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Immunohistochemical expression of androgen receptor and prostate-specific antigen in breast cancer

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Abstract: AR (androgen receptor) and PSA (prostate-specific antigen) are involved in the pathogenesis of breast cancer, but their role is not clearly defined. The purpose of this study was to analyze by immunohistochemistry the AR and PSA (prostate-specific antigen) expression in 156 female breast carcinomas and to correlate the results with some histopathological parameters, like ER (estrogen receptor), PR (progesterone receptor), HER2/neu, nodal and metastasis status, histological type and grade. ARs and PSA were expressed in 112/156 (72%) and respectively in 61/156 (39%) of cases and we found a positive correlation between AR and PSA expression in breast carcinomas ($p<0.0002$). We also found an association between the histological type of the tumor and AR ($p<0.001$), respectively PSA ($p=0.01$) and between AR and the grade of differentiation ($p=0.007$) and the nodal status ($p=0.02$). No correlations were found between the metastasis status and AR or PSA. 47.3% (53/112) of AR-positive cases and 46% (28/61) of PSA-positive cases were ER-negative. High frequency of AR (87.5%) and PSA (75%) expression was found in medullary carcinomas and 53% of lobular invasive carcinomas co-expressed AR and PSA. We found an inverse correlation between HER2/neu and PSA ($p=0.05$). Although most of the PSA-positive carcinomas were lymph node-negative, well and moderately differentiated, we did not find any statistically significant correlations between these parameters and PSA expression. Our study confirms that ARs are commonly expressed in breast cancer and the expression of PSA and AR are highly correlated. Moreover, all the lobular carcinomas and the majority of medullary carcinomas co-expressed AR and PSA, the majority of AR-positive carcinomas were lymph node-negative, well and moderately differentiated, and large number of ER-negative carcinomas expressed AR and PSA.

Key words: Androgen receptor - Prostate-specific antigen - Breast cancer

Introduction

The role of estrogen receptors (ERs) and progesterone receptors (PRs) in breast carcinomas is well established, but the function and clinical significance of androgen receptors (ARs) are still not clearly defined. Steroids and their nuclear receptors play crucial roles in the development and maintenance of normal functions of the human mammary gland. In addition to estrogen receptors-α, estrogen receptors-β and progesterone receptors, androgen receptors are present in both normal and tumoral breast tissue [50]. Despite the potential of testosterone and dehydroepiandrosterone to be aromatized to estrogen, it has been shown that androgens exhibit growth-inhibitory and apoptotic effects in some, but not in all breast cancer cell lines, suggesting that testosterone may serve as a natural, endogenous protector of the breast. These differences between cell lines appear to be due primarily to the variations in concentrations of specific coregulatory proteins at the receptor level [18, 35, 41].

The PSA is not exclusively synthesized by the human prostate gland, but it is also produced by the breast, ovary, liver, kidney, pancreas, lung, adrenal and parotid glands [11, 12, 36, 37]. The molecular weight [11] and mRNA sequence [33] of breast PSA were found to be identical to seminal PSA. PSA was found in 73% of tumor extracts [7], in milk of lactating women and in nipple aspirates [39, 43]. Sauter *et al.* [39] demonstrated that PSA expression in nipple aspirates was inversely associated with the presence of breast cancer and PSA levels decreased in tumors with more advanced disease stage, larger tumor size and nodal involvement. In general, PSA better predicted disease involvement in premenopausal women. Yu *et al.* [46] reported PSA-positivity in 65% of benign breast tumors, in 33% of normal breast tissue and in 28% of breast cancers.

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Table 1. Applied protocols and antibody characteristitics

Antibody against	Clone (Dako)	Antigen retrieval	Dilution	Incubation time with primary antibody	Working system	Positive control
PSA	polyclonal	Microwave HIER* pH=6.6, 5-10 min	Ready-to-use	30 min	EnVision	Prostate
AR	AR441	Microwave HIER* at 95-99°C, 25 min pH=9	1:30	60 min	LSAB2	Prostate
ER	1D5	Microwave HIER* at 90-99°C, 20 min	Ready-to-use	30 min	LSAB2	Breast
PR	PgR 636	Microwave HIER* at 90-99°C, 20 min	Ready-to-use	30 min	LSAB2	Breast
HER2/neu	HercepTest polyclonal	Microwave HIER* (95-99°C), 40 min	Ready-to-use	30 min	EnVision	Dako positive slides

*HIER= heat induced epitope retrieval

PSA gene expression in breast tumors appears to be under hormonal control, because in the steroid hormone receptor-positive breast cell lines T-47D and BT-474, PSA production can be induced by androgens, progesterone, mineral corticoids and glucocorticoids [49]. Magklara *et al.* [27] examined the expression of various known co-activator/co-repressor proteins in different breast cancer cell lines and found that the mRNA levels of steroid receptor co-activator 1, a known co-activator of the AR, were the highest in the breast cancer lines with the greatest PSA production and the lowest in the cell lines that secreted less PSA. This raises the possibility that the relative levels of specific co-activators/co-repressors might differentially modulate AR transcriptional activity within the promoter/enhancer region of KLK3 (PSA) of different breast cancer cell lines. DNA sequencing confirmed that no mutations were present in the coding region of PSA gene in breast tumors, but multiple mutations/polymorphisms were detected in the promoter and enhancer region. These mutations/polymorphisms may alter the steroid hormones regulation of the gene, affecting the PSA expression level [4, 28, 44].

Elevated levels of PSA in breast tumors have been shown to be a favorable prognostic indicator in breast cancers, but up to now the potential significance of PSA in breast cancer is not defined. The literature data concerning the correlations between PSA and sex steroid receptors, tumor grade, histological type and prognosis are controversial and raise the question whether PSA could play a role in the hormonal therapeutic strategies.

The purpose of this study was to evaluate AR and PSA immunoexpression in female breast carcinomas and to correlate the results with ER/PR status, HER2/neu status and some histopathological features, like histological type and grade, nodal and metastasis status.

Materials and methods

Breast cancer specimens. We studied 156 surgical specimens from female patients with breast cancer, collected in 2004. Clinical features of the patients were obtained from the archives of the hospitals. The cases with unknown nodal and metastasis status were excluded. Ethical approval was obtained and all patients gave informed consent. The samples were formalin-fixed and paraffin-embedded, according to the routine procedure. The pathological diagnosis and grading were done on hematoxylin-eosin-stained samples and were based on the Standard recommendations by AFIP in 2004 and Elston and Ellis modified Scarff-Bloom-Richardson grading system [15].

Immunohistochemistry. Immunostaining was performed to study the correlations between AR, PSA, ER, PR, HER2/neu expressions in human breast cancer. The slides were dewaxed and rehydrated. Antigen retrieval was performed before applying the antibodies. The endogenous peroxidase was blocked using 3% hydrogen peroxide in deionized water. The specific features of the method and the characteristics for each antibody are summarized in Table 1. We used the clones: AR441 for AR, 1D5 for ER, PgR636 for PR, HercepTest for HER2/neu and polyclonal PSA, all from Dako. The final product of the reaction was visualized with 3,3'-diaminobenzidine (DAB) and the nuclei were stained with Lillie's modified hematoxylin.

For semiquantitative evaluation of AR, ER, and PR immunoreactivity we considered the percentage of positive cells; samples were considered positive when at least 10% of nuclei were immunoreactive, irrespective of the intensity of the immunostaining. For AR, we considered also nuclear labeling in more than 10% neoplastic cells as the cut-off point for positivity, similar to standardized criteria used for other steroid hormone receptors [32]. For quantitative evaluation of ARs we used Lucia G image analysis software. Nikon Eclipse E600 microscope was coupled with a video camera that transmitted the image to the computer. Three representative × 20 fields were chosen to reflect the overall immunostaining of the tumor. The areas selected for image analysis showed immunostaining intensity allowing unequivocal differentiation between nuclei and the background.

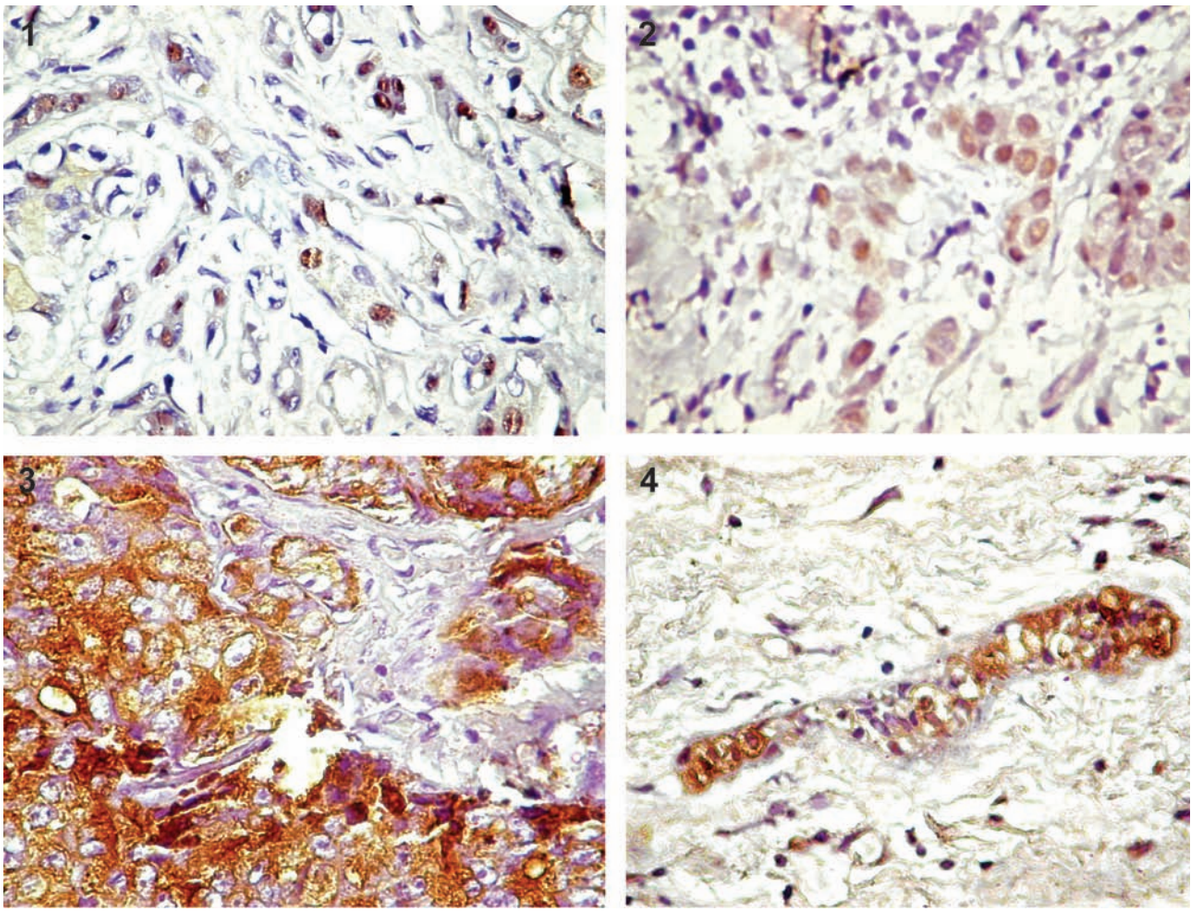

Fig. 1. Androgen receptor nuclear staining in moderately differentiated invasive ductal carcinoma (× 400). **Fig. 2.** Medullary carcinoma with a moderate androgen receptor nuclear immunoexpression in the tumoral cells and lack of immunoreaction in the stromal cells and lymphocytic infiltrate (× 400). **Fig. 3.** Strong immunoexpression of PSA in invasive, moderately differentiated ductal carcinoma (× 400). **Fig. 4.** Intense immunoreaction for PSA in invasive lobular carcinoma with typical Indian file pattern of growth (× 400).

We quantified the results for PSA according to a histoscore modified after Alanen *et al.* [2], calculated from the estimated percentage of the PSA-positive cancer cells multiplied by the staining intensity category. The results were estimated as negative (-), weakly positive (+1), moderately positive (+2) and strongly positive (+3) in the following manner: 0 - 0.04 = negative; 0.05 - 0.74 = (+1); 0.75 - 1.4 = (+2); 1.5 - 3 = (+3). For the determination of HER2 overexpression, we evaluated only the membrane staining as presence and intensity. The score +2 was interpreted as weakly positive, +3 as strongly positive and the scores 0 and +1 were reported as negative [13].

Positive controls included normal breast tissue surrounding the tumors for ER and PR, cases of prostate adenocarcinoma and prostate benign hyperplasia for AR and PSA and Dako positive slides for HER2/neu. Sections incubated without primary antibodies served as negative controls.

Statistical analysis. Chi-square test was used. P-value <0.05 was considered to be significant.

Results

The distribution of breast cancer samples according to the histopathological type and grade is presented in tables 2 and 4. Lymph node involvement was present in 72/156 (46%) cases and metastasis in 13/156 (8.33%) cases.

AR was expressed in 112/156 cases (71.8%), mostly (95.8%) represented by ductal invasive carcinoma (Fig. 1) and DCIS (90.9%). High frequency of AR expression was also found in the medullary carcinomas (87.5%) (Fig. 2) and lobular invasive carcinomas (53%) (Table 2). The majority of AR-positive carcinomas were well (78.8%, 26/33) and moderately differentiated (75.5%, 71/94) (Table 3). 48.2% (54/112) respectively 7% (8/112) of AR-positive carcinomas were nodal and metastasis positive. 51% (57/112) AR-positive cases were concomitant PSA-positive, but only 9% (4/44) AR-negative cases were PSA-positive and the majority of AR-negative carcinomas were also PSA-negative (40/44, 91%) (Fig. 5). We had a relatively small number of cases with apocrine morphology, but all these cases were concomitantly AR- and PSA- positive. The semi-quantitative evaluation was coupled with quantitative

Table 2. AR, PSA, ER/PR, HER2/neu immunoexpression in breast carcinomas

Histopathological type	AR+	PSA+	ER+	PR+	HER+
Ductal invasive (n=72)	69 (95.8%)	21 (29.16%)	59 (82%)	53 (73.61%)	33 (45.8%)
Lobular invasive (n=34)	18 (53%)	18 (53%)	14 (41.17%)	15 (44.11%)	12 (35.3%)
Ductal in situ (n=11)	10 (90.9%)	4 (36.36%)	7 (63.63%)	8 (72.73%)	4 (36.36%)
Lobular in situ (n=14)	3 (23.07%)	8 (57.14%)	8 (57.14%)	10 (71.43%)	3 (21.4%)
Medullary (n=8)	7 (87.5%)	6 (75%)	0	0	0
Mucinous (n=4)	1 (25%)	2 (50%)	4 (100%)	4 (100%)	1 (25%)
Undifferentiated (n=10)	2 (20%)	1 (10%)	2 (20%)	1 (10%)	5 (50%)
Neuroendocrine (n=3)	0	1 (33.33%)	2 (66.67%)	2 (66.67%)	0
Total cases (n=156)	112 (71.8%)	61 (39.1%)	87 (55.77%)	93 (59.61%)	58 (37.3%)

Table 3. Immunohistochemical expressions of AR, PSA, ER, PR, HER2/neu correlated to histological grade

Histological grade	AR +	PSA+	ER+	PR+	HER+ (+2,+3)
G1 (n=33)	26 (78.8%)	14 (42.42%)	25 (75.75%)	26 (78.8%)	10 (30.3%)
G2 (n=94)	71 (75.5%)	37 (39.4%)	54 (57.45%)	58 (61.7%)	30 (32%)
G3 (n=29)	15 (51.7%)	10 (34.48%)	8 (27.5%)	9 (31.03%)	18 (62%)
Total (n=156)	112 (72%)	61 (39.1%)	87 (55.77%)	93 (59.6%)	58 (37.18%)

computer image analysis of the cases and the results concurred; the medullary, ductal and lobular invasive carcinomas and apocrine metaplasia showed an increased number of positive signals.

In breast carcinomas, PSA was found in the cytoplasm of the tumor cells, with granular pattern and focal or diffuse distribution. It was absent from stromal cells. PSA immunostaining was also seen in normal mammary gland tissue and in some benign lesions, especially apocrine metaplasia adjacent to the carcinomas. We noticed that, unlike in the prostate, in the normal mammary gland only the acini were constantly positive, whereas the ducts were generally negative. PSA was expressed in 61/156 (39.1%) cases, with a higher frequency in the cases of well and moderately differentiated carcinomas (Fig. 3) (42% G1, 39.4% G2) (Table 3), as well as lobular (53%), medullary (75%) and mucinous carcinomas (50%). The lobular carcinomas (Fig. 4) (18/34, 53%) expressed PSA with higher frequency than ductal carcinomas (21/72, 29%) (Table 2**,** Fig. 5). The relationships between PSA and the other parameters investigated are presented in Table 4. 57/61 (93.4%) of PSA-positive carcinomas co-expressed AR and only 9% (4/44) of AR-negative carcinomas expressed PSA.

ERs and PRs were expressed in 54.5% (87/156) and 60.25% (93/156) of cases, respectively, mostly represented by ductal and well differentiated carcinomas. 47.3% (53/112) and 40% (45/112) of AR-positive carcinomas were respectively ER- and PR-negative; 46% (28/61) and 36% (22/61) of the PSA-positive carcinomas were respectively ER- and PR-negative. Overexpression of HER2 was found in 37.18% of cases, with higher frequency in poorly differentiated (G3) cancers (18/29, 62%) (Table 3, Fig. 6) and in ductal invasive carcinomas (33/72, 45.8%, Table 2). Most of PSA-positive carcinomas were HER2/neu negative (72%). The relevant histopathological data are reported in Tables 2-4 and Figures 5, 6.

Statistical analysis of all data showed a significant association between AR expression and PSA expression ($p<0.0002$), as well as between AR expression and histological grade ($p=0.007$) and histopathological type ($p\leq0.001$). We also found an inverse association between AR expression and nodal status of the tumor ($p=0.02$), most of AR-positive carcinomas being lymph node-negative. We did not find any association of AR expression with ER/PR status, HER2 status, and metastasis (Table 5).

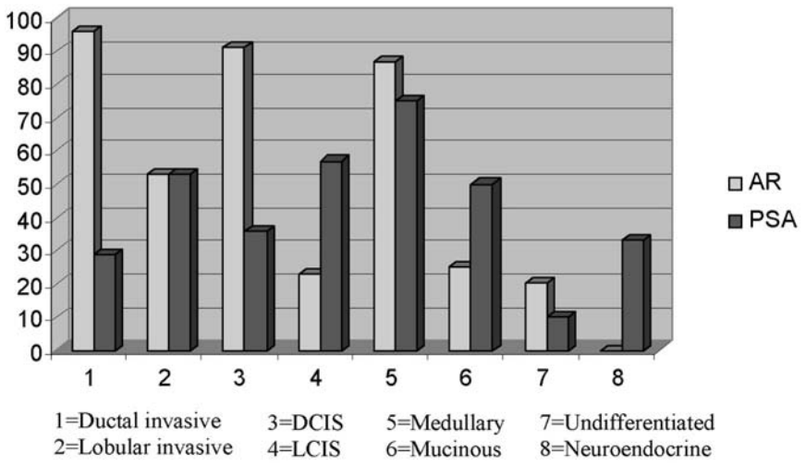

Fig. 5. Immunoexpression of AR and PSA in different histological types of breast carcinomas.

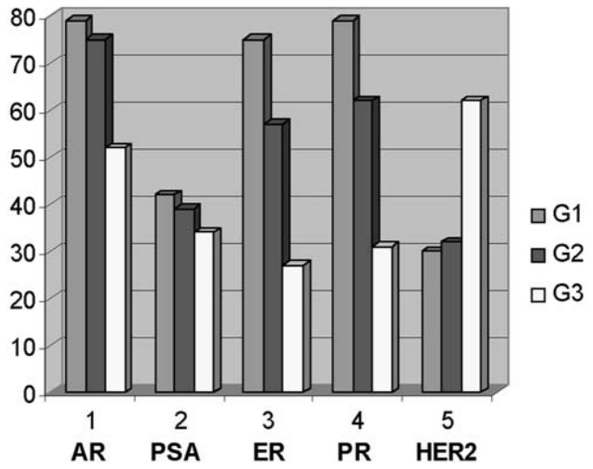

Fig. 6. Comparison of AR, PSA, ER, PR and HER2 immunoexpression in well (G1), moderately (G2) and poorly (G3) differentiated breast carcinomas.

When the material was divided into PSA-positive and PSA-negative cases, besides the highly significant correlation with AR immunoexpression, statistical analysis showed a significant association between PSA and the histological type of the tumor (p=0.01) and an inverse correlation between PSA-positive cases and HER2 overexpression (p=0.05). None of other parameters considered, including grade, ER/PR expression, nodal and metastasis status showed any significant correlation with PSA immunoreactivity.

Discussion

It is well known that sex steroids are involved in the growth of breast cancers, and the high majority of breast carcinomas express estrogen, progesterone and androgen receptors.

Immunohistochemical studies have shown that in breast tumors, AR is expressed with a comparable or higher frequency (70-90%) than ER and PR (60-80 and 50-70% respectively) [5, 14, 23, 24]. AR was identified as the only sex steroid receptor expressed in approximately 25% of breast cancer metastases [3]. On the other hand, the expression of PSA, an androgen regulated protein, in breast cancers is well established, ranging from 9.3% to 49% of cases [2, 31, 34, 42, 46], but the role of breast PSA appears to be complex and poorly understood. It has been suggested that PSA may act as a growth factor or a regulator of growth factors and it could be a marker of endogenous hormone balance between androgens, progesterone and estrogens [42, 47]. Some studies showed that PSA is a favorable prognostic indicator for breast cancers [7, 8, 30, 44, 48] while others reported that PSA is not useful for prognostic evaluation of breast carcinomas [20]. Yu *et al.* [48] reported no correlation with histological type and grade, but a significant correlation between PSA levels, PR levels, incipient stage of the lesion, small tumors, reduced risk of relapse and longer survival, making PSA a favorable prognostic indicator for women with breast cancer. We did not find any significant correlation between PSA and nodal, metastasis and ER/PR status, or the grade of differentiation. According to our knowledge, investigations of PSA in combination with HER2/neu were not accomplished up to now. In this context, we studied the relationship between PSA and HER2/neu and we found a relatively small statistical significant association between PSA and HER (p=0.05), the majority of PSA-positive breast carcinomas being HER2- negative. In another study (unpublished data) we found a statistically significant correlation between

Table 4. Expression of ER, PR, PSA, HER2, nodal and metastasis status in the two groups of AR/PSA-positive and -negative carcinomas

Parameter	AR +(112)	AR -(44)	PSA+ (61)	PSA- (95)
ER+	59 (52.6%)	26 (59%)	33 (54%)	52 (54.7%)
ER-	53 (47.3%)	18 (41%)	28 (46%)	43 (45.3%)
PR+	67 (60%)	27 (61.4%)	39 (64%)	55 (57.9%)
PR-	45 (40.2%)	17 (38.6%)	22 (36%)	40 (42.1%)
HER-	71 (63.4%)	27 (61.4%)	44 (72%)	54 (56.8%)
HER+	41 (36.6%)	17 (38.6%)	17 (28%)	41 (43.2%)
N+	54 (48.2%)	14 (31.8%)	26 (42.6%)	42 (44.2%)
N-	58 (51.7%)	30 (68.2%)	35 (57.4%)	53 (55.8%)
M+	8 (7%)	5 (11.36%)	7 (11.5%)	6 (6.3%)
M-	104 (92.8%)	39 (88.64%)	54 (88.5%)	89 (93.7%)

HER2/neu negativity in premenopausal women and PSA expression (75% of cases were PSA-positive and HER2-negative), that could suggest a subset of malignancies with better prognosis. In contrast, in the present study we did not find any significant correlation between AR expression and HER2 overexpression. This finding remains in agreement with the results of some studies [5, 22] but other studies suggest HER2/neu overexpression in G3, AR-positive carcinomas [1, 26, 32]. A common expression of AR was reported in mammary Paget's disease, while PR was always negative and ERs were positive only in a few cases. In mammary and extramammary Paget's disease, a coexpression of HER2/neu and AR was found in 88% and 52% of cases, respectively [26]. Regarding the nodal status, probably the most reliable prognostic factor in breast cancer, in accordance with some authors we found a positive association between AR immunoexpression and lymph node-negative carcinomas [5], but we did not find a correlation with the presence of distance metastasis. Some recent studies demonstrated no correlations with the stage of the tumor [38].

The apocrine feature was associated with the presence of AR immunoexpression and loss of ER/PR expression in DCIS [25, 40] and AR positivity was proposed as a marker of apocrine differentiation [17]. We had a relatively small number of cases with apocrine differentiation, but they showed a similar pattern, being AR-positive and ER/PR negative. In all these cases with apocrine differentiation, PSA was positive. These results correspond with the findings of Mannello *et al*. [29] who reported high concentration of PSA in apocrine cysts and suggested that PSA may play a role in malignant transformation. In addition, Hall *et al*. [19] found that positive immunoreactions for both PSA and GCDFP-15 (gross cystic disease fluid protein) was highly dependent on AR-status (98% of PSA-positive and 92% of GCDFP-15-positive tumors were AR-positive).

Table 5. Correlations between AR immunoexpression and other clinical and histopathological features: ER/PR, HER2/neu, PSA, histological grade and type, nodal status and metastasis

Parameter	AR+(112)	AR-(44)	*P*
ER			
+	59	26	
-	53	18	*0.4*
PR			
+	67	27	
-	45	17	*0.86*
HER2/neu			
-(0,1)	71	27	
+(+2,+3)	41	17	*0.8*
PSA			
+	57	4	
-	55	40	*<0.0002**
Grade			
G1	26	7	
G2	71	23	
G3	15	14	*0.007**
Histology			
Ductal**	79	4	
Lobular ***	21	26	
Other types	10	16	*≤0.001**
Nodal status			
+	54	14	
-	58	30	*0.02**
Metastasis			
+	8	5	
-	104	39	*0.4*

*Statistically significant
**DCIS are included
***LCIS are included

We had only a few cases of mucinous and neuroendocrine carcinomas, but like others authors [21, 45] we found a positive PSA staining in these histological types. The lobular and medullary carcinomas expressed more frequently PSA (53%, respectively 75%) and these cases co-expressed AR (53%, respectively 87.5%) It is known that lobular, medullary and mucinous carcinomas are associated with a low percentage of immunostaining for HER2/neu and these types are associated with a favorable prognosis. Riva *et al*. [38] reported a strong AR positivity in the majority of lobular carcinomas (87%). We found a higher frequency of AR expression for ductal carcinomas, but the difference could be partly explained by the fact that our study included different types of breast carcinomas and the ductal carcinoma represented the most frequent histological type. In contrast with other studies [22, 24, 38, 32], we did not find a statistically significant association between AR, ER and PR expression. Most of AR-positive carcinomas were ER/PR positive, but a significant number of AR-positive cases were negative for ER/PR (47.3%/40.2%). It has been suggested that AR expression could be used

for subdivision of ER-negative tumors into more and less favorable prognostic groups, because ER-negative but AR-positive tumors had significantly better disease-free survival [1]. In accordance with other studies [1, 5, 22, 32, 38], in our study, most of the AR-positive carcinomas were associated with a low or intermediate histological grade (G1, G2) (p=0.007), but a significant number of AR-positive breast cancers were poorly differentiated (G3, 15/29, 51.7%). Moreover, these cases were generally PSA-positive but we had a relatively small number of poorly differentiated cases to perform a statistical analysis only for these cases.

Some studies were focused on the relation between hormone therapy and PSA. Diamandis *et al.* [10] reported that 50% of the patients receiving megestrol acetate had a significant increase in PSA plasma level and this fact was correlated with poor prognosis. According to Foekens *et al*. [16], high tumor PSA levels are associated with a reduced risk of relapse and death and better prognosis, whereas high PSA levels after tamoxifen are associated with poor response to treatment. On the other hand, it has been suggested that tumor PSA values might identify a subset of estrogen-negative tumors, which would respond to endocrine therapy [7, 48]. Furthermore, the effects of tamoxifen [50] and medroxyprogesterone acetate [6] are mediated by AR. A recent study showed that reduced levels of AR or impaired AR function contribute to the failure of medroxyprogesterone acetate therapy, potentially due to abrogation of the inhibitory effect of AR on ER signaling [9]. These findings may indicate AR as a marker for the efficiency of the endocrine therapy and for new hormonal therapeutic strategies in women with estrogen-negative cancers.

We can conclude that ARs are commonly expressed in breast cancer and that expressions of PSA and AR are highly correlated ($p<0.0002$). In the present study, all the lobular carcinomas and the majority of medullary carcinomas co-expressed AR and PSA. The majority of AR-positive breast carcinomas were lymph node negative, well and moderately differentiated. Further investigations of AR and PSA could provide more information concerning the involvement of steroid receptors in the pathogenesis of breast cancer and perspectives of new hormonal therapeutic strategies.

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