THE ROLE OF ANDROGEN RECEPTORS FOR NEW THERAPEUTIC STRATEGIES AND THE PROGNOSTIC OUTCOME IN PATIENTS WITH BREAST CANCER

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ABSTRACT. It has been suggested that AR could be prognostic markers for breast cancers but their role in the pathogenesis of breast carcinomas is far to be clear. The purpose of this study was to asses by immunohistochemistry the AR expression in female breast carcinomas and to correlate the results with the immunoexpression ER/PR status, HER2/neu status and some histopathological features of carcinomas, like histological type and grade, nodal and metastasis status. We have found a positive correlation between AR and the histological type of the tumor and the grade of differentiation, most of AR-positive carcinomas being well and moderate differentiated. A negative association was found between AR and the nodal status, the majorities of AR-positive cases being lymph node negative. We did not find a statistical significant correlation with HER2, ER/PR and metastasis status. We can conclude that AR could be useful for establishing new therapeutic strategies and for evaluating the prognostic outcome in patients with breast cancer.

Keywords: breast cancer, estrogen receptors, progesterone receptors, androgen receptors, HER2/neu

INTRODUCTION

The role of estrogen receptor (ER) and progesterone receptor (PR) in breast carcinomas is well established, but little is known about the function and clinical significance of androgen receptor (AR) in breast carcinomas. 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 receptor-α, estrogen receptor-β and progesterone receptors, androgen receptors are present in both normal and tumoral breast tissue and hormone stimulation of mammary epithelial proliferation and apoptosis are important in tissue homeostasis (Zhou J et al., 2000; Dimitrakakis C et al., 2002). Androgens exert a variety of effects associated with worsening breast cancer risk and disease, including: preferential binding to circulating binding proteins, thus increasing free estradiol available to breast cells; increasing total estrogen levels through aromatic conversion of testosterone; direct stimulation of breast cells through androgen receptors; stimulation of other growth factors (Sauter ER et al., 2002). With these indirect estrogenic actions, androgens potentially increase breast cancer risk. On the other hand, androgens have been shown to regulate the proliferation of AR-positive breast cancer cell lines in culture (Hackenberg R and Schultz KD, 1996). So, the androgens exhibit growth-inhibitory and apoptotic effects in some, but not all, breast cancer cell lines. In rodent breast cancer models, androgen action is antiproliferative and proapoptotic, and it is mediated via androgen receptor, despite the potential for testosterone and dehydroepiandrosterone to be aromatized to estrogen. The results from studies in rhesus monkeys suggest that testosterone may serve as a natural endogenous protector of the breast and limit mitogenic and cancer-promoting effects of estrogen on mammary epithelium (Somboonporn W and Davis S, 2006). Epidemiological studies on women provided inconclusive results. Androgens have been associated with increased risk for breast cancer especially on postmenopausal women (Berrino F and Micheli A, 1996; Zeleniuch-Jacquotte A et al., 1997; Zeleniuch-Jacquotte A et al., 2004), but the risk on premenopausal females is controversial [9]. AR expression is a necessary requirement for androgenic effects on breast cancer cell proliferation, but the absolute levels of AR in cell lines are not definitely for these effects. Different effects between cell lines appear to be due primarily to variations in concentrations of specific coregulatory, stimulatory or inhibitory proteins at the receptor level or the structure of AR (Thomas HV et al., 1997) determine whether breast cancer cell proliferation is stimulated or inhibited in the presence of androgen (Suter NM et al., 2003); the effect is concentration dependent and depends also on the type of androgen (Birrell SN et al., 1998). It has been suggested that the effect of tamoxifen and effect of medroxyprogesterone acetate (Ortmann J et al., 2003) are mediated by AR. These findings suggest that AR determination may give additional predictive information on the response to endocrine treatments in breast cancer.

MATERIAL AND METHODS

We have studied 156 surgical specimens from female patients with breast cancer, during 2004 year. Clinical features of the patients were collected from the archives of the hospitals. The cases with unknown nodal and metastasis status were excluded.

The samples were formalin-fixed and paraffin-embedded, according to the routine procedure. From each representative paraffin block, we cut 4 μm sections. The pathological diagnosis and grading were done on hematoxylin-eosin samples and were based on the Standard recommendations by AFIP in 2004 and Elston and Ellis modified Scarff-Bloom-Richardson grading system. In this system, tumors are classified into 3 grades of decreasing differentiation (1, 2 and 3) according to the extent of glandular differentiation, nuclear pleomorphism and mitotic activity (Birrell SN et al., 1995). Grade 1 tumors are well differentiated, with minimal nuclear pleomorphism and mitotic activity, whereas grade 3 tumors are the least differentiated, with minimal or no gland formation, marked nuclear pleomorphism and prominent mitotic activity. Aditional sections from each paraffin block were immunostained for AR, ER, PR, HER2/neu, using the avidin-biotin immunoperoxidase technique. Briefly, the slides were dewaxed and rehydrated and we blocked endogenous peroxidase using 3% hydrogen peroxide in deionized water. This step was followed by an antigen retrieval step using microwave in sodium citrate buffer. The slides were then incubated with the primary antibody. The dilutions and the specific features of the method for each primary antibody are summarized in the table 1. The secondary antibody (biotinylated antiserum) was then applied and then, after washing with TBS (Tris buffered saline), we incubated the slides with freshly prepared avidin-biotin peroxidase complex for another 45 minutes. The final product of the reaction was visualized with 3, 3'-diaminobenzidine (DAB) and the nuclei were stained with Lillie's modified hematoxylin.

The pattern of immunostaining for AR, ER and PR was nuclear. For semiquantitative evaluation of ER, PR and AR we considered the percentage of positive cells (samples were considered positive when at least 10% of nuclei were immunoreactive) and the intensity of the immunostaining according to the Quick Score method [26], as follows: I) intensity of staining-slides were assessed for the average degree of staining on low power (x10) and the following scores allocated: weak (1), moderate (2) or strong (3); II) the percentage of cells with positive nuclei were counted on high power (x40) and the following scores were allocated:10-25% (1), 25-50% (2), 50-75% (3), >75% (4). The scores from I) and II) were added together to give a final score ranging from 0 to 7, designated as negative or positive as follows: score of 0-3, negative; score 4-7, positive.

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 [15].

Positive and negative controls were included in each staining batch. As positive control, we used 5 cases of prostate adenocarcinoma and prostate benign hyperplasia for AR and breast sections known to be positive for ER/PR. For HER2/neu we used Dako positive slides. Negative controls included sections processed in parallel with omission of the primary antibody. **Statistical analysis**. The frequency distribution of lymph node status, metastasis, histological type and grade, estrogen, progesterone and HER2/neu status in AR-positive and AR-negative groups were compared using chi square test with odds ratio and 95% confidence intervals. A p-value of less than 0.05 was considered to be significant.

Table 1

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

**HIER= heat induced epitope retrieval*

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RESULTS

The histopathology of 156 breast samples was represented by 72 ductal invasive carcinomas (46%), 34 lobular invasive carcinomas (21.8%), 11 cases of DCIS (7%), 14 LCIS (9%), 11 undifferentiated (7%), 8 medullary (5%), 4 mucinous (2.5%), and 3 neuroendocrine (1.9%). The well-differentiated (G1) carcinomas (n=33) represented 21%, moderately (G2) differentiated (n=94) were 60.25% and poorly (G3) differentiated (n=29) were 18.6%. Lymph node invasion was present in 46% cases and metastasis in 8.33% cases.

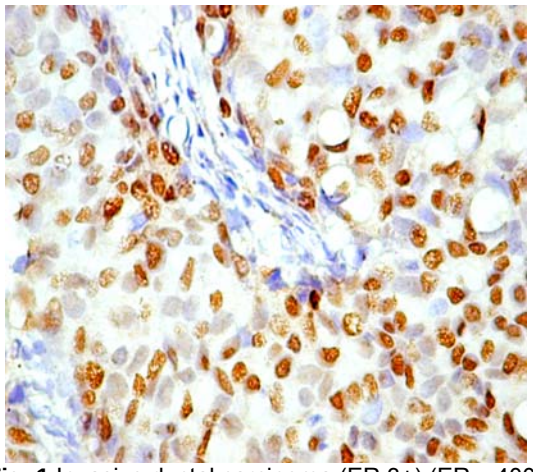

Fig. 1 Invasive ductal carcinoma (ER 3+) (ER, x400)

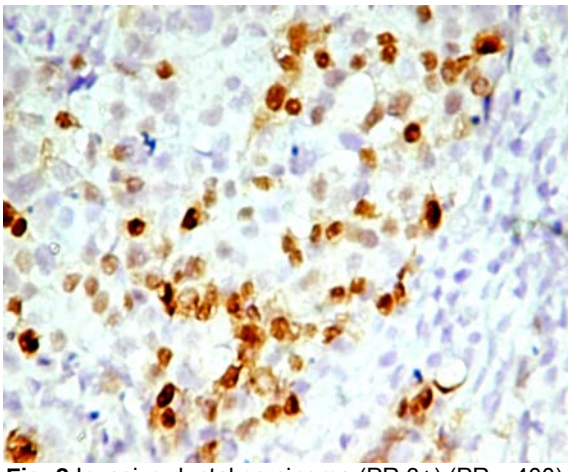

Fig. 2 Invasive ductal carcinoma (PR 3+) (PR, x400)

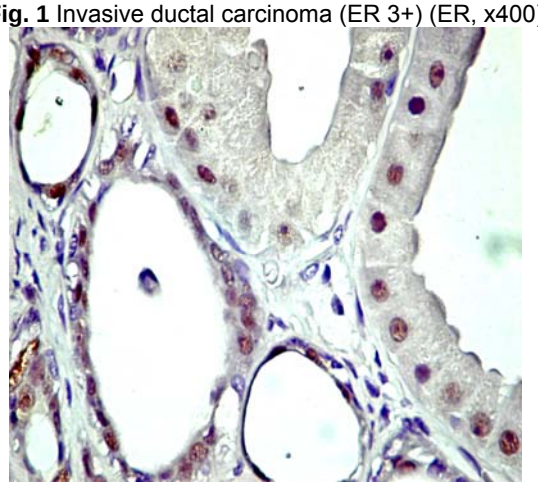

Fig. 3 Invasive ductal carcinoma with adjacent lesions of apocrine metaplasia. Androgen receptors are intensely expressed in the nuclei of both kinds of lesions (AR, x400)

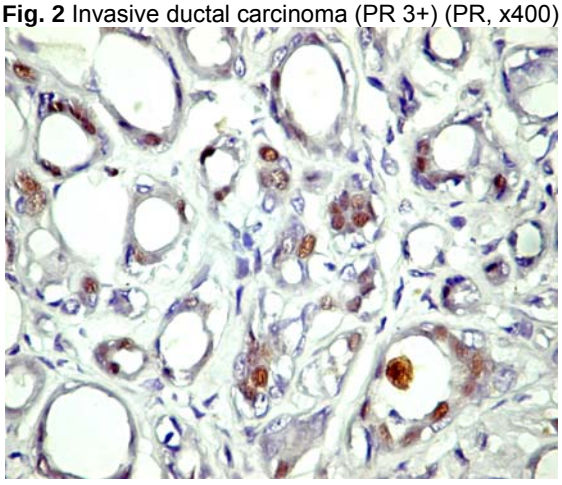

Fig. 4 Androgen receptor nuclear staining of moderately differentiated invasive ductal carcinoma (AR, x400)

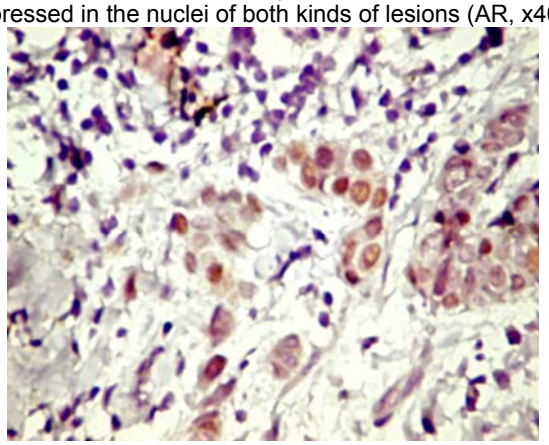

Fig. 5 Medullary carcinoma with an intense and moderate androgen receptor nuclear immunoexpression (AR, x400)

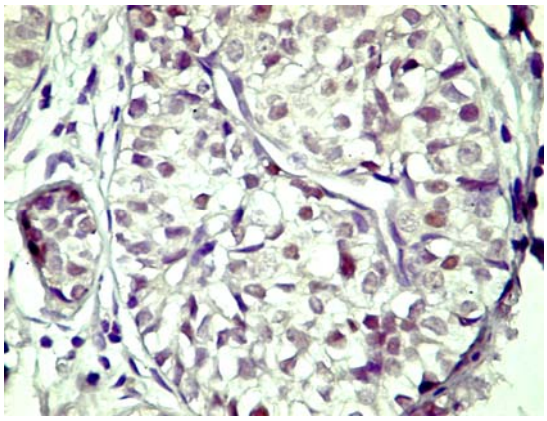

Fig. 6 Invasive lobular carcinoma (AR, 2+) (AR, x400)

AR nuclear staining varied between individual tumor cells, but generally it was of moderately and weak intensity and heterogeneous distributed. In cases with normal tissue present, staining of nuclei in normal ducts or lobules was taken as a positive internal control. AR was expressed in 112/156 cases (71.8%), most cases represented by ductal invasive carcinoma (95.8%) and DCIS (90.9%). High frequency of AR expression was also found in the medullary carcinomas (87.5%) and lobular invasive carcinomas (53%). Regarding the grade of differentiation, most of AR positive carcinomas were well (78.8%) and moderate

differentiated (75.5%). **ER** was expressed in 54.5% cases and **PR** in 60.25% cases. Regarding the **HER2** status, 37.18% of cases overexpressed HER2.

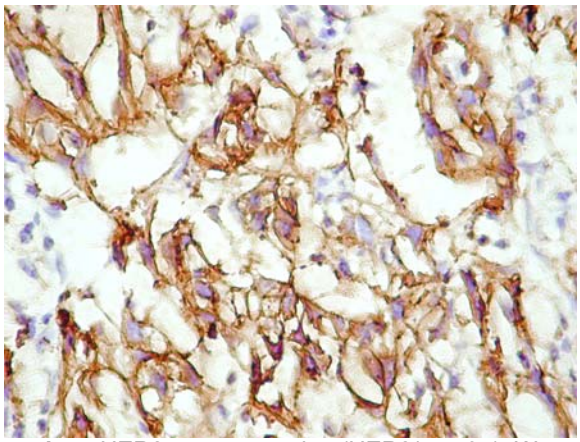

Fig. 7 HER2 overexpression (HER2/neu 3+). We observe an intense, continuous membrane staining for more than 10% of tumoral cells (x200)

When the material was divided into AR-positive and AR-negative groups we have found a statistical significant correlation between AR expression and the histological grade ($p<0.05$) and the histopathological type ($p\leq0.001$). We also found an inverse association between AR expression and nodal status of the tumor ($p=0.02$), the most of AR-positive carcinoma being lymph node negative. We did not find an association with ER/PR status, HER2 status, and metastasis.

DISCUSSIONS

In our study AR were expressed in 71.8% cases. Biochemical and immunohistochemical studies show that AR-positive tumors are more frequent (35-90%) than ER-positive and PR-positive tumors (60-80 and 50-70% respectively) (Ellis IO et al., 2000; Ellis LM et al., 1989; Kuenen-Boumeester V et al., 1996; Kimura N et al., 1993). Variations may be attributable to different methodologies and different fixatives, but a different case mix may also affect these studies. Regarding the correlations between the expression of AR and ER/PR in breast cancers, the results from different studies were not in line. In the present study, 47.3% of AR-positive carcinomas were ER-negative and 40% of the AR-positive tumors were PR negative. The results of other studies were not in line. So, Ellis et al observed a strong association only between the expression of AR and PR in invasive breast carcinoma. Isola (Kimura N et al., 2003), using frozen sections found AR expression in 79% of breast cancers and a significant positive association with ER. Most PR-negative tumors were also AR-negative, but significant proportions (38%) of AR-positive tumors were PR-negative. Unlike ER, AR was not associated with aneuploidy or erbB2 oncogene overexpression. Agoff et al. determined the prevalence of AR expression on subsets of ER-positive and ER-negative breast carcinomas and noticed that AR were positive in 89% of ER-positive, respectively 49% of ER-negative mammary cancers. In ER-negative tumors, AR was associated with increased age, postmenopausal status, tumor grade, tumor size and HER2 overexpression. In ER-positive tumors, AR was associated with progesterone receptor expression. Patients with ER-negative but AR-positive tumors had significantly better disease-free survival, and it was suggested that AR expression could be used for subdivide ER-negative tumors into more and less favorable prognostic groups.

We have found a statistical significant correlation between tumor Scarff-Bloom-Richardson grade and AR immunoexpression, in accord with other studies (Isola JJ et al., 1993; Agoff SN et al., 2003). Moinfar et al. identified AR immunoexpression in 60% of invasive carcinoma and 82% of DCIS. According to histological grade, 90% of grade 1 invasive and 95% of DCIS grade1 were AR-positive, whereas in grade 3 carcinomas only 46% of invasive and 76% of DCIS grade 3 were AR-positive. Among poorly differentiated carcinomas a significant number of cases were ER/PR negative but AR-positive. The apocrine feature was associated with the presence of AR immunoexpression and loss of ER/PR expression in DCIS (Agoff SN et al., 2003; Moinfar F et al., 2003; Leal C et al., 2001). In our study, we had a relatively reduced number of cases with apocrine differentiation, but they showed a similar pattern, being AR-positive and ER/PR negative. Regarding AR gene expression at the mRNA level, it was observed that AR was underexpressed in 18.3% and overexpressed in 34.4% cases relative to normal mammary tissue. There were observed links between AR status and age, menopausal status, histological grade, lymph node status and ER/PR status. High ARmRNA levels were negatively linked to MYC gene overexpression. It was not observed a relation with mRNA ERBB2. It has been observed that the majorities of breast carcinoma skin metastasis were AR positive and ER/PR negative, so AR immunohistochemistry could serve as a marker for identifying breast cancer in skin metastasis of unknown primary sites (Leal C et al., 2001).

It is well established that HER2/neu or c-erb B2 is an oncoprotein overexpressed in breast carcinomas with poor prognosis and actually, the HER2/neu immunohistochemical expression has a great value for the prediction of response to trastuzumab (Herceptin), a monoclonal antibody against this oncoprotein. In this study we did not find a significant correlation between AR expression and HER2 overexpression. This finding was in lines some studies (Kuenen-Boumeester V et al., 1996; Kimura N et al., 1993) but not all (Bieche I et al., 2001). Regarding the nodal status, probably the most reliable prognostic factor in breast cancer, we have found a positive association between AR immunoexpression and lymph node negative carcinomas, but we did not find a correlation with the presence of distance metastasis.

CONCLUSIONS

We have found a positive correlation between AR and the histological type, grade of differentiation, most of AR-positive carcinomas being well and moderate differentiated, and a negative association with nodal status. We did not find a statistical significant

correlation with HER2, ER/PR and metastasis status. AR could be useful for establishing new therapeutic strategies and for evaluating the prognostic outcome in patients with breast cancer.

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