Histological Factors Associated with Initial Bone Metastasis of Invasive Ductal Carcinoma of the Breast

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Bone is one of the most common sites of recurrence of breast cancer. Therefore, it would be clinically very useful if breast cancers with a high probability of bone metastasis (BM) could be identified by histopathological examination of the primary lesions. To elucidate histological characteristics associated with predisposition to initial BM, we examined nine histopathological parameters in the primary lesions of 110 invasive ductal carcinomas (IDCs) of the breast with 0 to 3 regional node metastases. These cases had recurrence between 4 months and 10.1 years after the initial radical surgery. The first metastatic site was bone in 24 cases, whereas other sites were involved in 86 cases. IDCs growing in a strand growth pattern or with fibrotic focus (FF) had a significantly higher frequency of initial BM than those growing in a non-strand growth pattern or without FF, respectively. Strand growth pattern was a significant predictor of the initial BM in multivariate analysis. In all 54 IDCs that developed BM during the follow-up period, osteolytic metastasis was significantly more frequent in the group with FF than in that without FF. This study demonstrated that strand growth pattern and the presence of FF are significant histopathological factors associated with initial BM. The combination of those predictive factors along with prognostic factors may provide a useful approach to identify patients at high risk for initial BM, enabling early treatment for the recurrent cancer.

Key words: Invasive ductal carcinoma — Bone metastasis — Fibrotic focus — Histology — Breast

Bone is one of the most common metastatic sites of breast cancer.[1] Bone metastasis (BM) is frequently responsible for serious clinical problems, such as intractable bone pain, frequent pathological fractures, nerve compression and hypercalcemia. Systemic chemotherapy, endocrine therapy or local radiation therapy for BM has been shown to be effective in a significant portion of patients. If cancers with a high probability of BM can be identified from the histopathological findings of the primary lesion, subsequent intensive survey for bone involvement may detect early metastasis and appropriate treatment might prolong patients' survival and maintain a higher quality of life. Several clinicopathological studies have demonstrated that certain biochemical and histological factors are associated with BM of breast cancers. For example, high estrogen receptor content,[2, 3] low histological grade[2] and expression of parathyroid hormone-related peptide[4] are risk factors for BM. In contrast, tumors with

squamoid character and involucrin expression have a low incidence of BM.[5]

Tumor metastases to the bone are classified into osteolytic and osteoblastic types. In the former, destruction of the lamellar bone is a typical finding, whereas the latter shows new bone formation within the foci of lamellar bone destruction. These two types probably have different biological characteristics and thus different clinical relevance.

The aim of this study was to identify histopathological findings which are correlated with initial BM of invasive ductal carcinoma (IDC) of the breast, because histopathological examination can be done in any hospital and is therefore clinically useful for the follow up of breast cancer patients. Furthermore, we examined histopathological factors that correlated with the osteolytic or osteoblastic type of BM.

MATERIALS AND METHODS

Cases In order to compare the clinical course among breast cancers at relatively localized stages, 110 primary IDCs with none or less than four lymph node metastases

were selected for this study. All these IDCs showed local recurrence or distant organ metastasis between 4 months and 10.1 years, despite the fact that they were regarded as being in a relatively early clinical stage at surgery. All the patients were Japanese women and underwent a standard or modified radical mastectomy at the National Cancer Center Hospital or the National Cancer Center Hospital East between January 1978 and April 1996. Synchronous or heterochronous bilateral breast cancer cases were excluded from the study. Clinical information was obtained from the patients' medical records.

The median follow-up was 3.9 years, ranging from 7 months to 18.3 years, with intervals of 1 to 2 weeks in the first year and 3 to 6 months in the second year or later. Patients were checked for local recurrences, and lymph node and liver metastases at the outpatient clinic by inspection and palpation. For the detection of lung metastasis, chest X-ray was taken every 3 or 6 months, and chest computed tomography was also frequently taken. According to whether the patients complained of pain, the presence of bone metastasis was surveyed by taking roentgenograms and/or scintigrams at intervals of 6 to 12 months. Abdominal ultrasonography and computed tomography were used for examination of the liver at intervals of 6 to 12 months. The median disease-free interval was 2.0 years, ranging from 4 months to 10.1 years.

Twenty-four (22%) of 110 IDCs developed BM as the initial site of recurrence. Other initial recurrence sites were liver in 19 (17%), lung in 37 (34%), and local in 30 (27%) IDCs. In addition to the 24 IDCs with initial BM, 30 IDCs developed BM during their follow-up period; overall, a total of 54 IDCs developed BM.

Histological examination of the primary tumor Surgically resected breast tissue was fixed in 10% formalin overnight at room temperature and each specimen was cut into slices at intervals of about 1 cm. Grossly, the size of the tumors was measured and the characteristics of the cut surface of the tumors were examined to determine whether there was tumor necrosis or hemorrhage, or whether the tumor invaded the skin or muscle tissue. Then, almost the entire tumor tissue was taken for histological examination, and the size of the tumor was reevaluated on the histological sections. The sections were processed routinely and embedded in paraffin. Sections of each tumor were cut from paraffin blocks, and one section was stained with hematoxylin and eosin, while another section was stained by Elastica staining to examine the presence of vascular invasion. The sections were examined pathologically to confirm the diagnosis and to evaluate the histological parameters. Factors examined were: 1) tumor size (≤20 or >20 mm), 2) nuclear atypia in Broom and Richardson's classification[6] (low, moderate and high), 3) the number of mitotic figures (≤10 or >10 per 10 high-

power fields), 4) tumor growth pattern (strand or non-strand) (Fig. 1), 5) fibrotic focus (FF)[7] (absent or present) (Figs. 2 and 3), 6) tumor necrosis (absent or present), 7) extra-mammary fat invasion (absent or present), 8) squamoid character[5] (absent or present) and 9) vascular invasion (absent or present). Since the size and number of FF differs from case to case, histological examination of the entire tissue was necessary to determine the presence of FF.[7] All the histologic sections were examined independently in a blinded fashion by three observers (TK, TH and HT).

Pattern of BM on roentgenography To classify the pattern of BM of IDCs, the roentgenographs of any BM from IDCs were examined and classified into osteolytic or osteoblastic type according to their dominant pattern. Roentgenographically, osteolytic bone metastasis was defined as metastasis having a motheaten or permeated bone destruction pattern, whereas osteoblastic bone
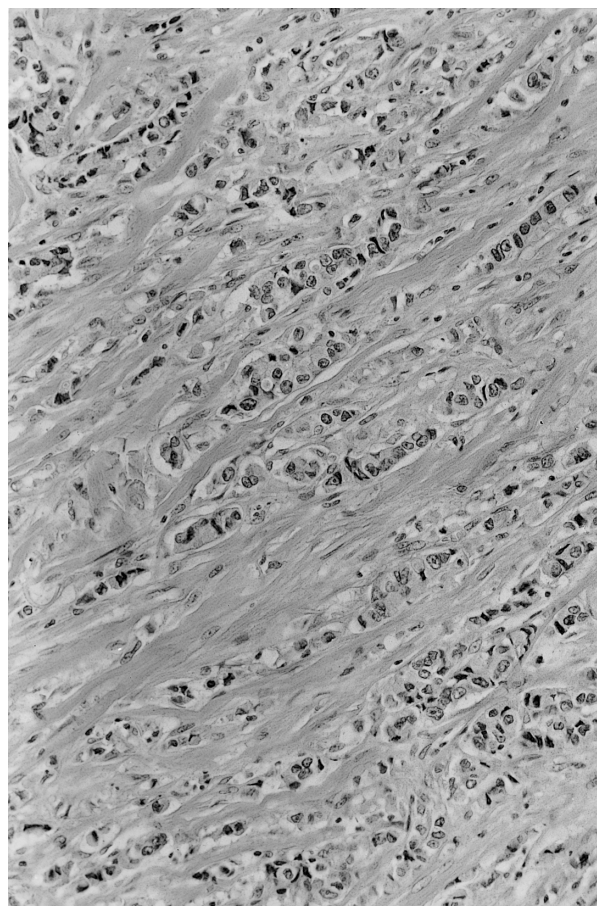

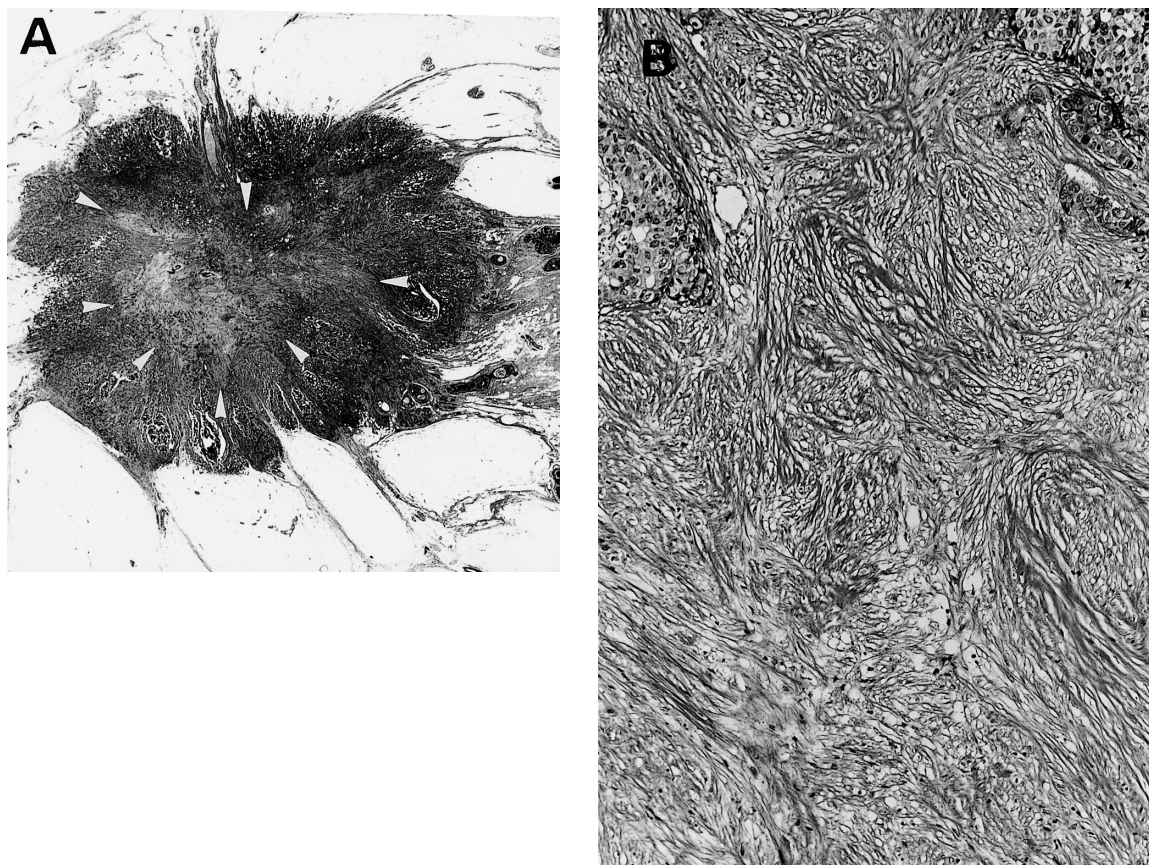Fig. 1. IDCs with strand growth. Tumor cells infiltrate in a strand fashion. (×200)

Fig. 2. IDC with FF. (A) Panoramic view of IDC with FF. An FF measuring 4.5×2.3 mm in size is seen within the tumor (arrowheads). (B) The FF is composed of fibroblasts and collagen fibers showing a storiform pattern, which is almost always found in FF. (×100).

metastasis consisted of solid, cloud-like or ivory-like mineralized matrix.

Statistical analysis The comparative studies using the categories described below were performed between the group showing initial BM and that without initial BM by Student's t test or the χ^2 test. Logistic regression model analysis was performed to examine the factors correlated with BM among the IDCs which developed recurrence. Then the data were submitted to multiple regression analysis using the step-down method until all the remaining factors which became significant at a P value below 0.05 were extracted. All analyses were conducted with Statistica Windows software (Stat Soft, Tulsa, OK).

RESULTS

Relationship between the initial BM and histopathological factors The mean age, menopausal status (pre- or post-), or the number of regional lymph node metastases (0 or 1–3) did not differ significantly between the patients with initial BM and those with initial recurrence at other sites (data not shown).

In univariate analysis, the percentage of the initial BM was higher in IDCs with strand growth or with FF than in those with non-strand growth or without FF. The risk ratio (RR) and 95% confidence interval (CI) of the strand growth were 3.18 and 1.22–8.33, respectively, and those of the FF were 3.74 and 1.03–13.60, respectively. The incidence of initial BM was significantly lower in the group with squamoid character than in the group without such character (RR 0.19, 95%CI 0.04–0.85) (Table I). Tumor size, nuclear atypia, mitotic counts, tumor necrosis, fat invasion and vascular invasion were not significantly correlated with the initial BM (Table I).

The multivariate analysis confirmed that strand growth was an independent factor associated with higher RR of initial BM, but the presence of FF or squamoid character showed no significant association.

Since the univariate analysis demonstrated that the strand growth pattern and FF were both significantly asso-
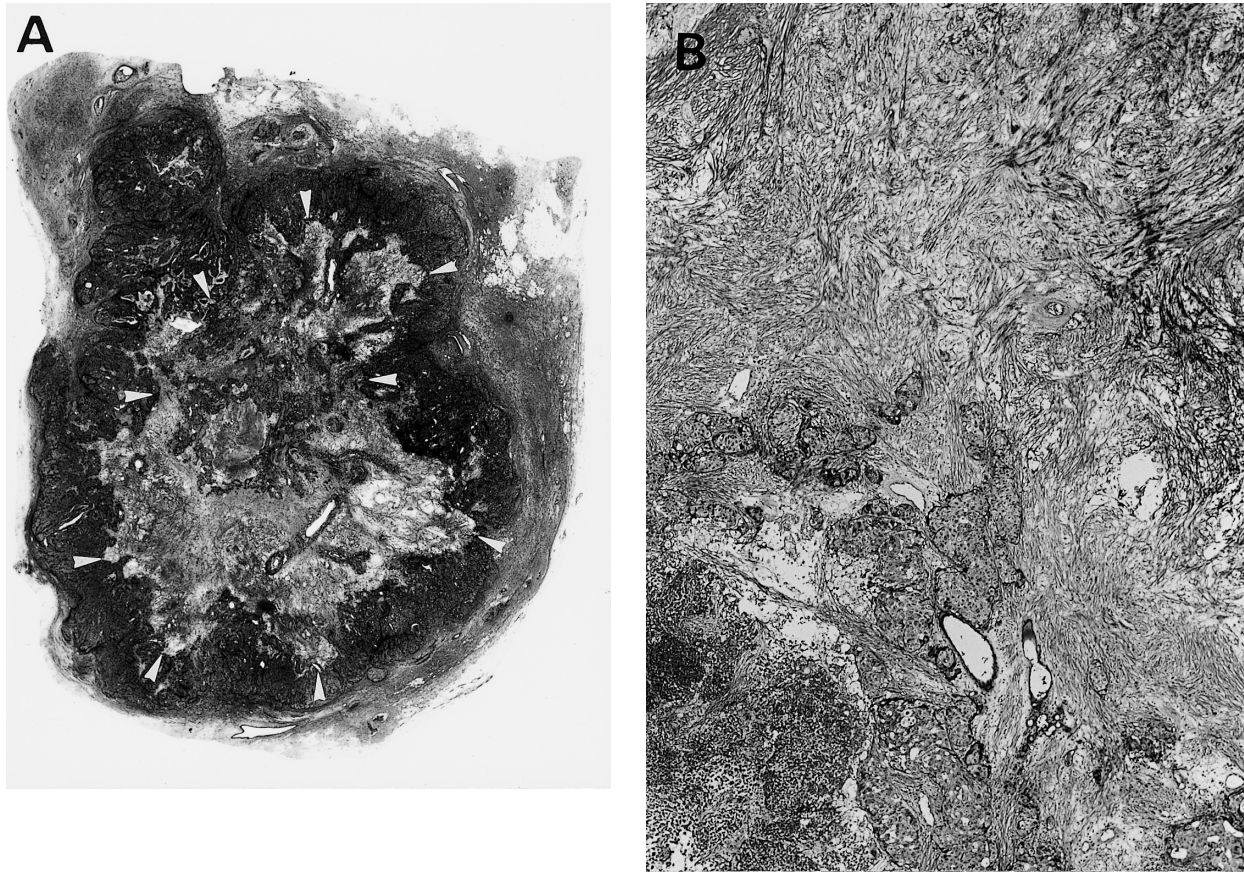
Fig. 3. IDC with FF. (A) Panoramic view of IDC with FF. An FF measuring 14.0×9.5 mm in size is seen within the tumor (arrowheads). (×100). (B) There are tumor cell islands within the FF.

ciated with initial BM, we determined whether the combination of these two factors increased the predictive accuracy for initial BM. Among 77 IDCs having FF, 22 cases showed a strand growth pattern. They had a higher rate of initial BM than those with FF and non-strand growth (RR 3.32, 95%CI 1.15–9.58) (Table II). When 54 cases which developed initial or late BM were analyzed, there were no histopathological factors which differed significantly between IDCs with any BM and IDCs without BM (data not shown).

Relationship between BM pattern and histopathological factors Among 54 IDCs with initial or late BM, 42 (78%) showed osteolytic BM, and 8 (15%) had osteoblastic BM. There were 4 (8%) IDCs whose BM pattern could not be classified as either osteolytic or osteoblastic.

The presence of FF was correlated with the osteolytic BM in the univariate analysis (RR 4.16, 95%CI 1.07–16.28) and multivariate analysis ($P=0.045$). In the univariate analysis, fat invasion was correlated with osteolytic BM (RR 3.63, 95%CI 0.89–11.41). Vascular invasion was also

inversely correlated with osteolytic BM (RR 0.17, 95%CI 0.02–1.16). No other factor showed any correlation with osteolytic BM (Table III).

DISCUSSION

This study demonstrated that two histological factors, strand growth pattern and the presence of FF in the primary lesion, were significantly associated with initial BM. In addition, we confirmed that squamoid character of the primary site was a negative predictive factor for initial BM. Although FF was not associated with initial BM in multivariate analysis, IDCs with both FF and strand growth pattern were considered to be the major subgroup predisposed to initial BM. Furthermore, the multivariate analysis showed that the strand growth pattern of tumor cells is an independent histological factor for predicting initial BM of IDCs. Therefore, strand growth pattern and FF may be valuable markers for the early detection of initial BM of IDCs.

Table I. Correlations between Histopathological Factors and Initial Bone Metastasis in 110 IDCs Analyzed by Univariate and Multivariate Analyses

	No. of patients	IBM[a](%)	RR[b]	95%CI[c]	P value[d] Uni-variate	P value[d] Multi-variate
All recurrent cases						
	(n=110)	24 (22)				
Tumor size(mm)						
≤20	34	6 (18)	1.0			
>20	76	18 (24)	1.45	0.53–3.97	0.482	—
Nuclear atypia						
Low or moderate	33	8 (24)	1.0			
High	77	16 (21)	0.82	0.33–2.01	0.688	—
Mitotic counts (per 10 high-power fields)						
≤10	41	10 (24)	1.0			
>10	69	14 (20)	0.79	0.30–2.07	0.616	—
Growth pattern						
Non-strand	81	13 (16)	1.0			
Strand	29	11 (38)	3.18	1.22–8.33	0.019	0.019
Fibrotic focus (FF)						
Absent	33	3 (9)	1.0			
Present	77	21 (27)	3.74	1.03–13.60	0.047	—
Tumor necrosis						
Absent	56	15 (27)	1.0			
Present	54	9 (17)	0.55	0.22–1.38	0.206	—
Fat invasion						
Absent	27	4 (15)	1.0			
Present	83	20 (24)	1.82	0.56–5.93	0.318	—
Squamoid character						
Absent	80	22 (28)	1.0			
Present	30	2 (7)	0.19	0.04–0.85	0.033	—
Vascular invasion						
Absent	93	23 (25)	1.0			
Present	17	1 (6)	0.19	0.02–1.51	0.120	—

a) IBM, initial bone metastasis.
b) RR, relative risk.
c) CI, confidence interval.
d) —, P value above 0.05 in multivariate analysis.

Table II. Association of Tumor Cell Growth Pattern with Initial Bone Metastasis in IDCs with FF Analyzed by Univariate Analysis

	No. of patients	IBM[a](%)	RR[b]	95%CI[c]	P value
IDC with FF	77	21 (27)			
Non-strand	55	11 (20)	1.0		
Strand	22	10 (45)	3.32	1.15–9.58	0.030

a) IBM, initial bone metastasis.
b) RR, relative risk.
c) CI, confidence interval.

In breast cancers with no axillary lymph node metastasis or less than 4 lymph node metastases, histological grade has been shown to be an indicator of tumor recurrence.[8, 9] Therefore, the combined examination of strand growth pattern, FF and histological grade may be useful to identify cases showing a high frequency of ini-

Table III. Correlations between Histopathological Factors and Osteolytic Metastasis in IDCs Analyzed by Univariate and Multivariate Analyses

	No. of patients	Osteolytic BM[a] (%)	RR[b]	95%CI[c]	P value[d] Uni-variate	P value[d] Multi-variate
All cases	54	41 (76)				
Tumor size (mm)						
≤20	14	12 (86)	1.0			
>20	40	29 (73)	0.44	0.09–2.27	0.333	—
Nuclear atypia						
Low or moderate	15	10 (67)	1.0			
High	39	31 (79)	1.93	0.51–7.32	0.332	—
Mitotic counts						
≤10	20	14 (70)	1.0			
>10	34	27 (79)	1.65	0.46–5.87	0.440	—
Growth pattern						
Non-strand	38	27 (71)	1.0			
Strand	16	14 (86)	2.86	0.54–14.88	0.216	—
Fibrotic focus (FF)						
Absent	13	7 (56)	1.0			
Present	41	34 (83)	4.16	1.07–16.28	0.045	0.045
Tumor necrosis						
Absent	31	25 (81)	1.0			
Present	23	16 (70)	0.55	0.16–1.92	0.353	—
Fat invasion						
Absent	11	6 (55)	1.0			
Present	43	35 (81)	3.63	0.89–11.41	0.079	—
Squamoid character						
Absent	41	32 (78)	1.0			
Present	13	9 (69)	0.63	0.16–2.53	0.522	—
Vascular invasion						
Absent	49	39 (78)	1.0			
Present	5	2 (40)	0.17	0.02–1.16	0.077	—

a) BM, bone metastasis.
b) RR, relative risk.
c) CI, confidence interval.
d) —, P value above 0.05 in multivariate analysis.

tial BM. In such a high-risk group, frequent bone survey is indicated for the early detection of BM, so that systemic and/or local therapy against the BM can be initiated as soon as possible.

IDCs growing in a strand pattern are accompanied with abundant fibrous stroma, while those growing in solid nests usually are not. It was reported that among IDCs with a strand growth pattern, IDCs with FF showed a significantly higher frequency of basic fibroblast growth factor (bFGF) expression than those without FF. However, there was no significant correlation between the presence of FF and a high frequency of bFGF expression in IDCs with a solid growth pattern.[10] In addition, fibroblasts forming FF in IDCs with strand growth showed a higher frequency of fibroblast growth factor receptor expression than those with solid growth.[10] From these observations, it seems plausible that an active interaction between the tumor and stromal cells mediated by fibroblast growth factor accounts for the higher potential of the tumors for BM.[11]

The presence of FF was associated with osteolytic BM in this study. Because IDCs with FF also tended to form FF in the sites of metastasis,[12] the mechanism of FF formation may be involved in the development of osteolytic metastasis. Osteolysis in BM is induced by osteoclasts within the lesion, or by soluble factors secreted by the tumor cells.[13] Osteoclasts within the tumor are activated by the parathyroid hormone-related peptide[3] and matrix metalloproteinases[14] produced by the tumor cells. The matrix metalloproteinases and certain cytokines or growth

factors[15, 16] secreted by the tumor cells or stromal cells presumably play an important role as soluble factors that mediate osteolysis. Therefore, it is reasonable to speculate that some of these molecules which act in the tumor and stromal cell interactions induce osteolytic BM in IDCs with FF.

In conclusion, this study clearly demonstrated that a strand growth pattern of the tumor cells and the presence of FF in IDCs are important histological factors predicting initial or osteolytic BM. This indicates that these histological factors are useful to identify a high-risk group for BM and to improve postoperative management of IDCs.

In the future, it seems very important to examine other factors correlated with BM, such as estrogen receptor content, and expression of parathyroid hormone-related peptide and involucrin, along with histological factors, in a larger number of patients.

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