

# Intraductal Spread of Invasive Breast Carcinoma Has a Positive Correlation with *c-erb* B-2 Overexpression and Vascular Invasion

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**BACKGROUND.** Studies of the histologic characteristics and biologic behavior of the intraductal spread of breast carcinoma are critically important in that they may lead to the identification of a unique spread pattern rather than a noninvasive lesion.

**METHODS.** Paraffin embedded specimens of 187 primary invasive breast carcinomas and 4 noninvasive ductal carcinomas, obtained by wide excision, quadrantectomy, total glandectomy, or mastectomy, were studied immunohistochemically. The overexpression of *c-erb* B-2, p53, *bcl-2*, and MIB-1, as well as the histologic characteristics of intraductal spread (such as histologic features and histologic grade), were assessed. Chi-square and Fisher exact tests were conducted to evaluate significant differences; the Macintosh for Expert StatView 4.0 system was used to conduct these tests.

**RESULTS.** The histologic characteristics of intraductal spread were similar to those of noninvasive ductal carcinoma. However, the expressions of *c-erb* B-2, p53, and other biologic markers of intraductal spread were similar to those of the main invasive tumor. The overexpression of *c-erb* B-2 protein was found more often in the group that was positive for intraductal spread than in the group that was negative ( $P < 0.01$ ). Intraductal spread was found more often in the group that was positive for lymphatic and venous invasion than in the group that was negative ( $P < 0.005$ ). Subnipple margin positive status was related closely to intraductal spread ( $P < 0.0001$ ).

**CONCLUSIONS.** The positive correlation between intraductal spread and *c-erb* B-2 overexpression as well as lymphatic, venous invasion was recognized, and it was determined that intraductal spread of invasive breast carcinoma possesses an invasive and metastatic potential that is distinct from noninvasive ductal carcinoma. *Cancer* 1999;86:439-48. © 1999 American Cancer Society.

**KEYWORDS:** breast carcinoma, intraductal spread, *c-erb* B-2, vascular invasion, metastasis.

Intraductal spread is a special histologic feature that is observed in certain patients with invasive breast carcinoma, and it is considered to be an important risk factor for local recurrence in breast-conserving therapy.<sup>1-4</sup> The role of biologic markers that may assist in evaluating the biologic behavior of intraductal spread in breast carcinoma has not been studied fully. Most of the reported studies indicate that the biologic markers *c-erb* B-2, p53, *bcl-2*, and MIB-1 in breast carcinoma are useful to some degree for evaluating patient prognosis.<sup>5-8</sup> In addition, histologic grade and tumor type have been considered to be essentially prognostic factors. We studied 187 patients with invasive breast carcinoma and 4 patients with noninvasive ductal carci-

noma to observe how different the biologic prognostic factors (*c-erb* B-2, p53, *bcl-2*, and MIB-1) were expressed between intraductal spread, positive and negative tumors, and intraductal and invasive components. We also studied whether such a difference in expression could explain the biologic behavior of intraductal spread. The correlations of intraductal spread and histopathologic prognostic factors of the tumor, such as histologic type and grade, lymphatic invasion, venous invasion, and lymph node status, also were analyzed.

## **MATERIALS AND METHODS**

### **Cases**

One hundred eighty-seven specimens from patients with invasive breast carcinoma, of which 159 patients had axillary lymph node dissection and 4 patients had noninvasive ductal carcinoma, were obtained from the Affiliated Kihoku Hospital of Wakayama Medical College from April 1996 to December 1997. The specimens were from patients who underwent wide excision, quadrantectomy, total glandectomy, or mastectomy. All of the specimens were prepared by routine fixation. Serial gross sections (5.0 mm thick) were cut by one of the authors (X. Jing). The subgross sections were recorded with a carbon-copy machine and were numbered consecutively. Selected samples containing nipple margins, lateral margins, main tumor, or satellite nodules as well as peripheral tissue adjacent to the main tumor or to satellite nodules were submitted for histologic examination. Eight to 25 blocks were selected according to the volume of the tumors and samples. The selected blocks were treated within serial graded ethanol and then routinely embedded in paraffin.

### **Histologic Study**

All 191 cases were diagnosed histologically according to the World Health Organization (WHO) Histologic Classification of Breast Tumors.<sup>9</sup> Invasive ductal carcinoma was subtyped according to the classification of the Japanese Breast Cancer Society.<sup>10</sup> Intraductal spread and noninvasive carcinoma were confirmed by alpha-smooth muscle actin ( $\alpha$ -SMA) and laminin immunohistochemical staining. Intraductal spread was defined as an extensive intraductal component (EIC)<sup>3,11</sup> outside the main tumor spreading either toward the nipple or toward the terminal lobules. The ratio of intraductal spread to invasive main tumor was  $\geq 1:5$ . The minimum distance of spread to qualify as intraductal spread was  $\geq 1$  low power microscopic field (LPF) from the borders of the main invasive tumor microscopically. Intraductal lesion within the main tumor or spread  $< 1$  LPF outside the main tumor

were not counted as intraductal spread, because it is the intraductal spread outside the main tumor that has the potential to play a role in recurrence for breast-conserving surgery. The patients with invasive ductal carcinoma that had a predominant intraductal component were classified into a special group of invasive ductal carcinoma, because this entity is restricted to patients in which the amount of the intraductal carcinoma is at least four times greater than that of the invasive component.<sup>9</sup> A positive subnipple margin was determined when neoplastic cells, such as cells from invasive carcinoma, intraductal spread, lymphatic invasion, or venous invasion, were detected at the subnipple margin, as marked during surgery. In addition, a composite morphologic grading score was determined by combining cell pleomorphism (variation in size and shape, nuclear:cytoplasmic ratio), nuclear polymorphism (variation in size and shape, bizarre nuclei, hyperchromatism increased nucleoli, and chromatin clumping), mitotic number, and atypical mitotic figures as well as the extent of tubule formation according to the Bloom and Richardson system,<sup>12</sup> with some modification. Tumors with pleomorphic nuclei, hyperchromatic nuclei, and marked variant nuclear and high mitotic figures ( $> 3$  per high power field) were classified as histologic Grade 3, and, apart from the group of patients with a Grade 3 classification, the other tumors were classified as another group of both Grade 1 and Grade 2.

The histologic identification of vascular dissemination (lymphatic or venous invasion) was made when the tumor cells or clumps within the lymphatic and venous vessels were lined by endothelium. Factor VIII-related antigen (von Willebrand) immunohistochemical staining was performed to help identify definite vascular invasion.

### **Immunohistochemistry**

For the immunohistochemical stains, 5- $\mu$ m-thick sections were cut from the formalin fixed, paraffin embedded blocks onto aminopropyltriethoxy silane-coated slides. Immunostaining of the *c-erb* B-2 (monoclonal CB11; 1:300 dilution; Novocastra Laboratories, Newcastle-upon-Tyne, United Kingdom), p53 (monoclonal DO7; 1:400 dilution; Novocastra Laboratories), *bcl-2* (monoclonal 100/D5; 1:500 dilution; Novocastra Laboratories), and laminin (polyclonal Z0097; 1:200 dilution; DAKO A/S, Glostrup, Denmark) were performed by using the avidin-biotin peroxidase complex method (Vector Laboratories, Burlingame, CA). The Ki-67 antibody was mouse monoclonal anti-Ki-67 clone MIB-1 (1:100 dilution; Immunotech S.A., Marseille, France).  $\alpha$ -SMA clone 1A4 from Chemical Credential ICN Biomedicals, Inc. (Costa Mesa, CA), von

**TABLE 1**  
**Immunohistochemistry in 187 Invasive and 4 Noninvasive Breast Carcinomas**

Histologic type	Cases	p53 (%)	<i>c-erb B-2</i> (%)	<i>bcl-2</i> (%)
I. Invasive ductal carcinoma				
1. Papillotubular type	25	7 (28.00)	14 (56.00)	12 (48.00)
2. Solid tubular type	41	25 (60.98)	28 (68.29)	16 (39.02)
3. Scirrhous type	68	18 (26.47)	42 (61.76)	40 (58.82)
II. Invasive ductal carcinoma with a predominant intraductal component	22	6 (27.27)	15 (68.18)	12 (54.54)
III. Special type				
1. Invasive lobular carcinoma	10	3 (30.00)	5 (50.00)	3 (30.00)
2. Mucinous carcinoma	4	0 (00.00)	3 (75.00)	3 (75.00)
3. Medullary carcinoma	11	5 (45.45)	7 (63.64)	4 (36.36)
4. Papillary carcinoma	1	0	1	0
5. Secretory carcinoma	1	0	0	0
6. Apocrine carcinoma	2	1	2	1
7. Carcinoma with spindle cell type	2	2	1	0
Invasive carcinoma total	187	67 (35.83)	118 (63.10)	91 (48.66)
Noninvasive ductal carcinoma	4	0 (00.00)	0 (00.00)	4 (100)
<i>P</i> value: I-1:I-2:I-3	—	0.0009	0.5896	0.1268
I:II:III	—	0.8102	0.8597	0.2555

Willebrand factor (factor VIII-related antigen; clone F8/86) from DAKO A/S (DAKO-vWf; 1:100 dilution), and MIB-1 were stained immunohistochemically by using an indirect method (peroxidase-conjugated goat antimouse immunoglobulins P 0447; 1:100 dilution; DAKO A/S). For laminin immunohistochemical staining, the slides were treated with 0.4% pepsin (in 0.01 N Hcl liquid), at 37°C for 1 hour. For von Willebrand factor staining, the slides were treated with 0.1% trypsin in Tris-buffered saline buffer. For antigen retrieval before *c-erb B-2*, p53, *bcl-2*, MIB-1, and  $\alpha$ -SMA immunohistochemical staining, the slides were immersed in a container with citrate buffer, pH 6.0, and microwaved on a high setting for 15 minutes (5 minutes, three times) (600-W with turntable; NE-A40; National Instruments, Baltimore, MD). Then, the container with slides was cooled for about 30 minutes in cool water before rinsing in deionized water and then placed in phosphate-buffered saline, pH 7.4. After the treatment described above, the slides were incubated overnight with each antibody, at 4°C in a humidity chamber and then processed by routine immunohistochemical staining. Methyl green was used for nuclear counterstaining.

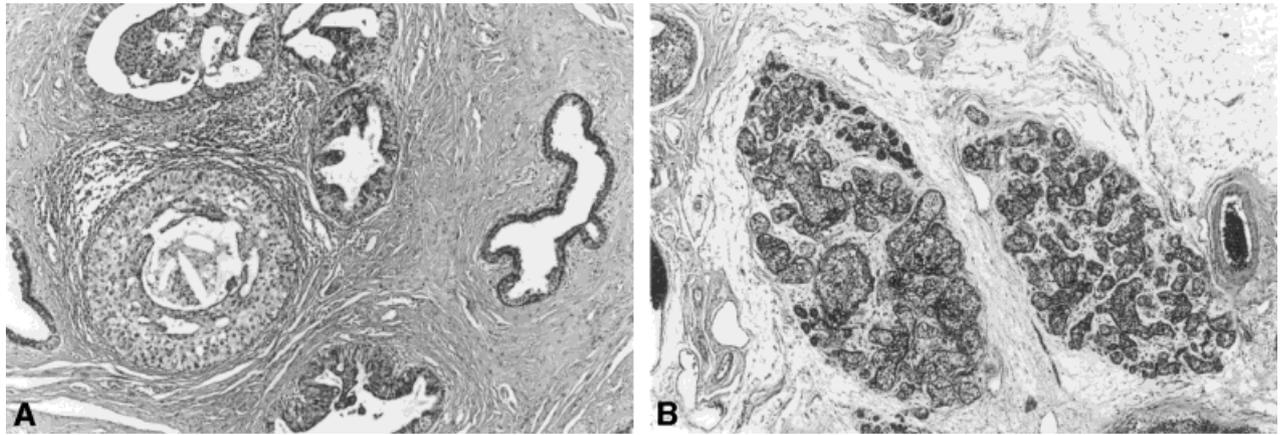
**Scoring of Immunohistochemical Results**

Nuclear staining of neoplastic cells was scored as positive for p53 and MIB-1. Staining of tumor cell membranes was scored as positive for *c-erb B-2*. Both cell membrane and cytoplasm staining was scored as positive for *bcl-2* protooncogene product. The intensity of staining was assessed as weak, moderate, or strong. The number of positive cells in tumor area per tissue

section was determined semiquantitatively. The total proportion of neoplastic cells that were stained positively at any intensity was scored as – (<10% positive neoplastic cells), + (<30% positive neoplastic cells), ++ ( $\approx$ 30–70% positive neoplastic cells), and +++ (>70% positive neoplastic cells). The threshold for positivity in this study was 10%. MIB-1 labeling scoring was performed according to the method described by Moriki et al.<sup>13</sup> The percentage of positive neoplastic cells was used for evaluating the MIB-1 index in our study. The MIB-1 index (S-phase fraction) was classified into three groups: <20%, 20–40%, and >40% (tumors with <20% MIB-1-labeled nuclei were considered the low proliferative activity group; tumors with >40% MIB-1-labeled nuclei were considered the high proliferative activity group; and tumors with proliferative activity between low and high were considered the moderate proliferative activity group).

**Statistics**

The chi-square test with Fisher exact test was applied to evaluate significant differences by using the Expert StatView system (version 4.0) on a Macintosh computer (Apple Computers, Cupertino, CA). Furthermore, multivariate analysis of logistic regression procedure with summary of stepwise procedure was applied to further identify variables correlated with the appearance of intraductal spread by using the SAS System (release 6.12; SAS Institute, Inc., Cary, NC). A *P* value of <0.05 was considered to be significant.



**FIGURE 1.** (A) Photomicrograph showing the intraductal component of invasive ductal carcinoma of the breast extending toward the nipple. The normal duct epithelium is replaced totally or partially by the neoplastic cells. An uninvolved duct is seen adjacent to the intraductal spread. (B) The intraductal component of the invasive ductal carcinoma extending toward the terminal lobules. The intraductal component can involve the whole lobule or part of it.

## RESULTS

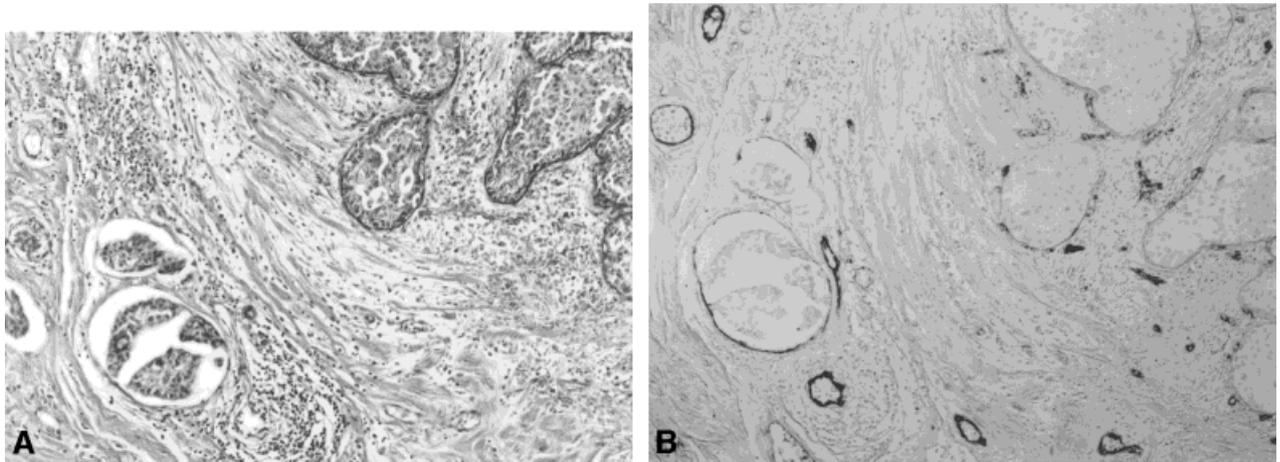
### Histologic Analysis

The 191 cases of breast carcinoma are summarized in Table 1. Three main groups were classified according to the WHO Histologic Classification of Breast Tumors.<sup>9</sup> They were invasive ductal carcinoma, invasive ductal carcinoma with a predominant intraductal component, and special types. The invasive ductal carcinomas were classified further into three subtypes—papillotubular, solid tubular, and scirrhous—according to the classification of the Japanese Breast Cancer Society.<sup>10</sup> The histologic features of the three subtypes of invasive ductal carcinoma have been well described by Ohtake et al.,<sup>14</sup> making a distinction between well differentiated and poorly differentiated tumors among the largest group of breast carcinomas. The subtypes were classified by predominant component when more than two histologic patterns appeared.

Intraductal spread was found in 86 of 187 cases (45.99%) of invasive carcinomas that included 11 medullary carcinomas by histologic examination in the selected sections. Intraductal spread by subtype of invasive ductal carcinoma (58.20%; 78 of 134) was as follows: 19 of 25 cases (76.00%) with the papillotubular type, 22 of 41 cases (53.65%) with the solid tubular type, and 37 of 68 cases (54.41%) with the scirrhous type. The incidence of intraductal spread showed no significant difference ( $P = 0.1209$ ) among the three subtypes of invasive breast carcinoma. The distances of extension varied among cases. The maximum extensive distance in our cases was 47 mm. An intraductal extension was found either toward the nipple or toward the terminal lobules (Fig. 1). The normal epithelial cells and myoepithelial cells of the duct and the

lobular gland were replaced totally or partially by neoplastic cells (Fig. 1B). Coexistent intraductal and intralobular components in a single duct lobular unit constituted one of the most special microscopic patterns of noninvasive spread. The main tumor in the index quadrant can spread to any of the other three quadrants or even can spread diffusely over the entire breast through the intraductal spread pattern. Multiple lobules can be involved by intraductal spread, resulting in multifocal lesions, because the quadrant divisions were man-made, and there are no actual dividing septa between the quadrants. Micropapillary, cribriform, solid, papillary, and comedo histologic types appeared commonly in the intraductal spread pattern. Solid and papillary types of intraductal spread, in general, were the same as the spread seen in main tumor of solid tubular and papillotubular invasive ductal carcinoma. However, micropapillary, cribriform, and comedo types were not correlated with the histologic type of the main invasive tumor.

Comparing the nuclear atypia and mitotic index between intraductal spread and main invasive tumor in each case, we found that the nuclear atypia in intraductal spread was similar to that in the main invasive tumor. Sixty-seven out of 187 cases invasive carcinoma were Grade 3, and 120 cases were Grade 1 or 2. Intraductal spread was found in 30 cases of Grade 3 invasive carcinoma (30 of 67 cases; 44.78%) and in 55 cases of Grade 1 or 2 invasive carcinoma (55 of 120 cases; 45.83%). The appearance of intraductal spread showed no significant difference ( $P = 0.8893$ ) with histologic grade. Lymphatic invasion was found in 93 out of the 187 (49.73%) invasive cases. Venous invasion was found in 14 out of the 187 (7.49%) invasive cases. Twelve cases were found positive for both lym-



**FIGURE 2.** (A) Photomicrograph showing vascular invasion of invasive ductal carcinoma of the breast (hematoxylin and eosin staining). Intraductal spread is located on the right, and lymphatic invasion is seen on the left. (B) Von Willebrand factor (factor VIII-related antigen) immunohistochemical staining of the same section shown in A. The endothelium of the lymphatics (left) is stained positive for Von Willebrand factor, but intraductal spread is negative.

**TABLE 2**  
The Relation between Intraductal Spread and Vascular Invasion

Status	Intraductal spread		Total
	Positive	Negative	
Positive for vascular invasion	55	40	95
Negative for vascular invasion	31	61	92
Total	86	101	187
<i>P</i> value			<0.005

phatic and venous invasion. Vascular invasion (lymphatic invasion or venous invasion) was found in 95 cases, which were identified and determined by both hematoxylin and eosin staining and von Willebrand factor immunohistochemical staining (Fig. 2), because we found that, in some cases, it was not easy to differentiate small foci of intraductal spread from vascular invasion. The relation between intraductal spread and vascular spread is shown in Table 2. Intraductal spread was found more often in the group that was positive for vascular spread ( $P < 0.01$ ). The agreement of vascular invasion and positive lymph nodes was 64.78% ( $P = 0.0001$ ), although the discrepancy was found in relatively large numbers ( $n = 56$  cases): 38 cases that were positive for vascular invasion had a negative lymph node status, and 18 cases that were negative for vascular invasion had a positive lymph node status. The relation between intraductal spread and lymph node status is summarized in Table 3. Intraductal spread was found more often in the group with a positive lymph node status compared with the group with a negative lymph node status. However, the difference was not significant statistically. A posi-

**TABLE 3**  
The Relation between Intraductal Spread and Axillary Lymph Node Status<sup>a</sup>

Status	No. positive for intraductal spread	%
Lymph node positive ( $n = 59$ )	31	52.54
Lymph node negative ( $n = 100$ )	44	44.00
<i>P</i> value	—	0.2973

<sup>a</sup> There were 28 patients who underwent surgery without lymph node dissection.

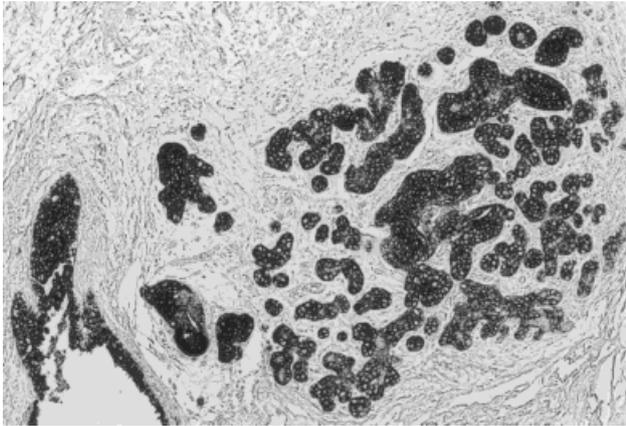
**TABLE 4**  
The Relation between Subnipple Margin and Intraductal Spread

Status	Intraductal spread		Total
	Positive	Negative	
Positive subnipple margin	29	7	36
Negative subnipple margin	57	94	151
Total	86	101	187
<i>P</i> value (positive:negative)	—	—	<0.0001

itive subnipple margin status was detected in 36 cases: Intraductal spread was found in the subnipple margin in 29 of 36 cases (80.55%) in the group with positive subnipple margins, and invasive lesions were found in the subnipple margin in 7 of 36 cases (19.44%) in the group with positive subnipple margins (Table 4;  $P < 0.0001$ ).

**Immunohistochemical Analysis**

The results of immunohistochemical staining in different types of breast carcinoma are shown in Table 1.



**FIGURE 3.** Strong *c-erb B-2* staining (3+) is seen in coexistent intraductal and intralobular components.

Positive staining for *c-erb B-2* immunohistochemistry was located in the tumor cell membrane. Positive staining could not be observed in nonneoplastic cells. In the positive cases, *c-erb B-2* oncogenic protein appeared in most of the tumor cells. Eighty-seven cases out of the 118 *c-erb B-2* positive cases showed that >30% of the tumor cells per tissue section were positive (46 cases were +++, and 41 cases were ++). The *c-erb B-2* positive incidence showed no significant difference among the different histologic tumor types. The main invasive tumor and the intraductal spread often showed the same *c-erb B-2* overexpression in each case (Fig. 3).

The location of p53 protein showed nuclear staining in all of the positive cases. The intensity of staining generally was homogeneous within each sample. There were two distributive patterns identified in the positive cases. One pattern was that almost all of the tumor cells were composed of p53 positive cells. In the other pattern, the tumor was comprised of both p53 positive and p53 negative cells, and the percentage of positive cells varied from one tumor to another. However, most of the cases (44 cases were +++, 8 cases were ++, and 15 cases were +) showed that >50% of the tumor cells were positive (++) . Positive staining could not be observed in the normal breast tissue. Statistical analysis showed that the over expression of p53 protein was more common (Table 1;  $P < 0.001$ ) in the solid tubular type of invasive ductal carcinoma in which poorly differentiated cases were seen more than in other subtype of invasive breast carcinoma. The p53 expression in medullary carcinoma was similar to that in solid tubular carcinoma.

The *bcl-2* protooncogene product was found in both the cell membrane and the cytoplasm. Immunohistochemical staining showed both normal epithe-

**TABLE 5**  
The Relation between Biologic Markers and Intraductal Spread

Intraductal spread	No.	p53 (%)	<i>c-erb B-2</i> (%)	<i>bcl-2</i> (%)
Positive	86	33 (38.37)	63 (70.08)	43 (50.00)
Negative	101	34 (33.66)	55 (51.43)	48 (47.52)
Total	187	67 (35.83)	118 (63.10)	91 (48.22)
<i>P</i> value		0.5035	0.0075	0.7357

lium and lymphocytes and tumor cells that were positive for *bcl-2*. The percentage of positive cells varied from one tumor to another. Lymphocytes and normal ductal epithelium always expressed *bcl-2*. However, endothelium and fibrous connective tissue did not express *bcl-2*. The positive incidence of *bcl-2* showed no significant difference among the different tumor types.

The immunohistochemical analysis of the biologic markers in invasive breast carcinomas that were positive for intraductal spread is shown in Table 5. We observed that both intraductal spread and the main tumor showed the same immunohistochemical expression of p53, *c-erb B-2*, *bcl-2*, and MIB-1 in each case. The expression of *c-erb B-2* was found more often in the group that was positive for intraductal spread group than that in the group that was negative ( $P < 0.01$ ; Table 5).

Immunohistochemical staining of MIB-1, compared with other biologic markers in each case, showed high proliferative activity in tumors that were positive for either p53 or *c-erb B-2* and in tumors that were negative for *bcl-2*, although we did not study the relation systematically. Among the 187 cases of invasive carcinomas, MIB-1 labeling was <20% in 100 cases (45 cases with intraductal spread), 20–40% in 42 cases (20 cases with intraductal spread), and >40% in 45 cases (21 cases with intraductal spread). All of the 4 cases of noninvasive ductal carcinoma showed <5% MIB-1 labeling and belonged to the low proliferative activity group. Proliferative activity did not differ significantly between the group that was positive for intraductal spread and the group that was negative. The MIB-1 labeling pattern showed that the positive nuclei were disseminated irregularly in the invasive tumor area that was examined. The percentage of MIB-1-labeled nuclei showed little difference between intraductal spread and the main invasive carcinoma in each case. There were greater numbers of MIB-1-labeled nuclei observed in the proximity of the stromal interface, and there were unlabeled nuclei in the central area of the intraductal spreading cells, which is in agreement with the results reported by Moriki et al.<sup>15</sup>

## DISCUSSION

Despite the fact that most malignant tumors are monoclonal in origin, their constituent cells are extremely heterogeneous by the time they become clinically evident.<sup>16</sup> Breast carcinoma is considered a morphologically heterogeneous tumor.<sup>17</sup> Usually, it is difficult to predict the patient's prognosis, because the biologic behavior of the tumor varies widely, and patients react differently to the tumor. Histologic grade has been described as a predictor of prognosis, but it is uncertain whether it can be used as a factor in the selection of breast-conserving therapy.<sup>18</sup> Intraductal spread, as a special spread pattern, signals the importance of screening for local recurrence after breast-conserving therapy.<sup>19,20</sup> Expression of estrogen receptor (ER) showed no significant difference between the EIC positive and EIC negative groups, as reported in our previous article.<sup>21</sup> The biologic characteristics of EIC are still unknown with regard to extensive spread. The aim of the current study was to ascertain any differences in the expression of *c-erb B-2*, p53, and *bcl-2* as well as differences in the MIB-1 index and histologic factors, such as vascular invasion and lymph node status, between tumors that are positive for intraductal spread and tumors that are negative as well as between the intraductal and invasive components. Determining these factors may assist clinicians with their postoperative management decisions after breast-conserving therapy.

Morphologically, intraductal spread is a noninvasive, relatively well-differentiated lesion, similar to noninvasive ductal carcinoma. It is accepted that most patients with this type of tumor have an equal or better prognosis compared with patients with infiltrating ductal carcinoma. However, at breast-conserving therapy, tumors with intraductal spread can leave residual islands of intraductal carcinoma that can result in recurrence. Holland et al.<sup>22</sup> reported that patients with tumors that contain an EIC more often have a large subclinical tumor burden in the remainder of the breast compared with patients with tumors that have no EIC, which indicates that intraductal spread may extend farther than invasive components. We found the same indication in our study. The tumor cells that spread through the duct may be different in their phenotypic attributes from the cells of the main invasive tumor. Generally, dissemination of cancers may occur through one of three pathways: 1) direct seeding of body cavities or organ surfaces, 2) lymphatic spread, and 3) hematogenous spread. Histologic evidence of penetration of small vessels at the site of the primary neoplasm is obviously an ominous feature, although such changes do not indicate the inevitable

development of metastasis. Vascular invasion is a critical step for a metastatic tumor, although only certain subclones possess the right combination of gene products to complete all of the steps of the metastatic cascade. Intraductal spread was found more often in the group that was positive for vascular invasion (Table 2;  $P = 0.0012$ ; chi-square test with Fisher exact test; multivariate analysis:  $P = 0.0164$ ; maximum likelihood estimates;  $P = 0.0055$ ; stepwise procedure), which supports the view that intraductal spread is possessed of invasive and metastatic potential. These neoplastic cells within a tumor are heterogeneous with respect to their metastatic potential.<sup>16</sup> Intraductal spread was observed extending to other quadrants far from the index quadrant of the main tumor, which provided further evidence that the subpopulations of cells within intraductal spread possessed invasive potential. They can spread farther through an intraductal spread pattern than the main invasive carcinoma. Therefore, we consider that intraductal spread is a unique spread pathway in breast carcinoma. It can enlarge the tumor volume, extend diffusely over the entire breast, and sometimes develops to form satellite neoplasms. In our previous study, 13 of 14 cases (92.86%) of multinodular breast carcinoma were related to intraductal spread ( $P < 0.0001$ ), which supports our view that intraductal spread is a unique spread pattern in breast carcinoma.<sup>21</sup> In addition, analysis (Table 3) of the relation between intraductal spread and axillary lymph node status showed that intraductal spread was slightly more frequent in the group with a lymph node status than in the group with a negative lymph node status, although there was no significant statistical difference. This result supports the view that intraductal spread may be possessed of metastatic potential.

Careful clinical and experimental studies of neoplasms reveal that increasing malignancy often is acquired in an incremental fashion.<sup>16</sup> This biologic phenomenon is related to the sequential appearance of subpopulations of cells. Intraductal spread was observed in about a half of the cases of invasive breast carcinomas. The incidence of intraductal spread in our study was substantially higher than the commonly reported average occurrence of 30% but lower than the incidence (80%) reported in the stereomicroscopic studies of subgross specimens.<sup>14</sup> This may be attributed to differences in study populations and investigative methods. The neoplastic cells in intraductal spread may have some phenotypic attributes, such as the ability to spread through ducts far from the main tumor and the ability to form satellite tumors.

In the current study, intraductal spread was found in 1 of 11 cases of medullary carcinomas (9.09%).

Rosen et al.<sup>23</sup> also found an intraductal component at the periphery of substantial numbers of medullary carcinomas. The appearance of intraductal spread in medullary carcinoma indicates either that the medullary carcinoma cells could invade breast duct and spread through the duct to the periphery tissue or that intraductal carcinoma, as a different component, coexists with the component of medullary carcinoma. In our study, because the morphologic features of the tumor cells with an intraductal component was the same as the features in the medullary carcinoma, the intraductal component is considered to be intraductal spread rather than a different component.

The results of *c-erb* B-2 immunostaining demonstrated high overexpression in the group that was positive for intraductal spread ( $P < 0.01$ ; chi-square test with Fisher exact test; multivariate analysis:  $P = 0.0475$ ; maximum likelihood estimates;  $P = 0.0142$ ; stepwise procedure). The high expression of p53 and *bcl-2* showed no significant difference between the group that was positive for intraductal spread and the group that was negative.

The product of the *c-erb* B-2 protooncogene is a cell surface growth factor receptor. It is widely accepted that overexpression of *c-erb* B-2 is associated with a biologically more aggressive form of breast carcinoma and relates to lymph node metastasis.<sup>24-26</sup> Until now, the expression of *c-erb* B-2 status in intraductal spread has not been evaluated fully. In the current study, the expression of *c-erb* B-2 was similar in both intraductal and invasive lesions from the same patient. These findings suggest that intraductal spread and invasive lesions may be highly related, which is in agreement with the results reported by Iglehart et al.<sup>27</sup> However, *c-erb* B-2 overexpression was found more often in the group that was positive for intraductal spread than in the group that was negative ( $P < 0.01$ ), which indicates that tumors that are positive for intraductal spread may possess greater invasive and metastatic potential than tumors that are negative for intraductal spread. DePotter et al.<sup>28</sup> reported that high grade ductal carcinoma in situ often was more extensive when it was positive for *c-erb* B-2 than when it was negative. One possible explanation for this result is a motility effect of the *c-erb* B-2 protein playing a role in the intraductal spread of breast carcinoma, as discussed by DePotter et al. Holland et al.<sup>22</sup> also reported the EIC positive tumors more often have a large subclinical tumor burden than EIC negative tumors, which supports the findings in this report.

The tumor suppressor gene p53 is the most commonly mutated gene identified in a variety of human malignant tumors, and it is thought to contribute to neoplastic transformation and tumor progression,

which indicates uncontrolled cell proliferation. Altered p53 expression in breast carcinoma could be associated with high proliferative activity,<sup>29</sup> *c-erb* B-2 protein overexpression,<sup>30</sup> and low *bcl-2* expression.<sup>31</sup> However, important differences can be found from one study to another.<sup>32-34</sup> We observed positive p53 staining in 35.83% of the invasive breast carcinoma cases, ranging from + to ++++. In most of the positive cases (50 of 67; 74.63%), greater than ++ positivity was seen in the main tumor. There was no significant difference found for p53 overexpression between the group that was positive for intraductal spread and the group that was negative ( $P = 0.8600$ ; Table 5). No difference was found in p53 expression between intraductal spread and main invasive tumor in any case in the group that was positive for intraductal spread. Therefore, p53 is not a suitable factor for the evaluation of intraductal spread status in breast carcinoma. However, greater p53 overexpression was found in the solid tubular type of invasive ductal carcinoma and in medullary carcinoma ( $P < 0.01$ ; Table 1) than in other types of breast carcinoma. Our results suggest that significantly high p53 overexpression is correlated with tumor differentiation, because 23 of 41 solid tubular tumors (78.05%) were poorly differentiated, and 10 of 11 medullary carcinomas (90.90%) also were poorly differentiated. Conversely, intraductal spread status showed no positive correlation to histologic differentiation.

Compared with most of the previously published reports, in the current study, the incidence of *c-erb* B-2 expression was high, and p53 expression was low. However, a higher incidence of *c-erb* B-2 expression (65%, 79%, and 89.2%)<sup>35-37</sup> and a lower incidence of positivity for p53 (17.6%)<sup>37</sup> were published in some reports. Variations in materials and antibodies may explain these differences.

The *bcl-2* gene is known to be a unique oncogene that plays an important role in carcinogenesis by blocking apoptosis. It has been reported that a higher fraction of *bcl-2* positive cells was observed significantly in small, ER positive, slowly proliferating, p53 negative tumors than in large, ER negative, rapidly proliferating, p53 positive tumors.<sup>38</sup> However, the results from the current study show that *bcl-2* protein was not related to those factors. The expression of *bcl-2* showed no correlation to tumors that were either positive or negative for intraductal spread, and there was no significant difference among different histologic types of breast carcinoma. Therefore, we do not believe that the expression *bcl-2* is an appropriate factor for the investigation of breast carcinoma with intraductal spread.

MIB-1 is superior as a proliferation marker com-

pared with other methods.<sup>39</sup> The MIB-1 labeling index showed no significant difference between the group that was positive for intraductal spread and the group that was negative. The percentage of MIB-1-labeled nuclei did not show as much difference as that seen between the intraductal spreading carcinoma and the main invasive carcinoma in each case. In intraductal spread, greater numbers of MIB-1-labeled nuclei were observed in the outer layer cells adjacent to the stroma, and there were unlabeled nuclei in the central area. One possible explanation for this phenomenon is that the outer layer cells can obtain more blood supply than those in the central area, which makes proliferation greater. An alternative explanation may be that the outer layer cells may have greater proliferative activity and can grow and spread quickly through the duct.

In summary, the expression of c-erb B-2, p53, bcl-2, and MIB-1 in the intraductal spread of breast carcinoma was similar to their expression in the main invasive carcinoma, which indicates that intraductal spread and invasive carcinoma within a unilateral breast are correlated highly. Breast tumor cells were found spreading farther through an intraductal spread pattern than direct stroma invasion from the main carcinoma. Breast carcinoma cells in intraductal spread may have greater invasive and metastatic potential, because greater overexpression of c-erb B-2 was found in the group that was positive for intraductal spread than in the group that was negative, and more frequent vascular invasion was seen in the group that was positive.

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