Research Journal of Biological Sciences 3 (3): 307-310, 2008
ISSN: 1815-8846

Evaluation of Androgen Receptor (AR) Expression in Females with Invasive Breast Carcinoma, Sari, Iran, 2002-2006

Z.H. Torabizadeh, F. Naghshvar and M. Ghahremani
Department of Pathobiology, Mazandaran University of Medical Science, Sari, Iran

Abstract: Several studies have demonstrated the biologic and therapeutic significant of Estrogen and Progesterone Receptors (ER and PR) in breast carcinomas. The aim of the current study, was to examine the presence of Androgen Receptors (AR) in breast carcinomas. One hundred ten cases of invasive breast carcinoma from May 2002 to May 2006 were examined using a monoclonal antibody against AR on formalin-fixed, paraffin-embedded archival material. The results were analyzed for correlations with inmmunohistochemically determined ER and PR. The mean age of the patients was 51.3 ± 10.83. Histologic grade was 16, 60 and 24% in grades of 1, 2 and 3, respectively. Forty eight of the 110 cases (43.6%) of invasive carcinoma were AR-positive according to internationally standardized guidelines. No significant association was found between AR expression and histologic grade and age of the patients.

Key words: Invasive breast carcinoma, androgen receptor, females, therapeutic

INTRODUCTION

The role of determining estrogen and progesterone receptor status in the management of breast carcinoma, particularly as a guide to identifying patients who are likely to respond to hormonal manipulations, is well established (Skinner *et al*., 1980). Estrogen and Progesterone Receptors (ER and PR) also have gained widespread acceptance as independent prognostic parameters in breast carcinoma (Chevallier *et al*., 1988). Several studies have shown that primary invasive breast carcinomas contain ER and PR in approximately 55-65% and 45-55% of cases, respectively (Stanford *et al*., 1986). The presence of both ER and PR in a breast tumor increases its likelihood of responding to hormonal manipulations from 55% as observed in patients with ER-positive tumors, to 75-80% (Wittliff, 1984). Studies have shown that PR status is at least as valuable in predicting the behavior of breast carcinoma as is ER status and the loss of PR by tumor cells is associated with a less favorable prognosis (McGurie and Clark, 1985).

Although numerous studies have examined ER and PR and their correlations with other prognostic indicators, surprisingly little is known about the role of Androgen Receptor (AR) and its prognostic value in breast carcinoma (Bryan *et al*., 1984). Previous studies have demonstrated AR expression in other malignancies, including endometrial carcinoma (Brys *et al*., 2002) Although determining ER and PR status on biopsy specimens before performing hormonal manipulations has become standard practice in the management of breast carcinoma, assessment of AR currently is not practiced.

The aim of the current study, was to investigate the expression of AR in a large series of breast carcinomas using immunohistochemical techniques. The results were analyzed for correlations with ER and PR expression, as determined immunohistochemically in tissue sections from paraffin-embedded archival material.

MATERIALS AND METHODS

From May 2002 to May 2006, 110 cases of breast carcinoma were retrieved from the files of the Department of Pathology at the University of mazandaran-Iran. Determination of tumor type and histopathologic grade was performed according to standardized guidelines (Tavassoli, 1998).

The invasive breast carcinoma series consisted of 106 Ductal Carcinomas (IDCs) and 4 Lobular Carcinoma (ILCs) of the 106 IDCs 17 were well differentiated (Grade 1 (G1)), 64 were moderately differentiated (Grade 2 (G2)) and 25 were poorly differentiated (Grade 3 (G3)). Among the ILCs, there were two G1, one G2 and one G3 tumors.

Formalin-fixed paraffin-embedded tissue blocks were cut into 3 micrometer thick serieal sections that were mounted on precoated slides. The sections were deparaffinized, rehydrated and rinsed in distilled water. Immunohistochemical assays for AR, ER and PR were

Corresponding Author: Z.H. Torabizadeh, Department of Pathobiology, Mazandaran University of Medical Science, Sari, Iran

perfomed on consecutive paraffin section using standardized aoutomated procedures (signet co). After incubation with the primary antibody, incubation with the secondary (link), biotinylated antibody was performed for 30 min. After washing, sections were incubated with streptavidin-peroxidase for 30 min. Finally, the enzyme was visualized after a 15 min incubation with diaminobenzidine. Counterstaining was performed with hematoxylin.

Samples were scored as positive when at least 10% of nuclei were immunoreactive (Moinfar, 2003). Positive controls included normal breast tissue surrounding the tumors; negative controls included substitution of the primary antibody with normal sera or phosphate-buffered saline, omission of the secondary antibody and incubation of the primary antibody solution with lymphoid tissue (Moinfar, 2003). All immunoslides were evaluated independently by at least two investigators.

Initial, exploratory statistical analysis were performed using SPSS software (Version 13; SPSS).

Next a stepwise agglomeration of variables into clusters was performed. In the first step of this agglomeration process, variable that are closest to each other were joined. Subsequently, variables at greater distances from each other were joined, until all variables has been merged. The steps of the analysis are represented as a horizontal dendrogram, which should be read from left to right.

RESULTS

All 110 cases contained normal breast tissue (ducts and lobules) adjacent to or set apart from thetumors. In all cases, ERs were identified in Normal Epithelial cells (NE). (The proportion of ER-positive NE ranged from 10-80% with an average of 45%) Myoepithelial cells (ME), however, were completely negative for ER in the vast majority of cases (90%). In rare cases, a small proportion of ME nuclei (1-20%) were positive for ER. Positive reactions for PR in NE were observed in all cases (average proportion of stained nuclei, 20% range, 10-50%). In contrast, no positive reaction could be identified in ME in most cases (88%), although some cases (12%) exhibited a level (1-20%) of PR-positivity. Positive reactions for AR in NE were observed in all cases (average proportion of stained nuclei, 40% ; range, 10-70%). ME were completely negative for AR in most cases (90%). In some cases (10%), a small proportion of ME nuclei (1%) exhibited AR-positivity. All stromal cells examined were completely negative for ER, PR and AR.

The means age of the patients was 51.3±10.83. Histologic grade was 16, 66 and 24% in grades of 1, 2 and 3, respectively.

Fourty eight (43%) of 110 cases of invasive carcinoma were AR positive according to internationally standardized guidelines. No significant association was found between AR expression and histologic grade and age of the patients. According to the fisher exact test and the chi-square test all pairwise correlations within the set of variables containing ER and PR were highly significant ($p<0.05$).

Sixty five of 110 invasive carcinomas Esterogen Receptor (ER) positive which 36 (55%) of them were AR positive. in contrast, 12 (27%) of 45 ER negative were AR positive. Fifty one of 110 invasive breast carcinomas were progesterone receptor positive which 32 (63% were AR positive. in contrast, 16 (27%) of 59 PR negative were AR positive. Also 9 (39%) of 23 patients with both ER and PR negative wer AR positive.

The results showed a strong positive correlation between ER and PR expression and androgen receptor expression.

DISCUSSION

Although several previous studies have shown the biologic and therapeutic significance of ER and PR in breast carcinoma (Manni *et al*., 980; Skinner *et al*., 1980; Chevallier *et al*., 1988; Stanford *et al*., 1986; Wittliff, 1984) few, to our knowledge have dealt with the role of AR in breast carcinoma. Studies that did focus on AR in breast carcinoma examination cultures of breast carcinoma cells (Miller *et al*., 1985) or frozen material using biochemical techniques (Bryan *et al*., 1984), to our knowledge, immunohistochemical determination of AR expression in breast carcinoma rarely has been performed using formalin-fixed paraffin-embedded material (Lea *et al*., 1989). Previous studies of AR in breast carcinoma dealt primarily with a small number of invasive carcinomas or examined the presence of AR in certain subtypes of breast carcinoma, such as apocrine carcinoma (Selim *et al*., 2002).

One notable finding in the current study was yielded by the comparison of AR with ER of invasive carcinomas were ER-negative but AR-positive.

Regarding apocrine differentiation of carcinoma cells, some studies have reported a characteristic constellation of AR-positive and ER-and PR negative immunoreactions (Leal *et al*., 2001). It is well known that apocrine metaplastic epithelial cells within cystic areas of nonneoplastic breast (i.e., fibrocsystic breast changes) characteristically are positive for AR but negative for both ER and PR (Leal *et al*., 2001).

Most examination invasive lobular carcinomas were AR-positive. The small number of cases of invasive lobular carcinoma, however, does not allow any statistically meaningful conclusions to be made in the current study. It must be noted that some of the previous studies of AR in breast carcinoma were based on biochemical assay that measure androgen binding in cytosolic fractions of tumor of homogenates (Bryan *et al*., 1984). Furthermore, steroid receptor assays that tissue homogenates are not capable of distinguishing between receptor-containing malignant and nonmalignant cells.

Using a monoclonal AR antibody on frozen sections of 76 primary breast carcinomas, Isola reported positive immunostaining in 79% of all tumors (Isola, 1993). Among the breast carcinomas examined by Isola, 7 cases (9%) were negative for ER and PR but positive for AR (Isola, 1993).

Moinfar reported 60% of invasive carcinoma were AR positive (Moinfar, 2003). A significant number of invasive carcinoma are ER and PR negative but AR positive (Moinfar, 2003).

In current study, using a monoclonal antibody against AR on formalin-fixed, paraffin-embedded archival material, we observed the presence of AR in 48 of 110 (43.6%) invasive breast carcinomas.

Statistical analysis of the results of the current study showed that invasive carcinomas, AR was expressed independently of ER, PR status.

The frequent expression of AR in breast carcinoma cells, as observed in the current study, raises the important question of the interaction between androgens and human breast carcinoma (Luthy *et al*., 1988). Studies have shown that androgens may affect the growth of breast carcinoma in animals (SPSS, 1999). Tumor proliferation in human mammary carcinoma also is significantly altered by androgen. Approximately 20% of patients with metastatic breast carcinoma may experience tumor regression after treatment with androgens (AMA, 1960). In contrast, studies analyzing the effects of androgens and antiandrogns on breast carcinomas in long-term tissue cultures indicated that some human breast carcinomas, at least *in vitro*, may be stimulated by androgens (Dilley *et al*., 1983). Furthermore, the results of adjuvant treatment with aromatse inhibitors, which block the conversion of adrenal steroids (mainly androgens) into estrogens, have been reported (Brueggemeie, 2002; Woner *et al*., 2002; Assikis and Buzdar, 2002). These studies also underscore the important role of androgens (albeit in an indirect way, through estrogens) in the stimulation of human mammary carcinoma growth (Assikis and Buzdar, 2002). Thus, androgens can have either stimulatory or inhibitory effects of tumor growth. These seemingly paradoxical effects may depend on carcinoma cell type and/or may be related to the presence or absence of other steroid receptors, such as ER and PR. In addition, the heterogeneity of carcinoma cells in terms of steroid receptor positivity and the proportional distribution of each steroid receptor among carcinoma cells may influence the activity of androgens in either a proliferative or inhibitory direction.

In summary, the current study demonstrates that androgen receptors frequently are expressed in breast carcinomas.

A side from ER and PR expression, the immunohistochemical assessment of AR expression may lead to new adjuvant hormonal treatment strategies for patients with breast carcinoma.

CONCLUSION

Androgen receptors are commonly expressed in invasive breast carcinoma. A significant number of invasive carcinomas are ER-negative and PR-negative but AR-positive. Immunohistochemical examination of AR would be desirable because it would provide additional information about steroid receptors in breast carcinomas and it seems to be helpful for hormonal therapy.

REFERENCES

AMA, 1960. Committee on Research. Androgen and estrogens in the treatment of disseminated mammary carcinoma. J. Am. Med. Assoc., 172: 1271-1274.

Assikis, V.J. and A. Buzdar, 2002. Recent advances in aromatase inhibitor therapy for breast cancer. Semin Oncol., 29: 120-128.

Brueggemeie, RW., 2002. Overview of the pharmacology of the aromatase inactivator exemestane. Breast Cancer Res. Treat., 74: 177-185.

Bryan, R.M., R.J. Mercer, R.C. Bennet, G.C. Rennie, T.H. Lie and F.J. Morgan, 1984. Androgen receptors in breast in breast cancer. Cancer, 54: 2436-2440.

Brys, M., A. Semczuk, W. Baranowski, J. Jakowicki and W.M. Krajewska, 2002. Androgen Receptor (AR) expression in normal and cancerous human endometrial tissues detected by RT-PCR and immunohistochemistry. Anticancer Res., 22: 1025-1031.

Chevallier, B., F. Heintzmann and V. Mosseri *et al*., 1988. Prognostic value of estrogen and progesterone receptors in operable breast cancer. Results of a univariate and multivariate analysis. Cancer, 62: 2517-2524.

Dilley, W.G., G.S. Leight, J.S. Silva, S. Ammirate, D.E. Haagensen, S.A. Wells, 1983. Androgen stimulation of gross cystic disease fluid protein and carcinoembryonic antigenin patients with metastatic breast carcinoma. J. Natl. Cancer Inst., 70: 69-74.

Isola, J.J., 1993. Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. J. Pathol., 170: 31-35.

Lea, O.A., S. Kvinnsland and T. Thorsen, 1989. Improved measurement of andogen receptors in human breast cancer. Cancer Res., 49: 7162-7167.

Leal, C., R. Henrique and P. Monterio *et al*., 2001. Apocrine ductal carcinoma in situ of the breast: Histologic classification and expression of biologic markers. Hum. Pathol., 32: 487-493.

Luthy, I.A., D. Begin and F. Labrie, 1988. Mediation by the androgen receptor of the stimulatory and antiandrogenic actions of 17 beta-estradiol on the growth of androgen-sensitive Shionogi mammary carcinoma cells in culture. Endocrinology, 123: 1418-1424.

Manni, A., B. Arafah and O.H. Pearso, 1980. Estrogen and progesterone receptors in the predicition of response of breast cancer to endocrine therapy Cancer, 46: 2838-2841.

McGurie, W.L. and G.M. Clark, 1984. Role of progesterone receptors in breast cancer. Semin Oncol., 12: 1-16.

Miller, W.R., J. Telford, J.M. Dixon and R.A. Hawkins, 1985. Androgen receptor activity in human breast cancer and its relationship with oestrogen and progestogen receptor activity. Eur. J. Cancer Clin. Oncol., 21: 539-542.

Moinfar, F., 2003. Androgen receptors frequently are expressen in breast carcinoma. Cancer J., 98: 705-711.

Selim, A.G., G. El-Ayat and C.A. Wells, 2002. Androgen receptor expression in ductal carcinoma in situ of the breast: Relation to ostrogen and progesterone receptors. J. Clin. Pathol., 55: 14-16.

Skinner, L.G., D.M. Barnes and G.G. Ribeiro, 1980. The clinical value of multiple steroid receptor assays in breast cancer management. Cancer, 46: 2939-2945.

SPSS, 1999. Categorical Principal Component Analysis (CATPCA). Chicago. SPSS Inc.

Stanford, J.L., M. Szklo and L.A. Brinton, 1986. Estrogen receptors and breast camcer. Epidermiol. Reu., 8: 109-118. s

Tavassoli, F.A., 1998. Pathology of the breast. Stamford, CT: Appleton and Lange.

Wittliff, J.L., 1984. Steroid-hormone receptors in breast cancer. Cancer, 53: 630-643.

Woner, E.P., C. Hudis and H..J Burstein *et al*., 2002. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for woman with hormone receptor-positive breast cancer: Status report. J. Clin. Oncol., 20: 3317-3327.