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High Positive Rate of pS2 Expression in Forefront Intraductal Cancerous Area in Breast Cancer

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Multiple sections of 40 consecutive cases with invasive ductal carcinoma of the breast, all of which had wide intraductal cancerous extension, were examined by immunohistochemical analysis for evaluation of hormone dependency in several areas of breast cancer tissues. In this study, we examined the expression of pS2 protein in the central invasive area (CIV), central intraductal cancerous area (CDC) and forefront intraductal cancerous area (FDC). pS2 staining was positive in 52.5% (21/40) of CIV and a significant correlation was found between pS2 expression in CIV and the estrogen receptor status (ER). pS2 staining was positive in 77.5% of CDC and 85.0% of FDC, respectively. A majority (68.4%) of the cases that were negative pS2 in CIV were positive for pS2 in FDC. Moreover, the cases with noncomedo intraductal carcinoma in premenopausal status showed a higher positivity of pS2 expression in FDC than the cases with comedo-carcinoma, though the number of cases of comedo-carcinoma was limited. These findings suggest that endocrine therapy may be useful after breast conserving treatment regardless of the ER status of the primary tumor.

Key words: Breast cancer — pS2 protein — Hormone dependency — Estrogen receptor — Breast conserving treatment

Breast conserving treatment (breast conserving surgery plus irradiation, BCT) has become a major treatment option for patients with stage I or II breast cancer.[1)] However, overall, the local recurrence rate in the ipsilateral breast following lumpectomy and irradiation is approximately 5% to 10% at 5 years and 10% to 16% at 10 years.[2,3)] It is widely accepted that an extensive intraductal cancerous component (EIC) is a major factor associated with local recurrence in the ipsilateral breast after lumpectomy.[4,5)] Previously, we demonstrated that most of the residual cancerous tissue after lumpectomy was an intraductal component.[6)] We also demonstrated that the forefront intraductal cancerous area was less aggressive than the primary invasive area.[7)] On the other hand, Inaji *et al.* reported that the hormone dependence of intraductal lesions was equal to or higher than that of invasive lesions.[8)] That is, the residual cancerous tissue after lumpectomy may have a less aggressive nature. pS2 protein is specifically regulated by estrogens in estrogen-responsive breast cancer cells,[9)] and it has been considered to be a marker of hormone dependence in breast cancer.[10)] Therefore, in this study, we examined the hormone dependence of the central invasive area (CIV), central intraductal cancerous area (CDC) and forefront intraductal cancerous area (FDC) of breast cancer cases with wide intraductal cancerous extension by performing immunohistochemical analysis of routine formalin-fixed sections.

In this study, we used an anti-pS2 polyclonal antibody, which can be detected in paraffin sections, as an antibody related to hormone dependence.

MATERIALS AND METHODS

In this study, we analyzed multiple sections of surgical materials from 40 patients, who had invasive ductal carcinoma with wide intraductal cancerous extension (accompanied with intraductal cancerous extension located more than 2.5 cm from the palpated tumor margin). The characteristics of these 40 patients are shown in Table I.

Breast tissue samples were fixed in 10% formalin, and multiple sections were prepared for hematoxylin-eosin analysis and immunohistochemical analysis. Paraffin-embedded tissue blocks were sectioned at a thickness of 4–5 μm. For the purpose of this study, one central invasive cancerous block and one forefront intraductal cancerous block from each patient were selected after reviewing hematoxylin-eosin stained sections. The histologic subtypes were diagnosed according to The General Rules for Clinical and Pathological Recording of Breast Cancer, established by the Japanese Breast Cancer Soci-

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ety.[11] Intraductal lesions were classified as "comedo" or "non-comedo" subsets.

The hormone dependency of the tumor was examined by the staining with anti-pS2 polyclonal antibody. The hormone dependencies in CIV, CDC and FDC were compared with each other. We defined CIV as the extraductal cancerous area in the center portion of the tumor, CDC as the intraductal cancerous area in the center portion of the tumor, and FDC as the intraductal cancerous area lying 2.5 cm or more from the palpated massive tumor.

Immunohistochemical analysis was performed by the previously described methods.[7] That is, sections were deparaffinized, endogenous peroxidase was blocked with 3% hydrogen peroxidase for 5 min and the sections were incubated with a polyclonal anti-pS2 antibody (diluted 1: 200, NCL-pS2, Novocastra, UK), as an antibody related to hormone dependence, overnight at 4°C in a moist chamber. The sections were incubated with avidin-biotin complex (LSAB) for 10 min at room temperature. To visualize the immunoreactivity, we used diaminobenzidine/H_2O_2 as the substrate. Regarding the evaluation of stained specimens, when more than 10% of the tumor cells were stained, the tumor was considered to be positive for pS2 protein expression. Estrogen receptor analysis was performed by an EIA method, with a cut-off of 10 fmol/mg protein.

Statistical analysis was performed by use of the chi-square test and Fisher's exact probability test.

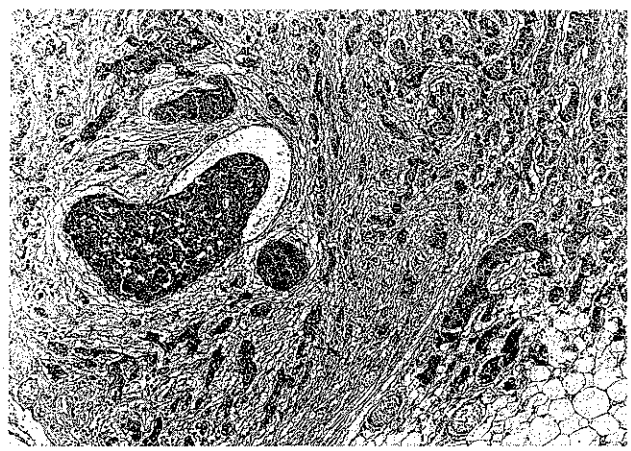

Fig. 1. Invasive ductal carcinoma, scirrhous type. Strong staining of some cancer cells and faint staining of the others with pS2. ABC immunoperoxidase stain. ×25.

RESULTS

pS2 expression in central invasive area, central intraductal area and forefront intraductal area pS2 expression was detected in the cytoplasm of breast cancer cells (Figs. 1–4). In most cases, heterogeneous staining was observed between tumor cells. In this study, we selected at least four clearly stained areas and evaluated the pS2 status as positive or negative. In CIV, CDC and FDC, pS2 staining was positive in 52.5% (21/40), 77.5% (31/40) and 85.0% (34/40), respectively.

Characteristics of patients and pS2 expression in central invasive area Table I shows the characteristics of patients and pS2 expression in CIV. A significant correlation was found between pS2 expression in CIV and the estrogen receptor status ($P<0.05$). There was no significant correlation between pS2 expression and menopausal

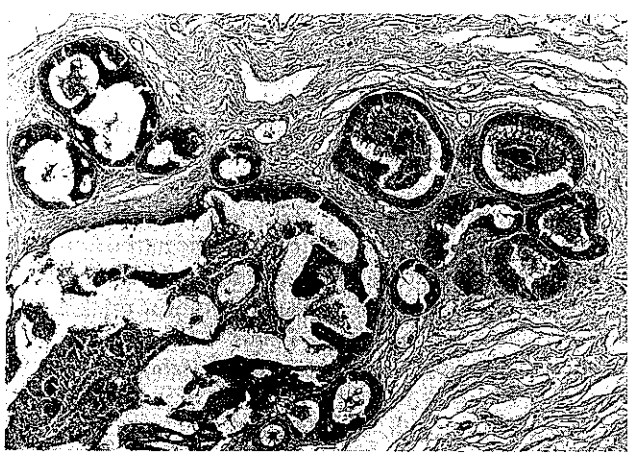

Fig. 2. Forefront intraductal cancerous area. Same staining pattern of the extensive intraductal cancerous component with pS2 as seen in Fig. 1. ABC immunoperoxidase stain. ×25.

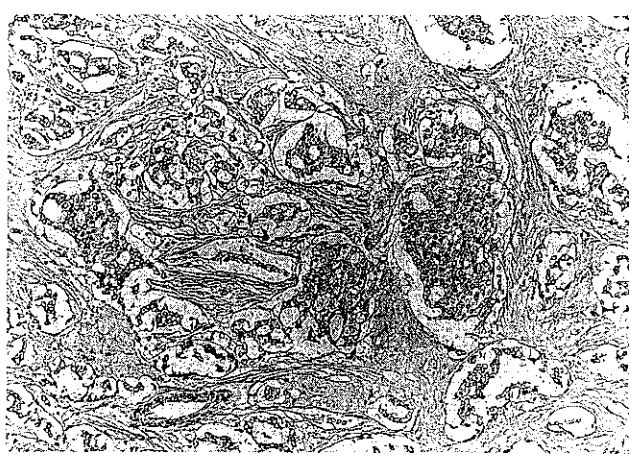

Fig. 3. Invasive ductal carcinoma, scirrhous type. Negative staining of cancer cells with pS2. ABC immunoperoxidase stain. ×25.

status, tumor size on palpation, lymph node status or histological subtypes.

Correlation of pS2 expression in central invasive area, central intraductal area and forefront intraductal area Table II shows the relationship of pS2 expression in the three areas (CIV, CDC and FDC) in the same cases. Among 21 cases positive for pS2 in CIV, all were positive for pS2 in both the CDC and FDC areas. Among 19 cases negative for pS2 in CIV, ten (52.6%) were positive in CDC, and 13 (68.4%) were positive in FDC. Namely, when pS2 expression was positive in CIV, both CDC and FDC were always positive for pS2 expression. On the other hand, when pS2 expression was negative in CIV, a majority of the cases were positive for pS2 in CDC and FDC. A significant difference was found between pS2 expression in the three areas ($P<0.05$).

pS2 expression between comedo-type and noncomedo-type Six cases (15.0%) had comedo-type in the intra-

Fig. 4. Forefront intraductal cancerous area. Strong staining of the extensive intraductal cancerous component with pS2. ABC immunoperoxidase stain. ×50. Fig. 3 and Fig. 4 show pS2 expression (CIV, negative; FDC, positive) from the same case.

Table II. pS2 Expression in CIV, CDC and FDC

	CIV pS2 (+), 21 cases		CIV pS2 (−), 19 cases	
	CDC		CDC	
	pS2(+)	pS2(−)	pS2(+)	pS2(−)
FDC pS2(+)	21	0	9	4
FDC pS2(−)	0	0	1	5

CIV vs. CDC: $P<0.05$.
CIV vs. FDC: $P<0.05$.
CDC vs. FDC: NS.
CIV vs. CDC vs. FDC: $P<0.05$.
CIV 52.5%(21/40), CDC 77.5%(31/40), FDC 85.0%(34/40).
CIV, central invasive area; CDC, central intraductal area; FDC, forefront intraductal area.

Table I. The Characteristics of Patients and pS2 Expression in CIV

	Total	pS2 (+), n=21	pS2 (−), n=19	
Menopausal state				
pre	24	13	11	NS[a]
post	16	8	8	
Tumor size[b]				
T1	24	14	10	NS
T2	14	6	8	
T3	2	1	1	
Nodal status				
0	24	12	12	NS
1–3[c]	12	7	5	
3<	4	2	2	
Histologic subtypes				
Papillo-tubular	16	7	9	NS
Solid-tubular	7	5	2	
Scirrhous	17	9	8	
ER status[d]				
ER(+)	16	13	3	$P<0.05$
ER(−)	14	4	10	
unknown	10	4	6	

CIV: central invasive area.
a) Not significant. *b*) Tumor size on palpation. *c*) Number of involved nodes. *d*) Ten fmol/mg cut-off (EIA).

ductal area, and 34 cases (85.0%) had noncomedo-type. In CIV, two (33.3%) of six cases with comedo-type were positive for pS2, while 19 (55.9%) of 34 cases with noncomedo-type were positive for pS2 expression. Three (50%) of six cases with comedo-type were pS2-positive in both CDC and FDC, while 27 (79.4%) of 34 cases with noncomedo-type in CDC and 29 (85.3%) of 34 cases with noncomedo-type in FDC were positive. However, there was no difference between the comedo-type and noncomedo-type in positivity for pS2 expression in the three areas (Table III). No difference in pS2 staining results was found among noncomedo histological subtypes.

Table III. pS2 Protein in Comedo-type and Noncomedo-type Carcinomas

		Comedo (n=6)	Noncomedo (n=34)	
CIV	pS2(+)	2	19	NS[a]
	pS2(−)	4	15	
CDC	pS2(+)	3	27	NS
	pS2(−)	3	7	
FDC	pS2(+)	3	29	NS
	pS2(−)	3	5	

CIV, central invasive area; CDC, central intraductal area; FDC, forefront intraductal area.
a) Not significant.

Table IV. pS2 Expression and Menopausal Status

		Premenopausal (n=24)	Postmenopausal (n=16)	
CIV	pS2(+)	13	8	NS[a]
	pS2(−)	11	8	
CDC	pS2(+)	19	11	NS
	pS2(−)	5	5	
FDC	pS2(+)	22	10	NS
	pS2(−)	2	6	

CIV, central invasive area; CDC, central intraductal area; FDC, forefront intraductal area.
a) Not significant.

pS2 expression in menopausal status We analyzed pS2 expression separately from menopausal status. There was no difference between premenopausal and postmenopausal patients in positivity for pS2 expression in CIV, CDC, and FDC (Table IV). Regarding comedo-type or noncomedo-type, in premenopausal patients, two (50%) of four cases with comedo-type were pS2-positive in both CDC and FDC, while 16 (80.0%) of 20 cases with noncomedo-type in CDC and all cases with non-comedo-type in FDC were pS2-positive. Among premenopausal patients, a significant correlation was found for pS2 expression in FDC between the comedo-type and non-comedo-type ($P<0.05$) (Table V).

DISCUSSION

Intraductal lesion of breast cancer is weakly agressive,[7] and highly hormone-dependent.[8] In this study, we examined the hormone dependence of CIV, CDC, and FDC areas in the same cases. We selected 40 cases with wide intraductal cancerous extension which was ascertained by analysis of multiple sections of surgical mastectomy materials. In this study, the definition of wide intraductal cancerous extension was the presence of intraductal cancerous extension more than 2.5 cm from the tumor palpated margin, because the tumor is resected with a normal breast tissue margin of 2 cm around the tumor in lumpectomy.

In regard to estrogen receptor analysis using immunohistochemical methods, although some authors reported hormone dependence for formalin-fixed, paraffin-embedded sections by immunohistochemical analysis using a monoclonal antibody (H222),[12,13] the use of several monoclonal antibodies is usually optimal for frozen sections.[14] However, frozen sections are not suitable for the analysis of small lesions. In this study, we

Table V. pS2 Expression and Menopausal Status in Comedo-type and Noncomedo-type Carcinomas

		Premenopausal			Postmenopausal		
		Comedo	Noncomedo		Comedo	Noncomedo	
CIV	pS2(+)	1	12	NS[a]	1	7	NS
	pS2(−)	3	8		1	7	
CDC	pS2(+)	2	17	NS	1	10	NS
	pS2(−)	2	3		1	4	
FDC	pS2(+)	2	20	$P<0.05$	1	9	NS
	pS2(−)	2	0		1	5	

CIV, central invasive area; CDC, central intraductal area; FDC, forefront intraductal area.
a) Not significant.

used an anti-pS2 polyclonal antibody for analysis of the hormone dependence of intraductal lesions (small lesions) on paraffin sections because pS2 protein is a good marker of the hormone dependency of breast cancer and the results of pS2 staining on paraffin sections are concordant with those on frozen sections.[8] The pS2 protein is a 6.45 kDa polypeptide secreted by MCF-7 cells and many other human breast carcinomas[15] and it has been considered to be a marker of hormone dependence in breast cancer.[10] It has also been reported that detection of pS2 expression by immunohistochemical analysis is a good prognostic marker[16] and predictive of response to hormonal therapy on relapse.[17] In this study, the expression of pS2 protein was heterogeneous in most cases, which conforms with the findings of others.[16, 17] We used a cut-off point of 10% of positively stained cells as representing positive pS2 expression, in accordance with the criterion of Schwartz *et al.*[18] Our results for the positivity for pS2 expression are surmised to reflect correctly hormone dependence because the pS2 positivity in CIV correlated significantly with a positive ER status by EIA analysis.

In this study, we demonstrated that pS2 staining was positive in 52.5% of CIV, 77.5% of CDC and 85.0% of FDC. When pS2 expression was positive in CIV, both CDC and FDC were always positive. Moreover, even if pS2 expression was negative in CIV, a majority of the cases were positive in CDC and FDC. The positive rate of ER in carcinoma *in situ* was found to be 75% to 91% by others.[11, 12] Inaji *et al.* examined intraductal lesions and invasive lesions and reported similar results to ours.[8] However, in our present study we examined three lesions (CIV, CDC and FDC), and FDC is considered to be the principal cancerous residue after lumpectomy. In this regard, we know of no report on hormone dependence in FDC. Bur *et al.* reported that ER expression in the intraductal area was similar to that in invasive lesions,[13] but Fabris *et al.* showed that ER expression of *in situ* carcinoma is more enhanced than that of invasive carcinoma,[19] and our results suggest that the hormone dependence in FDC may be higher than in CIV. The difference between the findings of Bur *et al.* and Fabris *et al.* may be caused by differences in the analytical methods and lesions, as well as the criteria for positive expression. Lagios *et al.* reported that comedo carcinoma had high malignant potential and locally recurred more frequently after BCT compared to noncomedo types.[20] Our results were that positivity for pS2 expression in noncomedo-type carcinoma is higher than that in comedo-type carcinoma, although the difference was not statistically significant. A significant difference was found for pS2 expression in FDC between the comedo-type and noncomedo-type in premenopausal patients but the number of cases of comedo-type was limited.

In conclusion, FDC, which is thought to be the principal cancerous residue after lumpectomy, shows higher hormone dependency than CIV and CDC. Hence, endocrine therapy after BCT may be useful regardless of the ER status of the primary tumor.

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