benign lesions and negative in the basal cells and in components of the stroma (Figure 1).

In prostate carcinomas all malignant cells were strongly positive, with granular diffuse pattern in the cytoplasm. In the normal breast tissue, benign and malignant tumors, the final reaction product has been observed only in the cytoplasm of the epithelial cells.

The stroma cells, the vascular wall, nervous fibre and adipose tissue were negative. The intensity of the reaction was significantly smaller in comparison with the outer positive control.

The distribution of the positive material was heterogeneous, equally in the normal tissue as in the tumor tissue. The normal breast tissue was found in 9 of the 24 cases, like little lobules, with intralobular and interlobular stroma, with all the normal microscopic features. The reaction was positive in 7 cases (77.7%). In 6 cases the intensity of the reaction was low and in

one case the intensity was moderate (Figure 2).

The distribution of the positive material was heterogeneous, whether in the same lobule or from one lobule to another. Isolated cells were strongly stained at the acinar level, but weak and moderate positive reaction was observed in the ductal cells. As a whole, weak reaction was detected in 6 cases and a moderate reaction in 1 case. In benign tumors, the reaction was positive in 54.5% of cases.

The lesions of mastopathy were negative, the fibroadenoma lesions were low positive in 6 from 9 cases of fibroadenoma, and the cystic dilatations were negative in all the 8 cases, either at the epithelial level as in the cyst content. The apocrine metaplasia was found in 10 cases, all strong positive; the final product of the reaction was uniform distributed in the cytoplasm, with granular pattern (Figure 3).

The adenosis and the intraductal papillomas were negative. In carcinomas, the reaction was positive in 46.5% of cases. Incidence of positive cases was greater for the intraductal and invasive well-differentiated carcinomas while for the less differentiated carcinomas; incidence of positive cases was reduced (Figure 4).

On the other hand, two from the three lymph node metastasis that we studied were positive (weak and moderate positive). The final product of the reaction was focally or diffuse distributed and the intensity of the reaction was variable from one zone of the tumor to another.

Table 1 – Distribution of the results after the lesions categories

The lesion	No. of cases	Negative	+1	+2
Normal breast tissue	9	2	6	1
Fibroadenoma	9	3	5	1
Cystic dilatations	8	8	–	–
Adenosis	1	1	–	–
Apocrine metaplasia	10	–	4	6
Mastopathy	2	2	–	–
Intraductal papilloma	2	2	–	–
Intraductal carcinoma	3	1	–	2
Invasive carcinoma	9	5	2	2
Lymph node metastasis	3	1	1	1

Since its discovery by Wang in 1978, PSA was considered over 15 years as a marker for secretory cells of the prostate. Only in the late five years, have been published a number of studies regarding the extraprostatic sources of PSA [5, 11, 12]. Of great interest was the localization of PSA in the female breast, in normal or pathological conditions.

Moreover, the involvement of the glandular cells of breast tissue in the PSA synthesis was demonstrated by detecting PSA mRNA in the cases with immunoreactivity [13]. The interest is due to the great incidence of the positive reaction in malignant tumors and to the possible correlations with the hormonal status. In this context, the problem was if this marker is present or not in the normal breast tissue and in benign tumors. PSA has been identified in the breast secretions and the cystic fluid, in biochemical detectable concentrations [8, 14–16].

Figure 1 – PSA immunoreactivity, positive in the secretory cells of benign lesions and negative in the basal cells and in components of the stroma

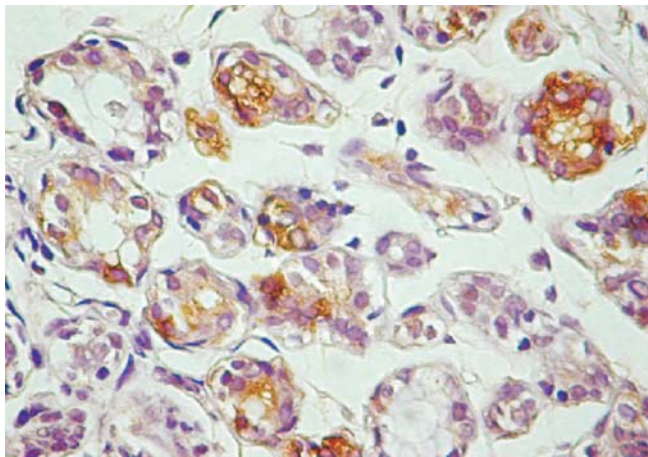
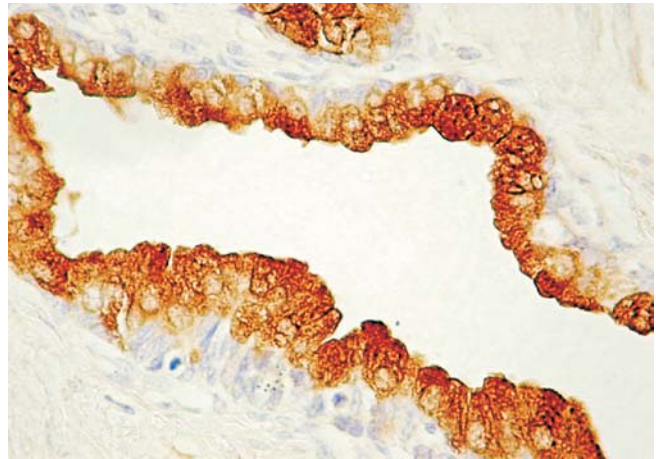


Figure 2 – Normal breast tissue, like little lobules, with intra- and interlobular stroma

Figure 3 – Apocrine metaplasia, in benign tumors; the final product of the reaction is uniform distributed in the cytoplasm, with granular pattern

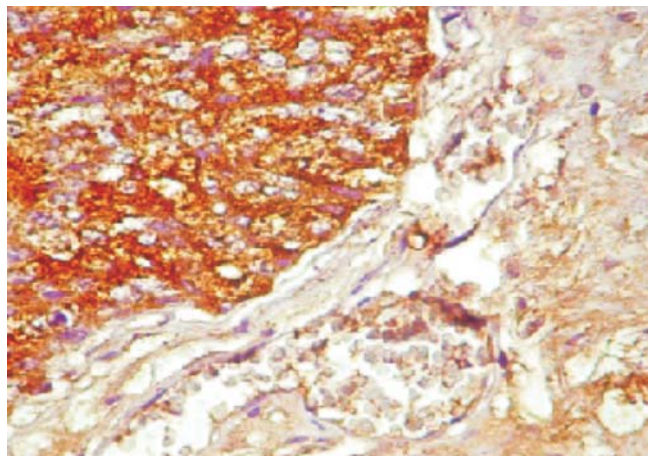
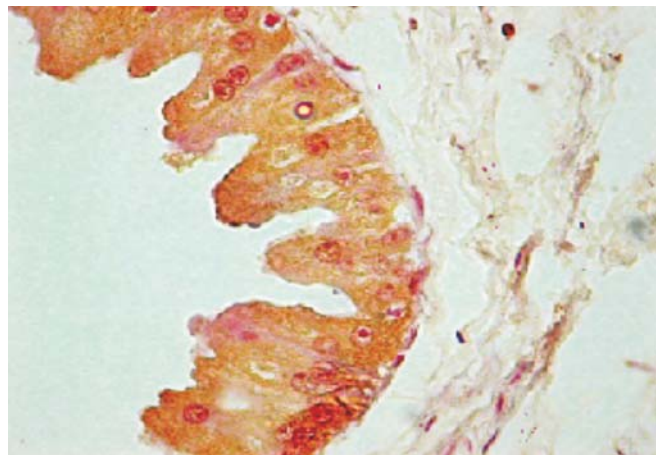


Figure 4 – The adenosis and the intraductal papillomas were negative. Incidence of positive cases was greater for the intraductal and invasive well-differentiated carcinomas

These findings raise some questions: is there a correlation between the PSA immunoreactivity and the expression of the hormone receptors? Has the PSA expression a prognostic value? Has it a predictive character for the adjuvant treatment?

In the next table are shown results obtained by different authors regarding the percent of positive cases.

Table 2 – Incidence of positive PSA immunoreaction in the normal, benign and malignant breast tissue

Authors	Year	No. of cases	Normal	Benign	Malignant
Yu <i>et al.</i>	1995	174	NI	NI	27%
Yu <i>et al.</i>	1996	273	33%	65%	28%
Boday <i>et al.</i>	1997	16	NI	NI	100%
Umekita <i>et al.</i>	1998	100	NI	10 %	NI
Alanen <i>et al.</i>	1999	171	NI	NI	32%
Heyl <i>et al.</i>	1999	100	NI	NI	49%

NI – not investigated

The mammary gland is the most important source of PSA in women. Serum variations were found during the ovarian cycle. Some studies revealed only 10% from the fibroadenoma positive for PSA.

The rate of the positive reaction that we obtained for the fibroadenoma was greater and we attribute these results to the EnVision technique that is very sensible. Other studies detected 100% PSA positivity for the breast carcinomas [17, 18].

Like others authors, we observed the intense positive reaction for the apocrine metaplasia and the hyperplastic ductal epithelium. In opposite of others studies (Howarth *et al.*, 1997), we cannot acknowledge the immunoreactivity in the cystic epithelium [19].

The results regarding the prognostic value of the PSA in breast cancer are controversial. On one hand, it is accepted the idea that the patients with breast cancer and positive immunoreactivity for PSA have a superior rate of survival [20].

The PSA expression in breast cancer correlates in part with the incipient state of the lesion [21, 22]. Our results confirm only in part these conclusions, because we observed a positive reaction also in two cases with lymph node metastasis. On the other hand, the incidence of negative cases was greater in the invasive well-differentiated carcinomas. Some authors did not observe significant survival differences regarding PSA expression and, in these conditions, the method has not a prognostic value [10, 23].

Very interesting are the observations regarding the correlation between the immunoexpression of PSA and the expression of the androgen hormone receptors. Between all the breast cancers that are PSA positive, 98% express androgen receptors. In the same studies it was not observed a significant correlation between PSA immunoexpression and the expression of estrogen hormone receptors [15].

They conclude that PSA expression is androgen-dependent and estrogen- and progesterone independent. However, Fokens *et al.* (1999) demonstrated that the PSA immunoexpression in breast cancer correlates with a poor response to the adjuvant therapy with Tamoxifen [24]. The hypothesis according that the PSA expression is induced by the oral contraceptives (Yu *et al.*, 1995)

requires evaluations on a large number of cases and the survey of the effects for a long time [21].

Until now, there are no data referring to the presence of PSA in the male breast. There were published only two cases of male breast tumors, but they were finally interpreted like prostate carcinoma metastasis [25].

The identification of PSA in male breast tissue would create serious problems of interpretation for the values of serum PSA, especially in the cases with values between 4 and 10 ng/ml. Even more, there are already argues for considerate skin and annexes, salivary glands, pancreas, like extraprostatic sources of PSA [26, 27].

Considering the presented data, it seems that PSA can no longer be considered like a specific marker for the prostate tissue, although, it keeps his great diagnostic value in the prostate adenocarcinoma. On the other hand, its expression and significance in breast carcinoma remains to be clarified.

✉ Conclusions

The study of the PSA immunoexpression in the benign and malignant breast tumors reveals a positive reaction in 54.5% of the benign tumors, respectively in 46.5% of the breast malignant tumors. Frequently positive were the normal breast tissue, the fibroadenoma and the apocrine metaplasia. All the cystic lesions were negative in our study. The greatest incidence of positive reaction was observed in well-differentiated malignant breast tumors.

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