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Expression of the Prolactin Receptor in Japanese Breast Cancer

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Prolactin（PRL）is associated with lactation, and also plays an important role in the development and progression of breast cancer（BC）. PRL exerts its effects via its receptor, the PRL-receptor（PRLR）, so it is beneficial to study the distribution and alteration in expression of PRLR in order to elucidate the biologic effects of PRL on BC. In 84 BCs, PRLR expression was examined by an immunohistochemical technique. PRLR immunoreactivity was detected mainly in the cytoplasm. And the overall positive rate（cut-off>10%）was 71%（60/84）, while invasive BC showed a significantly higher positive rate（75%, 57/76）than non-invasive BC（38%, 3/8）（p=0.0264）. The PRLR expression showed no correlation with age, primary tumor, nodal involvement, distant metastasis, or clinical stage; however, it was significantly correlated with estrogen receptor（ER）expression（p=0.0361）, especially in invasive BC（p=0.0098）, but not with the progesterone receptor（PgR）expression or HER-2 expression. PRLR was highly expressed in IDC（invasive ductal carcinoma）and correlated with ER expression, suggesting that PRL and PRLR may play important roles in the development and progression of ER（+）BC.

Key words: Breast cancer, prolactin, prolactin receptor

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INTRODUCTION

Prolactin（PRL）is a peptide hormone associated with lactation. PRL is synthesized and secreted by lactotropic cells in the anterior pituitary gland, and it is also produced in other tissues, such as the breast, decidua, central nervous system and immune system. PRL plays an important role in the proliferation and differentiation of the breast epithelium. Recent studies have suggested that PRL may also play an important role in the development and progression of breast cancer（BC）[1] PRL exerts its effects via its receptor, the PRL-receptor（PRLR）, so in order to elucidate the biologic effects of PRL in various tissues, it is beneficial to study the distribution and alteration in expression of PRLR. PRLR is a transmembrane receptor and belongs to the cytokine receptor superfamily [2]. Its locus is encoded by a gene, which is located on chromosome 5p13-14. PRLR consists of 3 domains: extracellular, transmembrane and cytoplasmic domains. When PRLR binds to PRL, it further dimmerizes with another PRLR, resulting in activation of a tyrosine kinase, JAK2, and a transcription factor, STAT5 [3].

PRLR is expressed in BC cells and PRL stimu-

lates the proliferation of BC cells [4,5]. A recent epidemiologic study indicated that serum PRL was modestly associated with an increased BC risk, particularly estrogen receptor (ER) positive (+) BC, over a wide range of ages [6]. Furthermore, recent in vitro studies indicate that estrogen up-regulates the gene expression of PRLR and stimulates breast tumor growth in BC cell lines [7,8]. Interestingly, it has been reported that a brief course of TAM treatment for 7 days reduced expression of PRLR mRNA in patients with ER (+) BC [9]. Therefore, PRL and PRLR are strongly associated with the generation and progression of BC.

As for the expression of PRLR in human BC, it is reported that PRLR mRNA can be detected in 80-100% of human BC [8,10], while PRLR expression can be detected at the protein level in only 13-100% of human BC: 13-72% expression by binding assay [11-16], and 70 - 94% expression by a recent immunohistochemical (IHC) technique [17-19]. On the other hand, the clinicopathological significance of PRLR expression is not fully elucidated in human BC. Furthermore, the expression of PRLR was examined only in one study using a binding assay, as far as we know [15].

The present study examined the clinicopathological significance of PRLR expression in Japanese BC using an IHC technique.

PATIENTS AND METHODS

Patients

We obtained informed consent regarding the genetic and histopathological background from the patients or their families.

Eighty-four female patients (34-85 years old; mean 58.3 years) with BC, who underwent mastectomy or breast-conserving surgeries between 1986 and 2005 at the Department of Surgery, Shimane Medical University and were followed up for more than 5 years after surgery, were randomly selected for the study. They included 76 invasive BCs and 8 non-invasive BCs. The patients' profiles are summarized in Table 1. The tumors were staged according to the UICC classification (TNM classification). Histologically, all specimens were verified to be BCs. None of the patients received any type of treatment prior to their surgical procedures. All patients were followed-up in our department, and the survival of the patients was surveyed on January 1, 2010. The postoperative survival was defined as the time that elapsed from the surgery to a cancer-related death.

Table 1. Patients' background

Age: 58.3 ± 12.4 (34 - 85)	
Surgery: breast-conserving surgery	77 (91.7%)
mastectomy	7 (8.3%)
Histology: invasive ductal carcinoma	65 (77.4%)
invasive lobular carcinoma	7 (8.3%)
other type of invasive carcinoma	4 (4.8%)
carcinoma in situ	8 (9.5%)
pT: 0 (carcinoma in situ)	8 (9.5%)
1 (~2.0cm in size)	35 (41.7%)
2 (2.1-5.0 cm in size)	26 (31.0%)
3 (>5.0cm in size)	10 (11.9%)
4 (invasion to skin or chest wall)	5 (5.9%)
pN: 0 (no nodal involvement)	54 (64.3%)
1 (1-3 positive nodes)	18 (21.4%)
2 (4-9 positive nodes)	3 (3.6%)
3 (10 or more positive nodes)	9 (10.7%)
M: 0 (no distant metastasis)	81 (96.4%)
1 (positive distant metastasis)	3 (3.6%)
Stage: 0	8 (9.5%)
1	24 (28.6)
2	36 (42.9%)
3	13 (15.5%)
4	3 (3.6%)

Antibodies

The anti-PRLR monoclonal antibody (mAb) (Prolactin Receptor Ab-1, clone B6.2, cat.# MS-1338) was purchased from LAB VISION, Fremont, CA, USA. The anti-ER mAb (1D5, code No. M7047) and anti-progesterone receptor (PgR) mAb (PgR636, code No. M3569) were purchased from Dako Corp., Carpinteria, CA, USA.

Immunohistochemistry

The specimens were immunostained primarily according to the labeled polymer method using a DAKO EnVision+™, Peroxidase Rabbit kit (DAKO Corp.), which is a goat anti-rabbit immunoglobulin conjugated to a peroxidase labeled-dextran polymer. Formalin-fixed, paraffin-embedded specimens were cut into 4-μm sections. The sections were deparaffinized in xylene for 5 min. 3 times, hydrated in 100%, 95% ethanol and finally in phosphate-buffered saline (PBS). Next, the sections were treated

with pepsin (DAKO) at 1 mg/ml Tris-HCl, pH 2.0 at 37°C for 10 min. for antigen retrieval. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 5 min. Then, they were incubated with a primary Ab for 30 min. at room temperature. For this series, the anti-PRLR mAb was diluted at 1 : 200, the anti-ER mAb (Code No. M7047) was diluted at 1 : 50 , and anti-PgR mAb (Code No. M3569) was diluted at 1 : 800. After incubation with mAb, we used a Labeled Polymer (Peroxidase labeled polymer conjugated to goat anti-mouse immunogloblins (K4000, Dako Cytomation) to visualize the antigen. Finally, the specimens were treated with a 0.05% 3,3'-diaminobenzidine solution for 5 min. at room temperature. After washing in distilled water, the specimens were counter-stained with hematoxylin.

Evaluation of IHC

The immunostaining was considered positive for PRLR only when strong cytoplasmic immunoreactivity in tumor cells was diffusely observed. On the other hand, ER and PgR expression was considered positive when strong nuclear immunoreactivity in tumor cells was observed. Those cases with only faint immunostaining were regarded as negative.

In the present study, a cut-off point of 10% or more positive cells was used for evaluation of PRLR, ER and PgR expression.

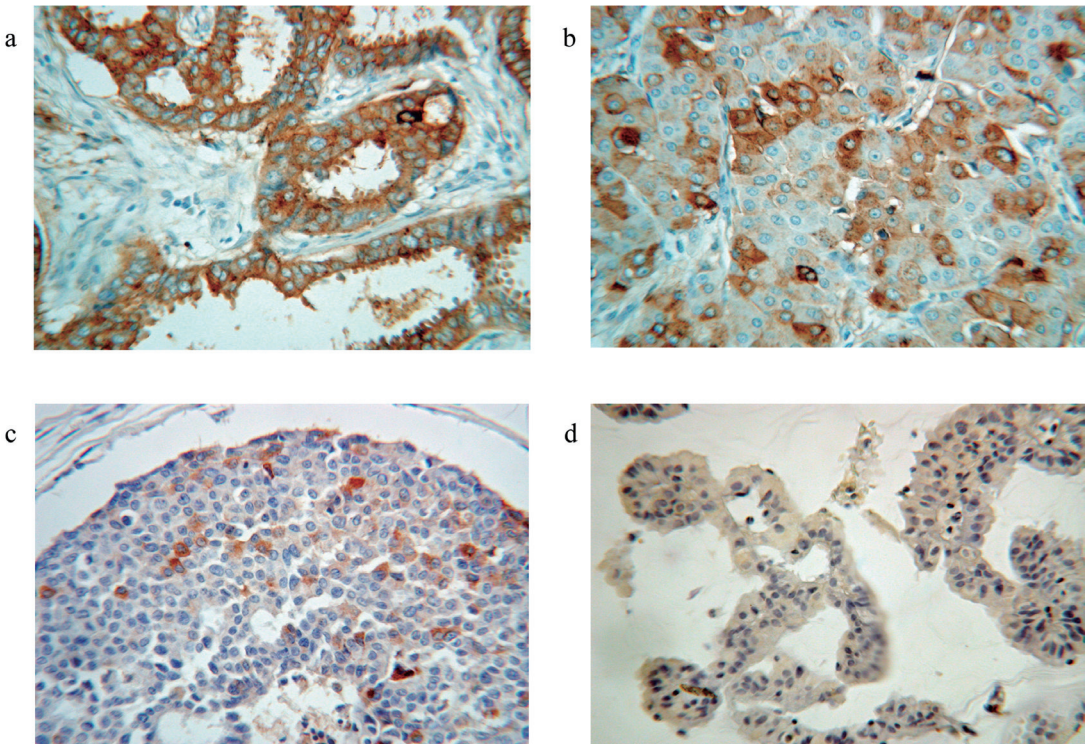
Statistical analysis

The correlations among PRLR, ER, and PgR expression, and clinicopathological factors were examined using Pearson's correlation coefficient and Mann-Whitney's U-test. All analyses were performed using StatView software (SAS Institute Inc., Cary, NC, USA), and a p-value less than 0.05 was considered statistically significant.

RESULTS

PRLR immunoreactivity was detected mainly in the cytoplasm (Figs. 1a-1d). When over 10% positive expression of tumor cells was set as a cut-off

Fig. 1. Immunohistochemical detection of PRLR in human breast carcinomas



a, Invasive ductal carcinoma, showing strongly positive cytoplasmic staining for PRLR in more than 90% of tumor cells.
b, Invasive ductal carcinoma, showing various intensities of positive cytoplasmic staining for PRLR in about 40% of tumor cells.
c, Non-invasive ductal carcinoma, showing positive cytoplasmic staining for PRLR in about 15% of tumor cells.
d, Mucinous carcinoma, showing negative staining for PRLR.

point, the overall positive rate was 71% (60/84), and PRLR expression was significantly higher in invasive BCs (75%, 57/76) than in non-invasive BCs (37.5%, 3/8) (p=0.0264) (Table 2).

Table 2. Expression of PRLR

	PRLR expression		
	(+)	(-)	Total
Invasive BC	57 (75.0%)	19 (25.0%)	76
Non-invasive BC	3 (37.5%)	5 (62.5%)	8
Total	60 (71.4%)	24 (28.6%)	84

BC, breast cancer
Invasive BC vs. non-invasive BC, p=0.0264 by Mann-Whitney's U-test

The correlation between the PRLR expression and clinicopathological factors for all cases is summarized in Table 3, The expression tended to correlate with the primary tumor (p=0.0619) and clinical stage (p=0.0865), but it was not correlated with other clinicopathological factors such as the patient's age, nodal involvement, distant metastasis, or clinical stage.

Table 4 summarizes the correlation between PRLR expression and ER or PgR expression. PRLR expression was significantly more highly expressed in ER (+) BCs than in ER (-) BCs (p=0.0361), but it was not correlated with the PgR expression. The association of PRLR expression with ER expression was more significant in invasive BCs (p=0.0098) (Table 4).

The effect of PRLR expression on the patient's survival was not statistically analyzed because only 3 patients died of BC recurrence.

DISCUSSION

The present study is the second report on the expression of PRLR in Japanese BC, and the first one using the IHC technique. Here, we demonstrated that [1] PRLR was highly expressed in BC, especially in invasive BCs; [2] PRLR expression was not statistically correlated with clinicopathological factors, including patient's age, stage, primary tumor, nodal involvement, and distant metastasis; and

Table 3. Correlation between PRLR expression and clinicopathological factors

	Correlation coefficient			
	Overall (n=84)		IDC (n=76)	
Variables	r-value (95% confidence)	p-value	r-value (95% confidence)	p-value
Age	-0.033 (-0.246 - 0.183)	0.7674	-0.045 (-0.268 - 0.182)	0.7005
pT	0.205 (-0.010 - 0.401)	0.0619	0.143 (-0.085 - 0.357	0.2188
pN	0.015 (-0.200 - 0.229)	0.8896	-0.038 (-0.261 - 0.189)	0.7467
M	0.122 (-0.095 - 0.328)	0.2709	0.117 (-0.111 - 0.334)	0.3151
stage	0.188 (-0.027 - 0.387)	0.0865	0.058 (-0.170 - 0.279)	0.6220

Table 4. Co-expression of PRLR and ER or PgR

		Total	ER+	ER-	p-value*	PgR+	PgR-	p-value*
I. Overall	PRLR+	60	52 (61.9%)	8 (9.5%)	0.0361	43 (51.2%)	17 (20.2%)	0.4145
	PRLR-	24	16 (19.0%)	8 (9.5%)		15 (17.9%)	9 (10.7%)	
	Total	84	68 (80.9%)	16 (19%)		58 (69.1%)	25 (30.9%)	
II. IDC	PRLR+	57	49 (64.5%)	8 (10.5%)	0.0098	40 (52.6%)	17 (22.4%)	0.1655
	PRLR-	19	11 (14.5%)	8 (10.5%)		10 (13.2%)	9 (11.8%)	
	Total	76	60 (79.0%)	16 (21.0%)		50 (65.8%)	26 (34.2%)	

*, by Mann-Whiteney's U-test

[3] it was significantly correlated with ER expression, but not with PgR expression.

The overall expression rate of PRLR was 71%, and it was compatible with the 69-100% expression rates in the results of previous reports using the IHC technique. The IHC study was more sensitive than the binding assay, and the expression rates of PRLR have been reported to be between 13 and 72% [11-16]. This study used the B6.2 clone as an anti-PRLR mAb for IHC, and the three studies using this clone reported that the expression rates were 100%, 69% and 73%, respectively [17,19,20]. On the other hand, studies using the clones U5 and U6 reported 93% for U5, and 97%, 87% for U6, respectively [10,18,21].

In terms of the relationship of PRLR expression to clinicopathological factors, there were no significant correlations with age, stage, primary tumor, nodal involvement or distant metastasis, although the expression rate was significantly higher in invasive BCs than in non-invasive BCs (p=0.0264) (Table 2). Other studies using the IHC technique also reported no correlation between PRLR expression and these clinicopathological factors [16,19], although one study using a binding assay reported that PRLR was higher in stage 3 BCs than in stage 1 and 2 ones [15].

PRLR expression was significantly related to that of ER, but there are controversial reports using the IHC technique or binding assay on the correlation between PRLR expression and ER expression: some studies reported a significant correlation between them [17,21], but others showed no correlations [16,18]. In one study, PRLR expression was strong in ER+ BC, but showed no significant correlation [19].

On the other hand, studies using mRNA analyses reported that in ER (-) and PgR (-) BCs, PRLR transcripts were expressed at low levels [10], and one study also reported that in Northern blot analysis, the PRLR expression in BC cell lines was linearly related to that of ER and PgR, but not to that of androgen receptors [8].

Interestingly, it was reported that tamoxifen (TAM) reduced expression of mRNA encoding the PRLR in a subgroup of breast tumors [9], although it was also reported that no interaction between PRLR and adjuvant TAM treatment was found [16]. As discussed above, recent studies using mRNA analyses and the IHC technique seem to indicate a mutual implication of PRLR and ER expression, although this issue is still controversial.

Regarding the influence of PRLR expression on the prognosis of patients with BC, it was not analyzed due to the low number of BC-related deaths in this series. It was reported that patients with PRLR (+) BC had a significantly worse survival than a PRLR (-) group [15]; however, no evidence was found for an independent prognostic role of PRLR on disease-free survival using the Cox model [16].

CONCLUSION

PRLR was highly expressed in IDC of the breast and correlated with ER expression, suggesting that PRL and PRLR may play important roles in the development and progression of ER (+) BC.

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