Abstract. Background: Recently, the immunohistochemical detection of estrogen receptor α (ERα) expression in breast cancer has become a prerequisite for therapeutic decision-making, however, it remains unknown whether ERα or progesterone receptor (PgR) expression in histologically normal breast epithelium (NBE) adjacent to carcinoma can be a reliable internal positive control. Patients and Methods: Tissues from a total of 220 breast cancer patients were investigated by immunohistochemistry of ERα and PgR expression in NBE adjacent to carcinoma, as well as in carcinoma. The expression pattern was divided into three groups: singular, one or two positive cells; scattered, scattered positive cells surrounded by negative cells; contiguous, ten or more positive cells in contact with each other. Results: In NBE adjacent to carcinoma, the positivity of ERα and PgR was 99% (217 out of 220) and 89% (195 out of 220), respectively. The expression pattern of ERα and PgR was as follows: singular – 13 and 42 patients, scattered – 116 and 100 patients, and contiguous – 88 and 53 patients, respectively. The contiguous expression pattern of PgR was more frequently noted in premenopausal patients in contrast with ERα (p=0.0004). PgR expression was more frequently seen in premenopausal than postmenopausal patients (p=0.0034). PgR expression in carcinoma was more frequently seen in premenopausal than postmenopausal patients (p=0.009). There was statistically significant correlation between PgR expression in carcinoma and NBE adjacent to carcinoma (p=0.0019). Conclusion: These findings suggest that more frequent PgR expression in NBE adjacent to carcinoma might be correlated with carcinogenesis in premenopausal breast cancer patients and that ERα expression, not PgR, in NBE adjacent to carcinoma could be a reliable internal positive control.

Patients and Methods

Specimens. Two hundred and twenty female patients with primary breast cancer who had undergone mastectomy or breast-conserving surgery at Sagara Hospital (Kagoshima, Japan) between January 2002 and March 2003 were selected. Their ages ranged from 24 to 82 years (median: 53 years). All tumors were histologically diagnosed as invasive ductal carcinoma according to the WHO classification (8).

Immunohistochemistry. Specimens were fixed in 10% neutrally buffered formalin for 24 to 48 h and embedded in paraffin. A representative block from each case containing an adequate tumor and normal breast tissue was selected. The DAKO ENVISION+ kit (DakoCytomation, Glostrup, Denmark) was used in conjunction with the DAKO Autostainer (DakoCytomation) according to instructions supplied by the manufacturer. Briefly, slides were deparaffinized in xylene and rehydrated in a graded series of ethanol/water rinses, then antigen retrieval was performed by heating slides in a water-bath to 95-99°C in Target Retrieval Solution. The DAKO ENVISION+ kit was used according to the manufacturer’s instructions. The primary antibodies were applied as follows: rabbit anti-human ERα (clone 6F11) and PgR (clone 1A6) antibodies were incubated for 1 h at room temperature. After washing, the Vectastain Elite ABC kit (Vector Laboratories) was applied for 30 min. The chromogen was 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories). The sections were counterstained with methyl green.

Key Words: Estrogen receptor, immunohistochemistry, breast cancer.
Solution High pH (DakoCytomation) for 40 min. After cooling for 20 min, the sections were treated with 3% hydrogen peroxide for 5 min followed by primary antibody for 30 min at room temperature. The monoclonal mouse anti-human ERα (clone 1D5; DakoCytomation) and PgR (clone PgR636; DakoCytomation) antibodies were used at 1:50 and 1:800 dilutions, respectively. Visualization using the LSAB2 System was accomplished using a biotinylated link antibody, peroxidase-streptavidin and 3,3’-diaminobenzidine tetrachloride (1 mg/mL) containing 0.1% hydrogen peroxidase (30% w/v). Non-immune serum instead of the primary antibody was used for negative controls.

Assessment of immunohistochemistry. For carcinoma, ERα- or PgR-positivity was defined as nuclear staining in more than 10% of cancer cells regardless of staining intensity (9). For NBE, the presence of nuclear-stained cells was considered as positive regardless of the number or staining intensity. The expression pattern in NBE was categorized into three groups: singular: one or two positive cells; scattered: scattered positive cells surrounded by negative cells; contiguous: ten or more positive cells in contact with each other.

Statistical analysis. All statistical analyses were performed using the Dr. SPSS software package (Release 8.0J; SPSS Japan Inc., Tokyo, Japan). Chi-square analysis was used to calculate the significance of differences between ERα- or PgR-positive and -negative groups. The cut-off for significance was taken as \( p = 0.05 \).

**Results**

In carcinoma, the positivity of ERα and PgR was 74% (162 out of 220) and 62% (137 out of 220), respectively. PgR expression was more frequently seen in premenopausal than postmenopausal patients \( (p = 0.009) \) (Table I). In NBE adjacent to carcinoma, the positivity of ERα and PgR was 99% (217 out of 220), 89% (195 out of 220), respectively (Table II). The expression pattern of ERα and PgR was divided as follows: singular – 13 and 42 patients, scattered – 116 and 100 patients and contiguous – 88 and 53 patients), respectively (Table III). A representative staining pattern is shown in Figure 1-3. PgR expression was more frequently seen in premenopausal than postmenopausal patients \( (p = 0.0034) \) (Table II). The contiguous expression pattern of PgR was more frequently noted than ERα in premenopausal patients \( (p = 0.0004) \) (Table III). There was statistically significant association between PgR expression in carcinoma and NBE adjacent to carcinoma \( (p = 0.0019) \) (Table IV).

**Discussion**

Since the emergence of a robust monoclonal antibody to ERα and PgR, there have been many reports investigating their expression in formalin-fixed, paraffin-embedded breast cancer tissue. In contrast, reports focusing on the expression of ERα in NBE are limited (4-7, 10-15). In premenopausal women, the proportion of ERα-positive cells ranges from 4 to 20%, depending on the phase of the menstrual cycle. These cells are distributed singly and are surrounded by
ERα-negative cells (2, 4, 10, 13). With increasing age, the number of ERα-positive cells increases (10, 11). Khan et al. (12) reported that the level of ERα expression in NBE tends to be consistent over a period of several years, fulfilling one of the conditions for high ERα expression to act as a risk factor for breast cancer. Furthermore, Lawson et al. (15) reported that the proportion of ER-positive breast epithelial cells was lower in women at low risk for breast
Few reports investigated ERα and PgR expressions in NBE adjacent to invasive breast carcinoma (6) and it remains unclear whether its expression could be a reliable internal positive control. We found that almost all NBE adjacent to invasive breast carcinoma expressed ERα, but not always PgR. Very few reports focused on the distribution pattern of ERα expression in NBE but suggested that ERα-positive cells showed a statistically significant increase with age, reaching a plateau after the menopause, and the increase was associated with a tendency for positive cells to become contiguous in patches of variable size (10). In contrast, the great majority of epithelial cells in preinvasive breast lesions such as atypical hyperplasia and carcinoma in situ are ERα-positive and in contiguity (10, 11). It is therefore conceivable that an increase in ERα-positive cells in the non-neoplastic breast, particularly if in contiguity, could represent a pre-cancerous change. We also showed that a contiguous pattern of ERα expression in NBE adjacent to carcinoma was more frequently seen in postmenopausal patients, however, PgR expression in NBE adjacent to invasive breast carcinoma was more frequently seen in premenopausal than postmenopausal patients. In addition, we revealed that PgR expression in carcinoma was more frequently noted in premenopausal than postmenopausal patients. To our knowledge, there is only one report focusing on PgR expression in NBE adjacent to carcinoma, in which PgR expression was significantly more common in NBE adjacent to invasive breast carcinoma in BRCA1-linked cases compared with sporadic cases (6). Although the precise mechanism is unclear, these results suggest that the increased expression of PgR in NBE adjacent to invasive breast carcinoma may be associated with carcinogenesis in premenopausal patients.

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References


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