J Cancer Res Clin Oncol (1996) 122: 495–498

ORIGINAL PAPER

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Aromatase in breast cancer tissue – localization and relationship with reproductive status of patients

Received: 12 June 1995 / Accepted: 19 February 1996

Abstract The precise place of local estrogen production in mammary cancer is still controversial. In this investigation localization of aromatase (the key enzyme of estrogen biosynthesis) was studied in breast cancer tissue by immunohistochemical method using polyclonal rabbit antibodies. The cytoplasmic staining was found in different cell types, but the most intensive specific staining was found in malignant cells and it was stronger ($P<0.01$) in postmenopausal patients than in patients of reproductive age.

Key words Aromatase · Breast cancer · Immunohistochemistry

Introduction

Estrogens play an important role in the development of hormone-dependent breast cancer. The key step in estrogen biosynthesis is aromatization of the A ring of an androgen precursor. This reaction is catalyzed by a microsomal enzyme from the cytochrome P-450 family called aromatase. For women of reproductive age aromatization takes place mainly in the ovaries; after menopause, peripheral tissues became the principal sites of aromatization, i.e. fat, muscle and some others (Longcope 1982; Siiteri 1982; James et al. 1987).

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Two main sources of estrogens in breast cancer tissue can be distinguished: the uptake of estrogens circulating in the blood and the local synthesis of estrogens in the tumor tissue itself (Adams and Li 1975; Miller and Forrest 1976; Varela et al. 1978). Besides the aromatization, two other reactions are involved in local estrogen production in breast tumors: the sulfatase reaction (conversion of estrone sulfate to estrone) and the 17β-hydroxysteroid dehydrogenase reaction (interconversion of estrone and estradiol) (Santen et al. 1986; Reed et al. 1991). As a result of these three reactions and the uptake of blood estrogens, the local level of estrogens in breast cancer tissue might be considerably higher than their blood concentration (van Landeghem et al. 1985; Vermeulen et al. 1986).

There is still some controversy in the literature regarding aromatization in breast cancer tissue. Practically all authors agree that local aromatization takes place in about one-half of breast cancer cases. In the majority of the other issues related to this problem, the opinions of authors often differ. Some authors demonstrate that local aromatization may be of particular significance in breast cancer pathogenesis (Miller 1991; Bolufer et al. 1992; Lu et al. 1995), others think that, despite the presence of the aromatase in breast cancer tissue, the aromatization does not play any significant role in the majority of breast cancer cases (Bradlow 1982; Siiteri 1982).

It has been shown that practically all the main types of cells that may be present in this tumor tissue (parenchymal cells, fat cells, fibroblasts) have a potential ability for aromatization (Perel et al. 1984; Simpson and Mendelson 1987), but it is not clear yet what their real relative input is into estrogen production in this tissue. Until recently the majority of investigators studied aromatization in breast cancer tissue by biochemical methods using tissue homogenates. This approach does not allow the evaluation of the distribution of aromatase within tissues. Only recently have a few groups of researchers started using the immunohistochemical method for studying this problem, but their conclusions have been controversial: some have reported that the aromatase in breast tumors is primarily present in stromal cells (Santen et al. 1994; Sasano et al. 1994) while

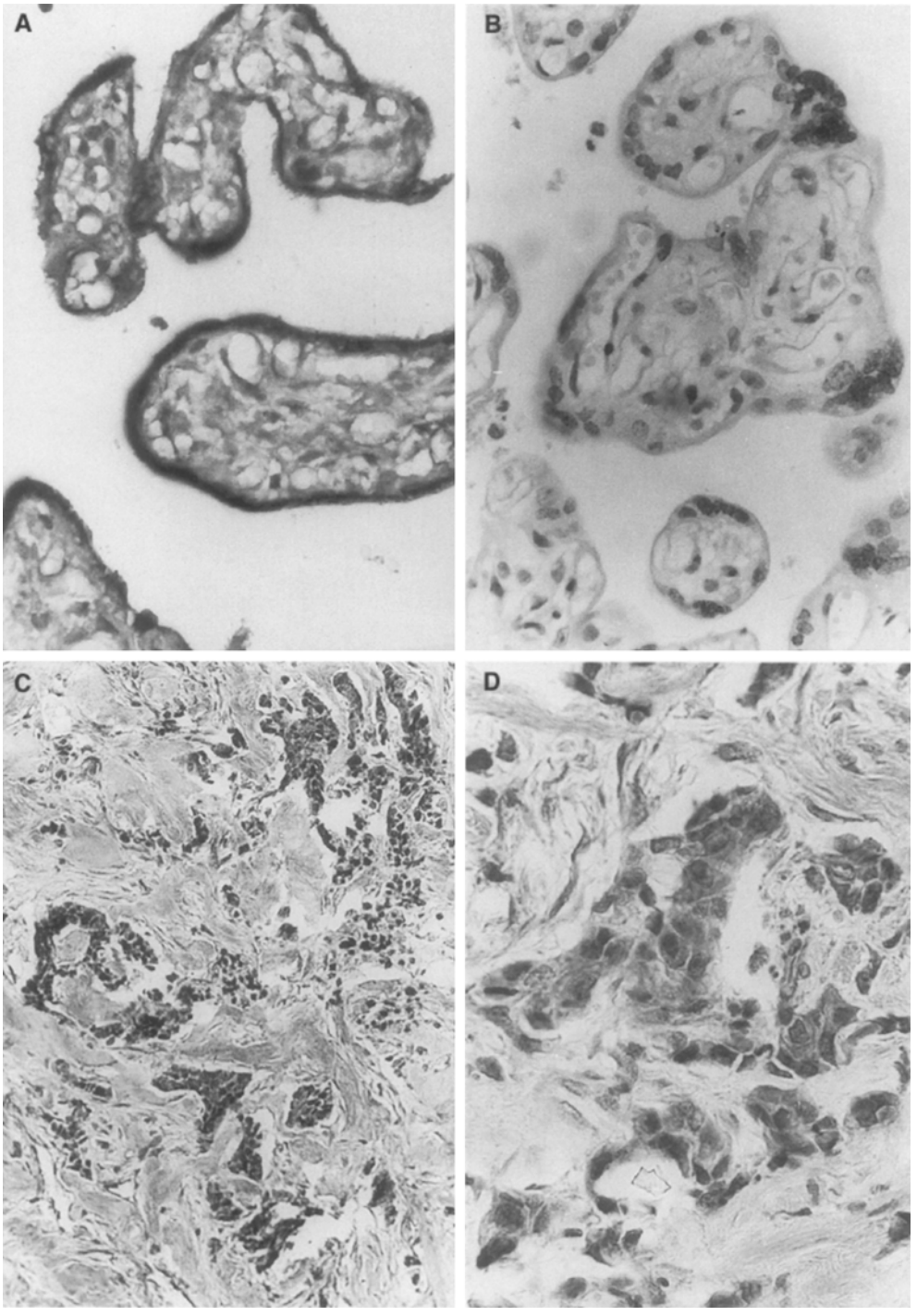

Fig. 1A–D Immunohistochemical staining with anti-aromatase antibody, counterstaining with hematoxylin. **A** (×200) Positive control section of human placenta (38 week of gestation). Specific staining in syncytiotrophoblasts. **B** (×200) Negative control section of the same placenta, primary antibody omitted. The staining in syncytiotrophoblasts is absent. **C, D** (×80, ×200) Invasive ductal breast carcinoma. The most intensive specific staining is present in malignant cells

others associated the immunoreaction mainly with cancer cells (Esteban et al. 1992). Because of these controversies we also decided to use the immunohistochemical method for studying this problem. Our preliminary data have been published in abstract form (Berstein et al. 1994).

Materials and methods

A total of 23 specimens of breast cancer tissue were studied: 10 from patients of reproductive age and 13 from postmenopausal patients. The specimens were taken from formalin-fixed, paraffin-embedded archival material 2–3 years old, and sections of 3–4 μm were mounted in poly-L-lysine-coated slides and subsequently deparaffinized and rehydrated in xylene and ethanol. Endogenous peroxidase was inhibited by 3% hydrogen peroxide solution. Specimens were treated with trypsin and were then stained by the peroxidase/antiperoxidase method using DACO corporation chemicals according to the recommendations of the company. The color was developed with diaminobenzidine and then slides were counterstained with hematoxylin.

Polyclonal rabbit anti-aromatase antibodies were kindly donated by Prof. E. Simpson (University of Texas, USA). Primary antibodies were used at a concentration of 20 μg/ml. The specific immunoreaction in placenta specimens that were stained with these antibodies was found in syncytiotrophoblasts where it is known that the aromatase is present

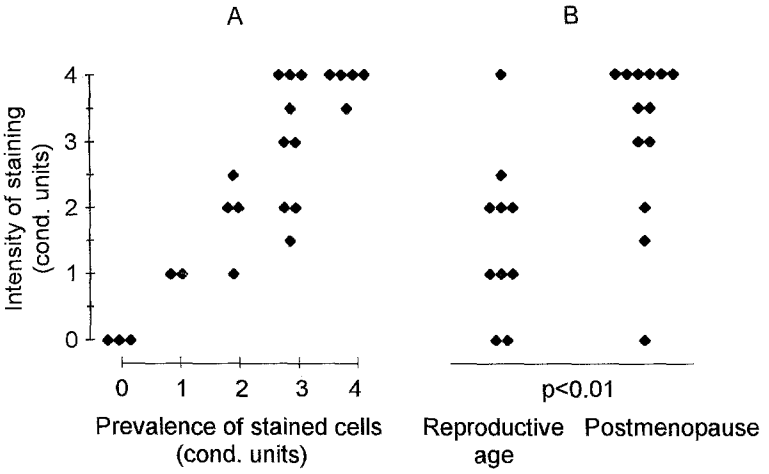

Fig. 2A,B Aromatase in malignant cells of breast cancer tissue (immunohistochemical study). Conditional units are explained in the text (see Materials and methods). **A** The allocation of the cases according to staining intensity and to quantity of stained cells. **B** Intensity of staining and reproductive status of patients (the comparisons were performed using the χ^2-test)

in significant quantity (Fig. 1A). In the control where the primary antibodies were omitted the staining in syncytiotrophoblasts was absent (Fig. 1B).

The quantity of stained cells was visually evaluated on a five-point scale: absence of stained cells, 0; isolated stained cells, 1; approximately 50% stained cells, 2; more than 50% stained cells, 3; about 100% stained cells, 4 units. The intensity of the staining was also visually semiquantified from 0 to 4+. The staining was assessed separately in different cell types (cancer cells, nonmalignant epithelial cells, fibroblasts, adipocytes, tumor-infiltrating lymphocytes and macrophages), which were identified by morphological criteria only.

The staining was compared with the age and reproductive status of the patients, TNM grade and some histological parameters of the tumors (scirrhus/solid structure, stroma/parenchyma ratio, expression of the intraductal component).

Nonparametric methods were used for statistical analysis (χ^2 and Spearman rank correlation).

Results

The most intensive specific immunoreaction was found in the cytoplasm of malignant cells (Fig. 1C,D). The staining was granular and was not present in all malignant cells in a slide. The intensity of staining also varied and the more cancer cells were stained in a slide the more intensive was their staining. The allocation of cases according to the staining intensity and to the quantity of stained cancer cells is shown in Fig. 2A. In some cases the specific immunoreaction was also found in nonmalignant epithelial cells. However, generally it was less intensive than in malignant ones.

Under the staining conditions that have been chosen, a nonspecific background staining of intercellular matrix and collagen fibers showed up on many slides. In some fibroblasts cytoplasmic staining was present but, as a rule, its intensity was no higher than background and was less than in malignant cells. Therefore, the nonspecific background staining did not allow us either to confirm or to reject the presence of the aromatase in fibroblasts. In some sections cytoplasmic staining was found in adipocytes, but fat tissue was present only in a small number of preparations.

The staining was also often found in tumor-infiltrating lymphocytes, in macrophages, and in mast and plasma cells. However, it is known that endogenous peroxidase is present in these cells and, despite being inhibited by 3% hydrogen peroxide solution, the nature of the staining of these cells did not exclude the possibility that it was nonspecific (though this question is a subject for further investigation).

While comparing the staining intensity of malignant cells with their clinical and morphological characteristics, significant associations were only found with reproductive status and age of the patients: for postmenopausal patients the staining intensity was higher than that for women of reproductive age ($P < 0.01$, Fig. 2B) and there was a weak but significant correlation between staining intensity and patients' age ($r = +0.46$, $P = 0.03$, $n = 23$). We did not find any associations between local aromatization and TNM grade or any of the histological parameters that were studied (see Materials and methods).

Discussion

As mentioned above, the aromatase has already been immunolocalised in breast cancer tissue in different cell types. Some authors (with data similar to ours) have detected aromatase mainly in malignant cells (Esteban et al. 1992). The presence of aromatase in cancer cells is confirmed by the positive correlation between the aromatization determined biochemically in tumor homogenates and tumor cellularity (Miller 1991). At the same time, others suggest that, despite the presence of the aromatase in malignant cells, the leading role in local aromatization belongs to stromal cells (Santen et al. 1994).

We suggest several possible explanations for this controversy. The conclusions of the last group of investigators were mainly based on the fact that there were more stained fibroblasts than malignant cells. At the same time they report that the intensity of cancer cells staining was not less than that in stromal cells. However, the relative role of fibroblasts may be overestimated in comparison with that of malignant cells when semiquantitative scores are based mainly on the number of cells, because the area of the stained cytoplasm is considerably less in fibroblasts than in malignant cells. In addition, there could be other reasons for

the above controversy. It may be noted that the specific staining was more expressed in stromal cells and less expressed in malignant cells when the antibodies were obtained from Dr. N. Harada et al. (Santen et al. 1994; Sasano et al. 1994) than when they were obtained from Dr. E. Simpson et al. (present paper; Lu et al. 1995) or Dr. P. Hall (Esteban et al. 1992).

The correlation between the patients' reproductive status and local aromatization in breast cancer tissue has been repeatedly studied (Varela and Dao 1978; Siiteri 1982; Bezwoda et al. 1987; Silva et al. 1989; Miller 1991; Esteban et al. 1992; Sasano et al. 1994). In the majority of studies there was no significant difference found between postmenopausal women and the women of reproductive age. However, when the authors presented the primary data, aromatization was usually insignificantly but nevertheless observably higher for postmenopausal women (Varela et al. 1978; Silva et al. 1989; Esteban et al. 1992) and there is one work in which (as with our data) this difference was significant (Bezwoda et al. 1987).

In conclusion it is necessary to re-emphasize that the literature on local aromatization in breast cancer tissue is rather controversial. In particular, it has been shown that aromatase may be found in stromal cells (Santen et al. 1994; Sasano et al. 1994). At the same time, on the basis of our results and on the results obtained by others (Esteban et al. 1992; Lu et al. 1995), we cannot exclude the possibility that malignant cells play a significant role in local aromatization. In addition to the reasons mentioned above, the autocrine estrogen secretion by malignant cells may be of particular significance in tumor growth stimulation because, in this case, estrogen molecules immediately find themselves near tumor cells and may induce a more significant effect than in the case of a paracrine mode of action.

Acknowledgements We sincerely thank Prof. E. Simpson for the generous gift of the anti-aromatase antibodies, and Mrs. I. Kovalenko and Mrs. T. Shamova for their help in preparing this paper.

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