

Expression of Estrogen Receptor α and Progesterone Receptor in Normal Human Breast Epithelium

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Abstract. *Background:* Recently, the immunohistochemical detection of estrogen receptor α (ER α) expression in breast cancer has become a prerequisite for therapeutic decision-making, however, it remains unknown whether ER α 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 ER α 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 ER α and PgR was 99% (217 out of 220) and 89% (195 out of 220), respectively. The expression pattern of ER α 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 ER α ($p=0.0004$). 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 ER α

expression, not PgR, in NBE adjacent to carcinoma could be a reliable internal positive control.

Estrogen is thought to be important in the pathogenesis of breast cancer and is associated with most of the epidemiological risk factors of breast cancer (1). Estrogen is also associated with epithelial proliferation in the non-cancerous breast during the menstrual cycle (2) and in pregnancy (3), and acts on cells via estrogen receptor α (ER α). In histologically normal breast epithelium (NBE), cells expressing ER α account for 4-15% of the epithelial cell population (2, 4, 5) and are present as single, scattered cells surrounded by cells without ER α expression. However, there are few reports investigating the frequency and expression pattern of ER α and progesterone receptor (PgR) in NBE adjacent to invasive breast carcinoma (6, 7). This was investigated in addition to the reliability of ER α or PgR expression in NBE adjacent to invasive breast carcinoma as an internal positive control for the evaluation of hormone receptor expression in breast carcinoma.

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

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Table I. Menopausal status and expression of ER α and PgR in carcinoma.

	ER α expression (n)			PgR expression (n)		
	positive	negative	p-value	positive	negative	p-value
Premenopausal	77	23	0.268	72	28	0.009
Postmenopausal	85	35		65	55	

n: number of patients.

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 ER α (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, ER α - 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 ER α - or PgR-positive and -negative groups. The cut-off for significance was taken as $p=0.05$.

Results

In carcinoma, the positivity of ER α 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 ER α and PgR was 99% (217 out of 220), 89% (195 out of 220), respectively (Table II). The expression pattern of ER α 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 ER α in

Table II. Menopausal status and expression of ER α and PgR in normal breast epithelium.

	ER α expression (n)			PgR expression (n)		
	positive	negative	p-value	positive	negative	p-value
Premenopausal	98	2	0.873	96	4	0.0034
Postmenopausal	119	1		99	21	

n: number of patients.

Table III. Menopausal stastus and expression pattern of ER α and PgR.

Expression pattern	ER α (n)			PgR (n)		
	singular	scattered	contiguous	singular	scattered	contiguous
Premenopausal	9	45	31	15	47	35
Postmenopausal	4	71	57	27	53	18

n: number of patients.

Table IV. ER α and PgR expression in carcinoma and normal breast epithelium.

Carcinoma	ER α expression (n)			PgR expression (n)		
	normal breast epithelium			normal breast epithelium		
	positive	negative	p-value	positive	negative	p-value
positive	159	3	0.701	129	8	0.0019
negative	58	0		66	17	

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 ER α 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 ER α in NBE are limited (4-7, 10-15). In premenopausal women, the proportion of ER α -positive cells ranges from 4 to 20%, depending on the phase of the menstrual cycle. These cells are distributed singly and are surrounded by

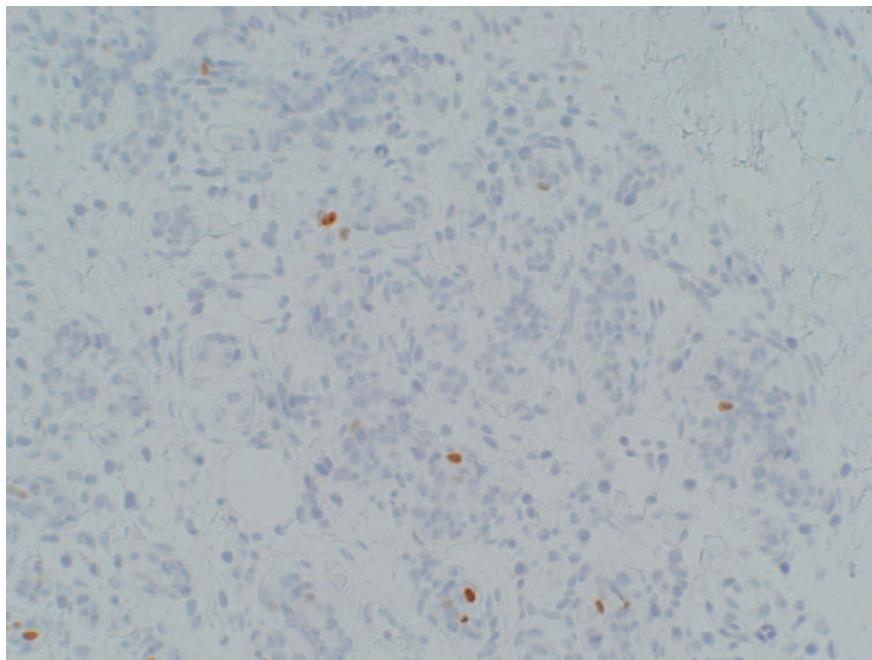


Figure 1. Singular expression pattern of PgR in normal breast epithelium adjacent to invasive breast carcinoma showing one or two positive cells (immunohistochemical staining, original magnification x 100).

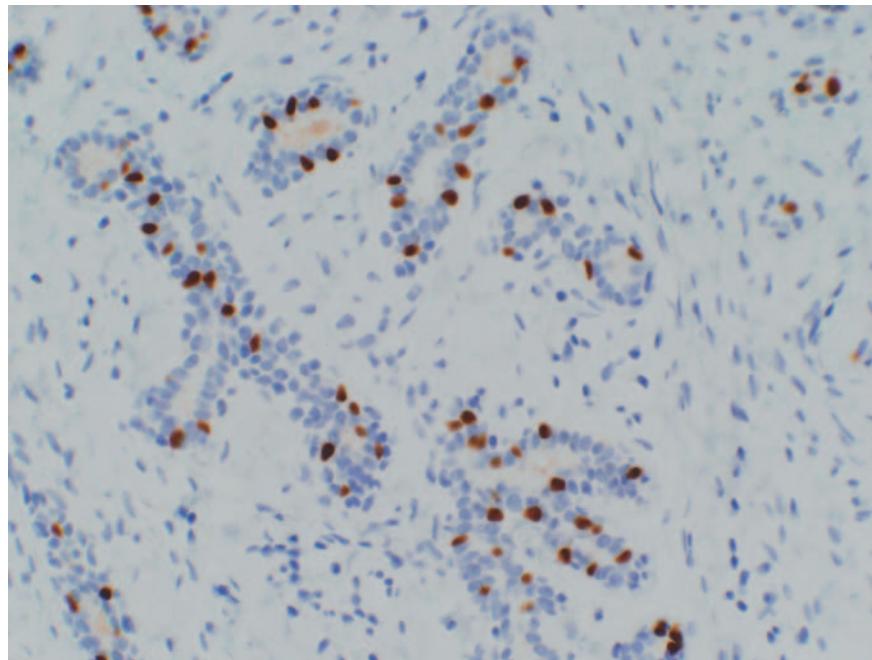


Figure 2. Scattered expression pattern of PgR in normal breast epithelium adjacent to invasive breast carcinoma showing scattered positive cells surrounded by negative cells (immunohistochemical staining, original magnification x 200).

ER α -negative cells (2, 4, 10, 13). With increasing age, the number of ER α -positive cells increases (10, 11). Khan *et al.* (12) reported that the level of ER α expression in NBE tends to be consistent over a period of several years,

fulfilling one of the conditions for high ER α 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



Figure 3. Contiguous expression pattern of PgR in normal breast epithelium adjacent to invasive breast carcinoma showing ten or more positive cells in contact with each other (immunohistochemical staining, original magnification x 200).

cancer. Few reports investigated ER α 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 ER α , but not always PgR. Very few reports focused on the distribution pattern of ER α expression in NBE but suggested that ER α -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 ER α -positive and in contiguity (10, 11). It is therefore conceivable that an increase in ER α -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 ER α expression in NBE adjacent to carcinoma was more frequently seen in postmenopausal than premenopausal 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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