Image of the Month

A Case of Ductal Carcinoma In Situ of the Breast

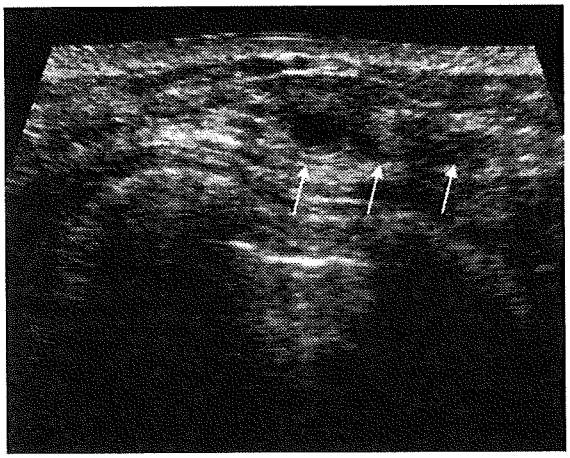

Figure 1.

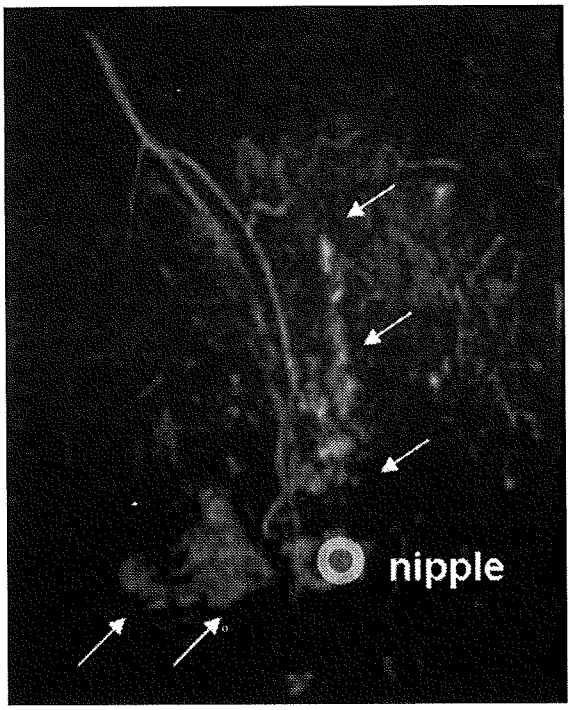

Figure 2.

A 55-year-old woman underwent a follow-up study 3 years after left mastectomy for ductal carcinoma *in situ* (DCIS). On ultrasonography (US), a line of small hypoechoic areas was found in the right breast (Fig. 1), which was not shown on mammography. On magnetic resonance imaging (MRI), an irregularly enhanced segmental tumor with a maximum length of 7 cm was demonstrated in the right upper outer quadrant (Fig. 2). Vacuum-assisted core biopsy of the tumor under US-guidance revealed DCIS. She underwent right mastectomy with sentinel node biopsy. The sentinel nodes were negative for cancer. The histopathological extension of the tumor was more precisely predicted on MRI than on US.

In review of 137 patients with DCIS, we also found that the histopathological extension of the tumor was more precisely predicted on MRI than on mammography or US. Although microcalcification on mammography is considered a key finding for detecting DCIS, MRI might be an essential imaging study for patients with DCIS.

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CASE REPORT

Primary small cell carcinoma of the breast

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Abstract Primary small cell carcinoma of the breast is a very rare disease, and only a few case reports have described small cell carcinoma of the breast that responds to chemotherapy. Here, we report a case of primary small cell carcinoma of the breast that was treated with surgery and chemotherapy for postoperative local recurrence in the chest wall and metastasis to the liver. The metastatic lesions showed a partial response (PR) to carboplatin and irinotecan, but did not respond to subsequent Taxotere and doxifluridine (5′-DFUR) treatment. We then treated the metastatic lesions with CBDCA and etoposide (VP-16), and were able to stop disease progression. Small cell carcinoma of the breast is as aggressive as its pulmonary counterpart. Therefore, the best therapy for primary small cell carcinoma of the breast may be surgery followed by adjuvant therapy similar to that recommended for small cell lung carcinoma.

Keywords Breast · Small cell carcinoma · Chemotherapy

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Introduction

Primary small cell carcinoma of the breast is a very rare disease, with fewer than 33 cases described in the literature [1–15, 27–29]. There are even fewer reports of this disease responding to chemotherapy [1, 3, 7–13, 15]. We report here a case in which a hepatic metastasis of primary breast small cell carcinoma showed a response to chemotherapy. We discuss treatment strategies for primary breast small cell carcinoma based on this case and previous reports.

Case report

A 60-year-old, post-menopausal Japanese woman presented at the hospital with a mass in her right breast that she had noticed 3 months earlier. Physical examination revealed a 2.2 × 1.5-cm firm non-tender tumor with irregular borders in the upper-outer quadrant of the right breast; the nipple-tumor distance was 5.5 cm. There was no nipple discharge, and bilateral axillary lymph nodes showed no abnormality. Laboratory data and tumor markers were within normal ranges [carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), and National Cancer Center-Stomach-439 (NCC-ST-439)]. A mammogram of the right breast showed a microlobulated mass without calcification. An ultrasonogram confirmed the heterogeneity of the mass and showed no intraductal component. MRI showed a distinctly contrasting mass of about 3.0 × 2.0 cm in the upper-outer quadrant of the right breast, and neither ductal spread nor multiple lesions were observed. Furthermore, a computed tomography scan revealed no obvious findings of lung tumors or distant organ metastasis. We diagnosed the mass as cancer by

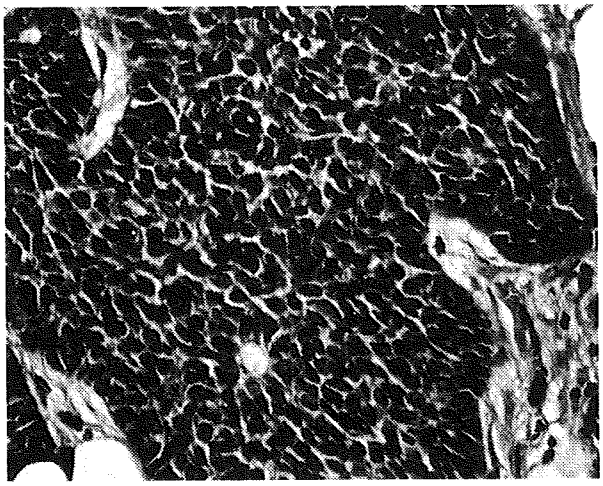

Fig. 1 Pathological findings (hematoxylin-eosin). Histopathological examination by hematoxylin and eosin staining showed that the neoplastic cells have scant cytoplasm and hyperchromatic nuclei. Some rosette-like structures are present in this nest

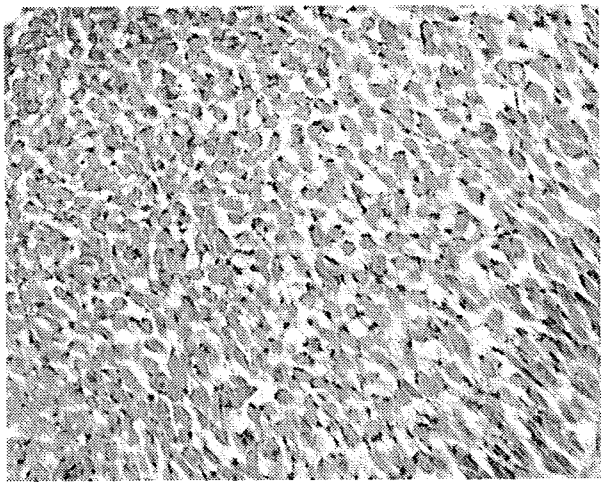

Fig. 2 Pathological findings (immunohistochemical staining for Grimelius). Grimelius staining was positive for narrow cytoplasm of neoplastic cells

needle biopsy. The breast cancer was preoperatively classified as T2N0M0 (UICC, 6th edition, 2002).

We planned conservation surgery with axillary lymph node dissection. However, this was changed to modified radical mastectomy because the margin of the removed specimen showed cancer invasion. The tumor was a solid yellow-white mass of 3.0 × 1.0 cm in the greatest cut dimension, and the margin was infiltrating with an indistinct border. No axillary lymph node involvement was observed.

Histopathological examination by hematoxylin and eosin staining showed that the tumor was composed of nests of small cells with round to fusiform shape, scant cytoplasm, finely granular nuclear chromatin and absent or inconspicuous nucleoli. Occasionally, rosette-like structures were observed. Foci in the ductal components were Pagetoid spread (Fig. 1). The results of immunostaining were as follows. The tumor cells were positive for Grimelius, cytokeratinAE1/AE3, neuron-specific enolase (NSE), CD56, bcl-2 and CD117 (c-kit), but were negative for Chromogranin, cytokeratin34BE12, synaptophysin, estrogen receptor, progesterone receptor and Her2/neu. The patient did not undergo adjuvant therapy.

A mass later appeared on the chest wall at the operative site. This was diagnosed as breast small cell carcinoma by cytodiagnosis. Multiple metastases were found on the liver by abdominal computed tomography, but no metastases were found in other organs. Chemotherapy was performed using a regimen for pulmonary small cell carcinoma: day 1 cisplatin at 60 mg/m^2 and days 1, 8 and 15 CPT-11 at 60 mg/m^2. However, we changed cisplatin to carboplatin, because the patient experienced grade 2 neutropenia, grade 2 leucopenia and grade 2 vomiting (NCI-CTC) following the first administration. The patient was treated with carboplatin (300 mg/m^2) on day 1 and CPT-11 (60 mg/m^2) on days 1, 8 and 15 every 3 weeks for five cycles. The patient experienced no grave side effects. The local recurrence disappeared during chemotherapy, and the metastatic lesions on the liver were reduced by 71% (Fig. 2). The second course of chemotherapy administered docetaxel (DTX) and 5′-DFUR; however, the hepatic tumors progressed during this course. The second course is a regimen often used to treat breast cancer and other regional carcinomas; however, this therapy was not efficacious in this case (Fig. 3). Therefore, we treated with a regimen of carboplatin (300 mg/m^2) on day 1 and etoposide (80 mg/m^2) on days 1 and 2 every 3 weeks for four cycles. The metastatic lesions on the liver were reduced by 5.8% during the third course of treatment (Fig. 3). Subsequently, the liver metastasis progressed, but the patient chose to stop chemotherapy. The patient passed away 26 months following the initial surgery.

Discussion

Herein, we report treatment of a primary small cell carcinoma of the breast.

The tumor was positive for Grimelius (Fig. 3), but negative for Chromogranin by immunostaining. Because the Grimelius staining was weak, we thought it was possible that the tumor was a small cell carcinoma of low secretory ability; this would also explain the lack of Chromogranin staining.

Extrapulmonary small cell carcinoma (EPSCC), a rare neoplasm, has been increasingly recognized as a clinicopathologic entity distinct from small cell carcinoma of the

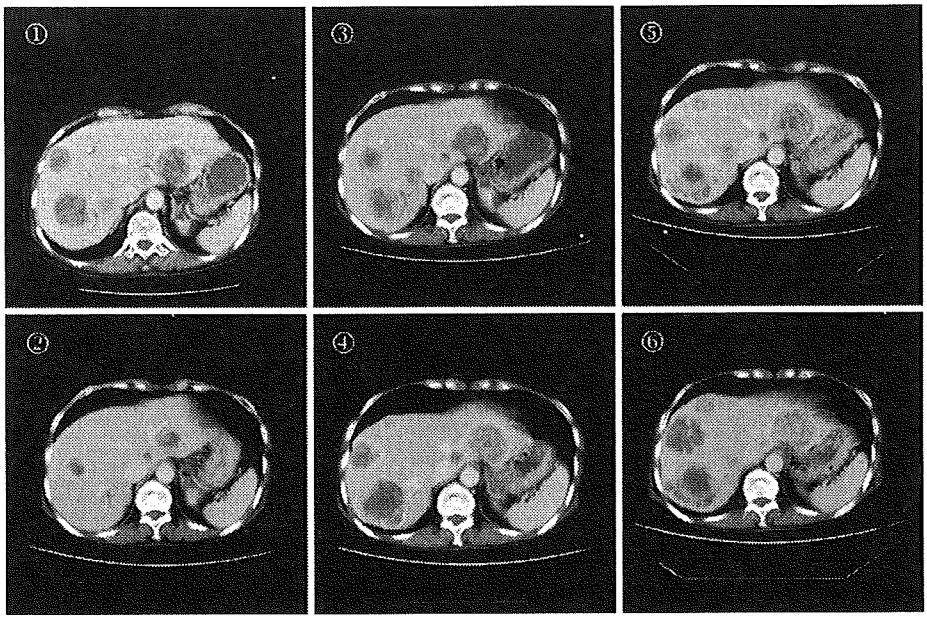

Fig. 3 Computed tomography (CT). Use of chemotherapy for hepatic metastasis and evaluation by CT. The patient was treated with a combination of carboplatin and CPT 11 for (1) to (2) period, and a partial response was achieved. The patient was treated with docetaxel and 5′-DFUR in periods (3) to (4), and progressive disease was observed. Carboplatin and VP-16 treatment was administered in periods (5) to (6), resulting in stable disease

Table 1 Reported cases of primary mammary small cell carcinoma: clinical summary

Authors	Neo-adjuvant (regimen)	Adjuvant (regimen)	Chemotherapy for MBC (regimen)	Response	Location of MBC	Follow-up (month)	Status
Stein et al. [13]	CDDP + VP16	ND		NC		24	Alive
Mariscal et al. [7]	CDDP + VP16	ND		CR		6	Alive
Samli et al. [9]	CEF	ND		Response		6	Alive
Sebenik et al. [10]	CDDP + VP16	CDDP + VP16		CR		33	Alive
Adegbola et al. [1]		CDDP + VP16				48	Alive
		CDDP + VP16				20	Died
		CDDP + VP16				6	Alive
Sridhar et al. [12]		AD + CDDP				18	Alive
Wade et al. [14]		AC + VCR				9	Died
Yamasaki et al. [15]		CMF				16	Alive
Papotti et al. [8]		TAM				44	Alive
		TAM				9	Alive
		ND	CMF	PD	HEP/BRA	14	Died
Francois et al. [3]		ND	AC + VP16	PR	LYM/PUL	21	Died
Kitakata et al. [27]		EC + DTX				22	Alive
Present case		ND	CBDCA + CPT11	PR	SKI/HEP	26	Died

ND not done, *MBC* metastatic breast cancer, *CDDP* cisplatin, *AD* adriamycin, *VCR* vincristine, *CBDCA* carboplatin, *CPT-11* irinotecan, *CMF* cyclophosphamide, methotrexate and fluorouracyl, *EC* epirubicin and cyclophosphamide, *DTX* docetaxel, *TAM* tamoxifen, *HEP* hepatic, *BRA* brain, *LYM* lymph nodes, *PUL* pulmonary, *SKI* skin

lung. It has been estimated that approximately 1,000 new cases of extrapulmonary small cell carcinoma are diagnosed annually in the US, with an overall incidence of 0.1–0.4% [17]. Approximately 2.5% of all small cell cancers occur in extrapulmonary sites [18]. Irfan [19] reported that the gastrointestinal system (45%), urinary bladder (27%) and uterus (9%) are the most common extrapulmonary sites of small cell carcinoma. There is no standard treatment for limited extrapulmonary small cell carcinoma. In recent years, surgery, if undertaken, was usually performed after induction chemotherapy. Chemotherapy for EPSCC usually follows regimens used to treat small cell lung carcinoma. Cisplatin, etoposide, cyclophosphamide and doxorubicin represent the backbone of most of the combinations used. The overall response rate in extensive disease, using cisplatin-based or cyclophosphamide/doxorubicin

with vincristine or etoposide chemotherapy, is 70–90% [17, 20–26].

Treatment for breast cancer typically involves both local and systemic treatment; however, as small cell carcinoma of the breast is extremely rare, treatment has not been established. In this case, chemotherapeutic regimens typically used to treat small cell lung carcinoma were effective against the breast small cell carcinoma.

In the literature, we were able to identify four reports of neo-adjuvant chemotherapy [7, 10, 13], ten reports of adjuvant therapy [1, 8, 10, 12, 14, 15, 27] and three reports of therapy for metastasis of breast small cell carcinoma [3, 8].

In three of the four neo-adjuvant cases, chemotherapy regimens for small cell lung carcinoma were used. In two of these cases, a complete response was observed.

In five of the ten adjuvant cases, chemotherapy regimens for small cell lung carcinoma were used, and in three of these cases, the patient survived.

Taken together, these cases suggest that chemotherapeutic regimens typically used to treat small lung cell carcinoma can be effective against small cell carcinoma of the breast (Table 1). The best treatment for primary small cell carcinoma of the breast may therefore be surgery followed by such chemotherapeutic regimens.

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Tumor histology in lymph vessels and lymph nodes for the accurate prediction of outcome among breast cancer patients treated with neoadjuvant chemotherapy

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The present study investigated fibrotic foci (FFs), the grading system for lymph vessel tumor emboli (LVTEs), and the histological characteristics of nodal metastatic tumors that were significantly associated with the outcomes of 115 patients with invasive ductal carcinoma (IDC) who had received neoadjuvant chemotherapy. We compared the outcome predictive power of FFs, the grading system for LVTEs, and the histological characteristics of metastatic tumors in lymph nodes with the well-known clinicopathological characteristics of tumor recurrence and tumor-related death in multivariate analyses. The presence of FFs, as assessed by a biopsy performed before neoadjuvant chemotherapy, significantly increased the hazard rates (HRs) for tumor-related death in all the cases and in cases with nodal metastasis. The grading system for LVTEs, which was assessed using surgical specimens obtained after neoadjuvant chemotherapy, was significantly associated with increasing hazard rates (HRs) for tumor recurrence and tumor-related death in all the cases and in cases with nodal metastasis. Moderate to severe stroma in nodal metastatic tumors and five or more mitotic figures in nodal metastatic tumors were significantly associated with elevated HRs for tumor recurrence and tumor-related death among all the cases. These results indicated that FFs, the grading system for LVTEs, and the histological characteristics of tumor cells in lymph nodes play important roles in predicting the tumor progression of IDCs of the breast in patients treated with neoadjuvant chemotherapy. (*Cancer Sci* 2009; 100: 1823–1833)

Traditionally, neoadjuvant chemotherapy has been used for the treatment of locally advanced or inoperable breast cancer.[1,2] More recently, neoadjuvant chemotherapy has been used for the treatment of patients with smaller tumors that would have previously been considered operable at the patient's initial presentation.[3] The purpose of neoadjuvant chemotherapy is to reduce the size of the primary tumor in the breast, so as to facilitate breast conservation surgery, and also to abolish or reduce the disease burden associated with micro-metastatic disease with the intention of prolonging the patient's overall survival.

Gene or protein expression profiles have recently been reported to be significant predictors of the outcome of patients receiving neoadjuvant chemotherapy.[4–6] However, identifying histological predictors of prognosis is very important because histopathological examinations of invasive ductal carcinomas (IDCs) can be routinely performed at any hospital and also are a very useful method for following IDC patients who received neoadjuvant chemotherapy clinically. Clinicopathological factors including age, residual invasive tumor size, histologic grade of the primary invasive tumors, axillary node status, and pathological response have been reported to be good predictors of prognosis among patients with IDC who have received neoadjuvant chemotherapy,[7–10] and we recently demonstrated that a grading system for lymph vessel tumor emboli (LVTEs) and the histological characteristics of tumor cells in lymph nodes are very important histological predictors of prognosis among IDC patients who did not receive neoadjuvant therapy.[11,12] These findings strongly suggest that a grading system for LVTEs or the histological characteristics of tumor cells in lymph node might also be very important histological predictors of prognosis among IDC patients who received neoadjuvant chemotherapy.

The purpose of this study was to investigate the histological characteristics of primary invasive tumors, the grading system for LVTEs, and the histological characteristics of nodal metastatic tumors that were significantly associated with the outcomes of IDC patients who received neoadjuvant chemotherapy. We found that the presence of fibrotic foci (FFs), as assessed using biopsy materials obtained before neoadjuvant chemotherapy; the grading system for LVTEs, as assessed using surgical specimens obtained after neoadjuvant chemotherapy; and several histological characteristics of tumor cells in lymph nodes, as assessed using surgical specimens obtained after neoadjuvant chemotherapy, had significant effects on outcome among IDC patients who received neoadjuvant chemotherapy.

Materials and Methods

Patients. The subjects of this study comprised 115 consecutive patients with IDC of the breast who had been surgically treated at the National Cancer Center Hospital between January 1997 and December 2002. The IDCs were diagnosed preoperatively by aspiration cytology, mammography, or ultrasonography. Clinical information was obtained from the patients' medical records after a complete histological examination of all the IDCs. All the patients were Japanese women, ranging in age from 30 to 71 years (median, 50 years). All the patients had a solitary lesion; 49 patients were premenopausal, and 57 were postmenopausal. A partial mastectomy had been performed in 14 patients, and a

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Table 1. Criteria used in the grading systems for lymph vessel tumor emboli in invasive ductal carcinoma (IDC)

Grading system for lymph vessel tumor emboli according to the number of mitotic and apoptotic figures in tumor cells of lymph vessel tumor emboli		
Grade 0	IDCs with no lymph vessel tumor emboli	
Grades 1, 2, and 3	IDCs with one or more lymph vessel tumor emboli	
	No. of mitotic figures	No. of apoptotic figures
Grade 1	Low-proliferative type	
1a	0	0
1b	0	>0
1c	>0	0
Grade 2	Intermediate-proliferative type	
2a	1 to 4	>0
2b	>0	1 to 6
Grade 3	High-proliferative type	
3a	>4	>6

modified radical mastectomy had been performed in 101. A level I and II axillary lymph node dissection had been performed in all the patients, and a level III axillary lymph node dissection had been performed in some of the IDC patients.

Of the 115 patients, 17 (12%) had only residual ductal carcinoma *in situ*, while 98 (88%) patients had residual IDC; none of the patients exhibited a pathological complete response (no tumor) to neoadjuvant chemotherapy. All the neoadjuvant chemotherapy regimens were anthracycline-based with or without taxane. No cases of inflammatory breast cancer were encountered in this series. All the tumors were classified according to the pathological International Union Against Cancer (UICC)-TNM (pTNM) classification.[13]

For the pathological examination, biopsy specimens obtained before neoadjuvant chemotherapy and surgically resected specimens obtained after neoadjuvant chemotherapy were fixed in 10% formalin and subsequently examined. The size and gross appearance of the surgically resected tumor specimens were recorded as the residual invasive tumor size. The tumor size of the surgically resected specimens was confirmed by comparison with the tumor size on histological slides; if more than one invasive focus was present, the size of the largest invasive focus was recorded as the residual invasive tumor size in this study.

Histological examination. Serial sections of the biopsy materials obtained before neoadjuvant chemotherapy, and serial sections of the tumor area in the surgically resected materials obtained after neoadjuvant chemotherapy were cut from paraffin blocks. One section of each biopsy or surgical specimen was stained with hematoxylin and eosin (H&E) and examined histologically to confirm the diagnosis, and another section was subjected to immunohistochemistry. The following eight histological features of the primary invasive tumors were evaluated in the biopsy materials obtained before neoadjuvant chemotherapy and the surgical materials obtained after neoadjuvant chemotherapy: (1) clinical invasive tumor size or residual invasive tumor size (≤20, >20 to ≤0, >50 mm); (2) histologic grade (1, 2, 3);[14] (3) tumor necrosis (absent, present);[15] (4) FF (absent, present) (Fig. 1a,b);[16,17] (5) blood vessel invasion (absent, present); (6) adipose tissue invasion (absent, present); and (7) skin invasion (absent, present). We also evaluated a grading system for LVTEs, as assessed using biopsy materials obtained before neoadjuvant chemotherapy and surgical materials obtained after neoadjuvant chemotherapy (Table 1, Fig. 1c,d).[11] Briefly, the number of tumor cell mitotic figures and the number of apoptotic figures in the lymph vessels were counted in 20 high-power fields of the surgical materials. In practice, for the surgical materials, we first examined all the slides of the IDCs containing both tumor areas and non-tumor areas to identify the LVTEs. Next, we selected the LVTEs, e.g. large LVTEs located far from the stroma–invasive tumor margin, and recorded the number of mitotic figures and the number of apoptotic figures in the tumor cells composing the LVTEs of the IDC. The mitotic and apoptotic figures were counted under a high-power field, and the largest number of mitotic figures and/or the largest number of apoptotic figures were recorded as the number of mitotic figures and apoptotic figures in the LVTEs of the IDC, respectively. The cumulative numbers of tumor cell mitotic figures and apoptotic figures in the LVTEs in all 20 high-power fields were not used. In IDCs containing a small number of LVTEs, the mitotic figures and apoptotic figures were counted in less than 20 high-power fields. For the biopsy materials, we examined the presence or absence of LVTE or LVTEs; when LVTE or LVTEs were observed in the biopsy material, an assessment similar to that described above was performed. We also evaluated the prognostic predictive power of the location of lymph vessel invasion,[18] the Fisher's neoadjuvant-chemotherapy-effect classification,[19,20] and the Japanese Breast Cancer Society (JBCS) neoadjuvant-chemotherapy-effect classification for surgical materials obtained after neoadjuvant chemotherapy.[21] Cases with non-invasive ductal carcinoma (NIDC) after neoadjuvant chemotherapy were classified as belonging to grade 3 of the JBCS neoadjuvant-chemotherapy effect classification.[21] None of the IDC cases exhibited the disappearance of all the tumor cells (invasive tumor cells and non-invasive tumor cells) after neoadjuvant chemotherapy in this series.

The following histological features of metastatic tumors in lymph nodes dissected at the time of surgery (after neoadjuvant chemotherapy) were examined:[12] (1) the maximum dimension of nodal metastatic tumors; (2) lymph nodes with extra-nodal invasion (absent, present); (3) extra-nodal blood vessel tumor emboli (absent, present); (4) number of mitotic figures in tumors in the lymph node (≤5, >5); (5) histologic grade of tumors in the lymph node (1, 2, 3); and (6) grade of stromal fibrosis of tumors in the lymph node (none, mild, moderate, severe) (Fig. 1e,f). Extra-nodal invasion was defined as the extension of tumor cells through the capsule of at least one lymph node into the perinodal adipose tissue. Nuclear atypia, structural atypia, and the number of mitotic figures were evaluated in the same manner as for the primary invasive tumors. The presence of metastases in the lymph nodes was evaluated using single sections of each node or half of each node stained with H&E.

Immunohistochemistry. Immunohistochemical staining for estrogen receptors (ERs), progesterone receptors (PRs), and HER2 products was performed using an autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA, USA). The antigen retrieval device of the Optimax Plus was autoclaved, and each specimen was immersed in citrate buffer and incubated at 121°C for 10 min. Immunoperoxidase staining was performed using a labeled streptavidin biotin staining kit (BioGenex) according to the manufacturer's instructions. The antibodies used were an

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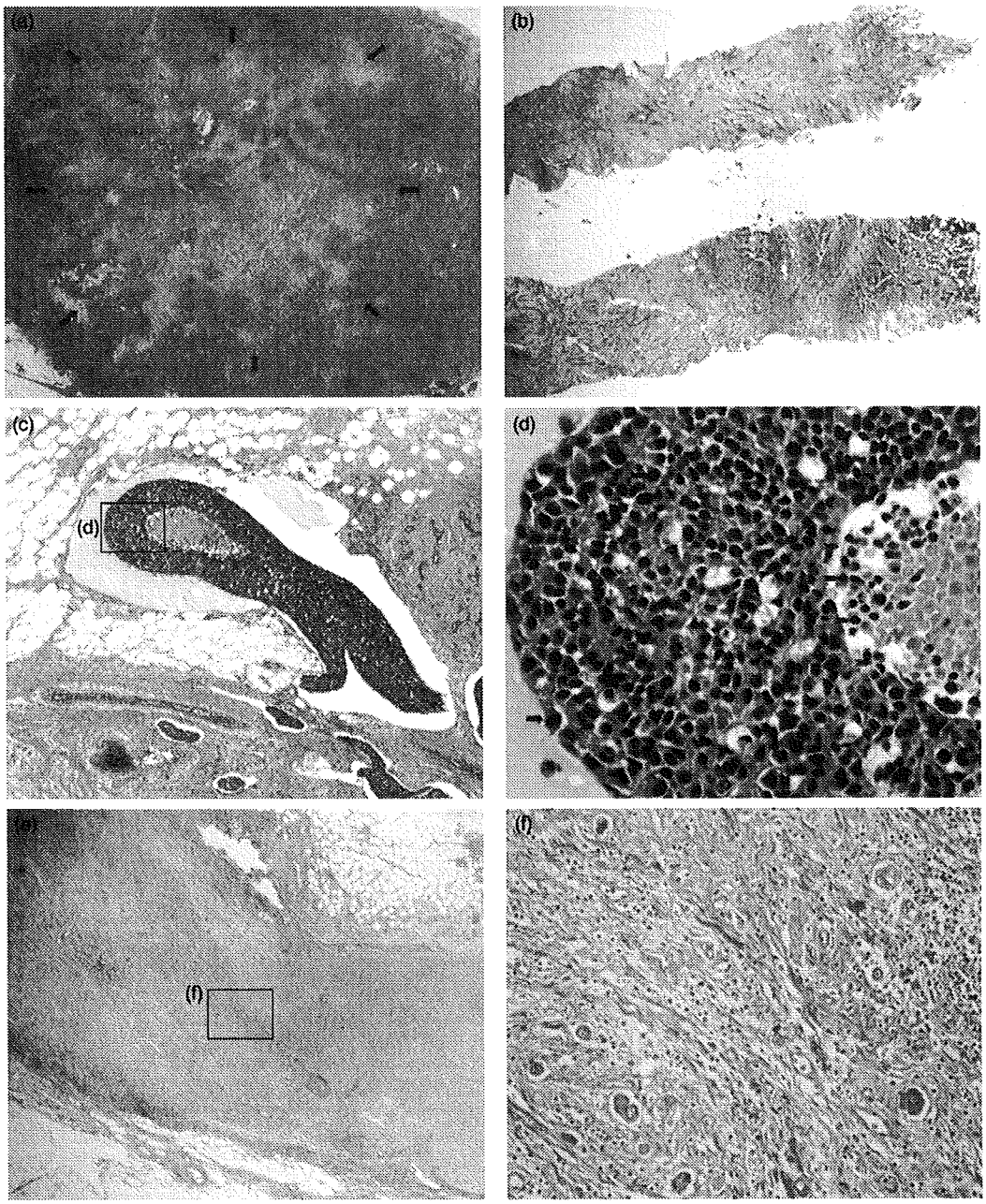

Fig. 1. Histological characteristics of fibrotic foci (FFs), lymph vessel tumor emboli, and nodal metastatic tumors. (a) An FF measuring 8.4 × 6.2 mm is visible within the tumor (arrows) in a surgical specimen. The FF has the appearance of a scar-like feature, and it is surrounded by invasive ductal carcinoma cells. The FF area consists of fibroblasts and collagen fibers arranged in a storiform pattern with tumor cell nests. (b) A core-needle biopsy specimen shows an FF consisting of fibroblasts and collagen fibers in a storiform arrangement intermingled with invasive tumor cells (fibrosis grade 3). (c) One large lymph vessel tumor embolus and five lymph vessel tumor emboli are shown. A necrotic tumor focus is visible in the large lymph vessel tumor embolus. (d) Several apoptotic bodies and apoptotic tumor cells are visible (arrowheads), and six mitotic tumor cells (arrows) can be seen in the lymph vessel tumor embolus. The apoptotic bodies are small, variously shaped pyknotic bodies that resemble sesame seeds, and the apoptotic tumor cells were identified as tumor cells containing eosinophilic or amphophilic cytoplasm and irregularly shaped pyknotic nuclei. (e) Metastatic tumor in the lymph node exhibiting dense stromal fibrosis. (f) Tumor cells with light eosinophilic cytoplasm and irregularly shaped nuclei exhibiting scattered growth in dense fibrous stroma of a metastatic tumor in a lymph node.

anti-ER mouse monoclonal antibody (mAb), ER88 (BioGenex); an anti-PR mAb, PR88 (BioGenex); and an anti-HER2 mAb, CB11 (BioGnex). ER88, PR88, and CB11 were already diluted. After immunostaining, the sections were counterstained with hematoxylin. Sections of IDCs positive for ER, PR, and HER2 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobin. An IDC with nuclear staining for ER or PR in 10% or more of its tumor cells was assessed as ER-positive or PR-positive. The HER2 status of the tumor cells was semi-quantitatively scored on a 0 to 3 scale according to the level of HER2 protein expression.[22]

One author (N.T.) assessed all the characteristics of the primary tumors, the tumors in the lymph vessels, and the nodal

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Clinical invasive tumor size (mm)					
≤20	0		0.004		0.088
>20 to ≤50	72	14 (19)		8 (11)	
>50	43	18 (42)		8 (19)	
Histologic grade of primary invasive tumor					
1	25	8 (32)	0.352	2 (8)	0.890
2	70	21 (30)		13 (19)	
3	20	3 (15)		1 (5)	
Fibrotic focus					
Absent	96	25 (26)	0.285	10 (11)	0.010
Present	19	7 (37)		6 (32)	
Tumor necrosis					
Absent	80	23 (29)	0.769	10 (13)	0.499
Present	35	9 (26)		6 (17)	
Grading system for lymph vessel tumor emboli					
Grade 0	106	28 (26)	0.079	12 (11)	0.009
Grade 1	5	1 (20)		1 (20)	
Grade 2	4	3 (75)		3 (75)	
Grade 3	0				
ER and PR status (n = 106)					
Negative	42	12 (29)	0.707	7 (17)	0.590
Positive	64	17 (27)		7 (11)	
HER2 status (n = 109)					
0 to 2	82	21 (26)	0.422	8 (10)	0.155
3	27	9 (33)		7 (26)	

ER and PR status negative, ER and PR both negative; ER and/or PR status positive, ER positive or PR positive, or both positive. ER, estrogen receptor; PR, progesterone receptor.

Table 2. Association of clinicopathological factors assessed using biopsy materials obtained before neoadjuvant chemotherapy with tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma who received neoadjuvant chemotherapy

metastatic tumors as well as the immunohistochemical parameters of the biopsy and surgical materials, and another author (T.H.) identified the characteristics of all the IDCs or the immunohistochemical parameters to confirm the tumor cell characteristics in these tumor components and the immunohistochemical characteristics recorded by N.T. Whenever a discrepancy occurred, the authors re-examined the slides to reach a consensus.

Patient outcome and statistical analysis. Survival was evaluated using a median follow-up period of 52.3 months (range, 4.9 to 84.6 months) until February 2007. At that time, 83 of the 115 patients who had received neoadjuvant chemotherapy were alive and well, 32 had developed tumor recurrences, and 16 had died of their disease. The recurrence-free and overall survival periods were determined beginning at the time of surgery. Tumor relapse was considered to have occurred whenever evidence of metastasis was first observed.

We analyzed the outcome predictive power of a grading system for LVTEs assessed using biopsy or surgical materials, the seven histological factors of primary invasive tumors assessed using biopsy or surgical materials, six histological factors of metastatic tumors in lymph nodes assessed using surgical materials, ER and PR expression in primary invasive tumor cells assessed using biopsy or surgical materials, the category of HER2 expression in primary invasive tumor cells using biopsy or surgical materials, the Fisher's classification for neoadjuvant chemotherapy,[19,20] the classification of the JBCS for neoadjuvant chemotherapy,[21] age (≤39 years and >39 years), the UICC-pathological nodal status (UICC pN: no nodal metastasis, N0; 1 to 3 nodal metastases, N1; 4 to 9 nodal metastases, N2; and 10 or more nodal metastases, N3), the UICC-pTNM stage classification[13] for tumor recurrence, and tumor-related death using univariate analyses with the Cox proportional hazard regression model.[23] Factors significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses using the Cox proportional hazard regression model[23] according to nodal status. The step-down method was applied until all of the remaining factors were significant at a P-value of less than 0.05. Since the following factors were examined using both biopsy materials obtained before neoadjuvant therapy and surgical materials obtained after neoadjuvant chemotherapy, to be able to accurately assess the prognostic value of each of these factors using multivariate analyses, their mutual influence on the outcome was avoided by analyzing the prognostic predictive power of the biopsy materials obtained before neoadjuvant chemotherapy and that of the surgical materials obtained after neoadjuvant chemotherapy separately (model 1, factors examined using biopsy materials; model 2, factors examined using surgical materials): (1) invasive tumor size; (2) histologic grade; (3) FF; (4) tumor necrosis; (5) grading system for LVTEs; (6) blood vessel invasion; (7) ER and PR status; and (8) HER2 status. In IDC patients without nodal metastasis, since tumor recurrence was observed in three patients, and tumor-related death was observed in only two patients, we were unable to perform multivariate analyses for tumor recurrence or tumor-related death. The survival curves were drawn using the Kaplan–Meier method.[24] All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK, USA).

Results

Factors significantly associated with tumor recurrence and tumor-related death. The univariate analyses of data for biopsy materials obtained before neoadjuvant chemotherapy showed that the clinical invasive tumor size and skin invasions were significantly associated with tumor recurrence, while the presence of FF (Fig. 2a) and the grading system for LVTEs were significantly associated with tumor-related death (Table 2). None of the

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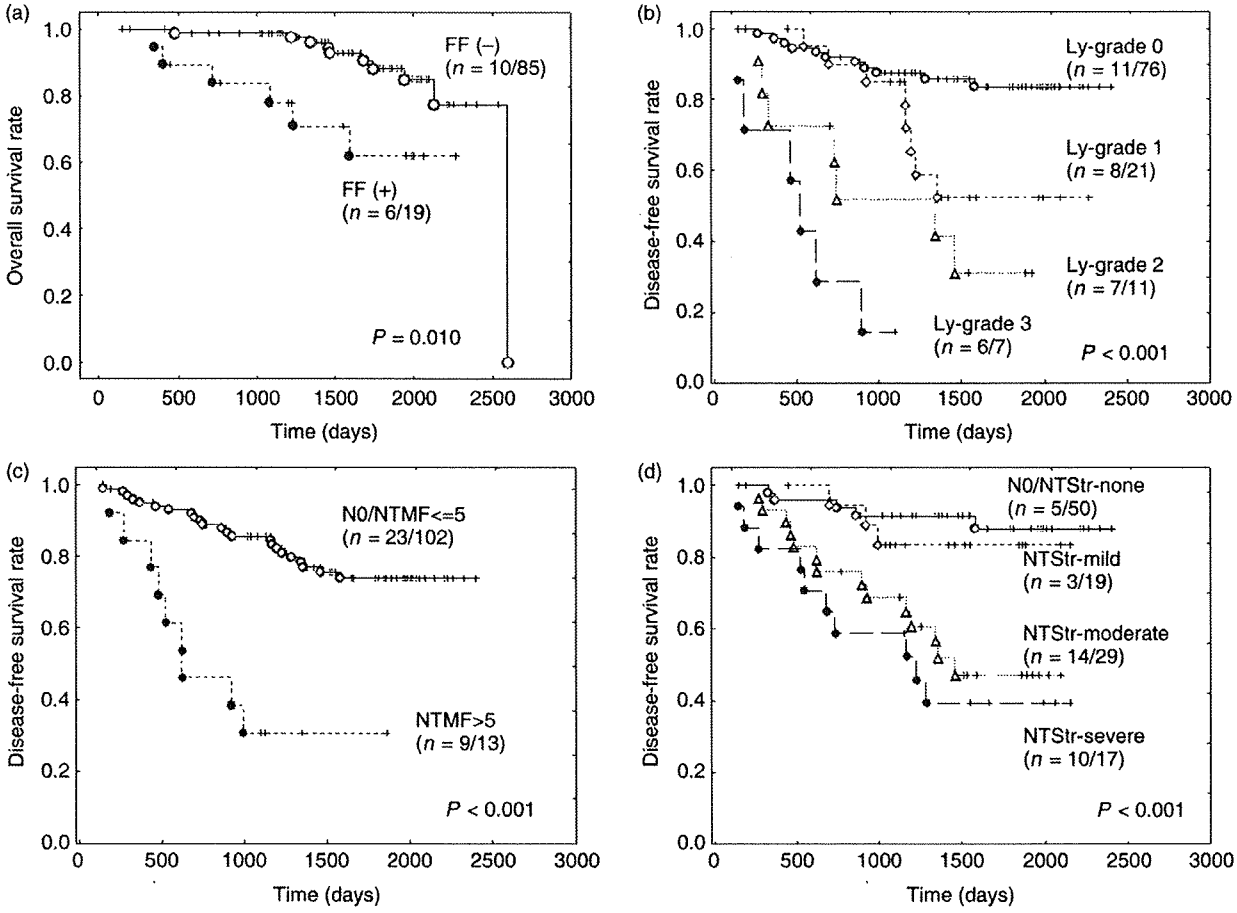

Fig. 2. (a–d) Overall survival curves and disease-free survival curves of invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy. (a) Patients with IDCs exhibiting fibrotic foci (FFs) assessed using biopsy specimens obtained before neoadjuvant chemotherapy have a significantly shorter overall survival period than patients with IDCs that do not exhibit FFs, as assessed using biopsy specimens obtained before neoadjuvant chemotherapy. (b) The disease-free survival of IDC patients classified according to a grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy decreases significantly according to the grade. Ly, lymph vessel tumor embolus or emboli. (c) The disease-free survival of IDC patients with nodal metastatic tumors containing five or more mitotic figures is significantly shorter than that of IDC patients without nodal metastasis or those with nodal metastatic tumors containing less than five mitotic figures. N0, no nodal metastasis; NTMF, mitotic figures in nodal metastatic tumor. (d) The disease-free survival of IDC patients classified according to the tumor stroma of nodal metastatic tumors decreases significantly according to the degree of fibrosis in the nodal metastatic tumors. NTStr, nodal metastatic tumor stroma.

biopsy materials obtained before neoadjuvant chemotherapy exhibited blood vessel invasion.

The univariate analyses of data for surgical materials obtained after neoadjuvant chemotherapy showed that skin invasion, the histologic grade of the primary invasive tumors, tumor necrosis, the grading system for LVTEs (Fig. 2b), the UICC pN category, nodal metastatic tumor stroma (Fig. 2d), five or more mitotic figures in nodal metastatic tumors (Fig. 2c), the histologic grade of the nodal metastatic tumors, the presence of a node with extranodal blood vessel tumor emboli, the presence of a node with extranodal invasion, and the UICC pTNM stage classification were significantly associated with tumor recurrence and tumor-related death (Table 3). Residual invasive tumor size, the presence of lymph vessel tumor emboli in the advanced area of primary invasive tumors, and the presence of lymph vessel tumor emboli in the non-tumor areas of primary invasive tumors were significantly associated with tumor recurrence but not tumor-related death, while the other factors were not significantly associated with tumor recurrence or tumor-related death in the univariate analyses (Table 3).

Overall, five or more mitotic figures in nodal metastatic tumors, and nodes with extranodal invasion were significantly associated with elevated hazard rates (HRs) for tumor recurrence and tumor-related death (Table 4, model 1). Clinical invasive tumor size, the presence of tumor necrosis (assessed using surgical materials), and severe nodal metastatic tumor stroma were significantly associated with elevated HRs for tumor recurrence (Table 4, model 1). Grade 2 LVTEs (assessed using biopsy materials) and the presence of FF (assessed using biopsy materials) were significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 4, model 1). In model 2, the grading system for LVTEs (assessed using surgical materials), five or more mitotic figures in nodal metastatic tumors, moderate to severe stroma in nodal metastatic tumors, and the presence of tumor necrosis (assessed using surgical materials) were significantly associated with elevated HRs for tumor recurrence; among these factors, grade 2 LVTEs (assessed using surgical materials), five or more mitotic figures in nodal metastatic tumors, and severe stroma in nodal metastatic tumors were also significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 4).

In patients with nodal metastasis, five or more mitotic figures in the nodal metastatic tumors was significantly associated with elevated HRs for tumor recurrence and tumor-related death,

Table 3. Association of clinicopathological factors using surgical materials obtained after neoadjuvant therapy with tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma (IDC) who received neoadjuvant chemotherapy

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Age, years					
≤39	20	4 (20)	0.332	2 (10)	0.622
>39	95	28 (30)		14 (15)	
Adjuvant therapy					
No	10	1 (10)	0.234	0	0.195
Yes	105	31 (30)		16 (15)	
Fisher's classification					
NIDC cases	17	2 (12)	0.095	1 (6)	0.162
IDC cases	98	30 (31)		15 (15)	
Grade classification of neoadjuvant chemotherapy according to the Japan Breast Cancer Society classification					
Grade 0	3	0	0.364	0	0.368
Grade 1a	48	14 (29)		6 (13)	
Grade 1b	31	10 (32)		6 (19)	
Grade 2	14	4 (29)		3 (21)	
Grade 3	17	2 (12)		1 (6)	
Residual invasive tumor size (mm)					
NIDC cases	17	2 (12)	0.014	1 (6)	0.163
≤20	33	9 (27)		7 (21)	
>20 to ≤50	45	11 (24)		4 (9)	
>50	20	10 (50)		4 (20)	
Skin invasion					
Absent	86	20 (24)	0.030	8 (9)	0.006
Present	29	12 (41)		8 (28)	
Histologic grade of primary invasive tumor					
NIDC cases	17	2 (12)	0.012	1 (6)	0.003
1	27	5 (19)		0	
2	48	17 (35)		11 (23)	
3	23	8 (35)		4 (17)	
Fibrotic focus					
Absent	88	23 (26)	0.417	12 (14)	0.687
Present	27	9 (33)		4 (15)	
Tumor necrosis					
Absent	88	20 (23)	0.010	10 (11)	0.038
Present	27	12 (44)		6 (22)	
Grading system for lymph vessel tumor emboli					
Grade 0	76	11 (14)	<0.001	7 (9)	<0.001
Grade 1	21	8 (38)		2 (10)	
Grade 2	11	7 (64)		5 (45)	
Grade 3	7	6 (86)		2 (30)	
Lymph vessel tumor emboli in the advance area					
Absent	91	20 (22)	0.003	10 (11)	0.328
Present	24	12 (50)		6 (30)	
Lymph vessel tumor emboli in the non-tumor stroma area					
Absent	88	18 (20)	<0.001	10 (11)	0.051
Present	27	14 (52)		6 (22)	
Blood vessel invasion					
Absent	6	1 (17)	0.510	1 (17)	0.757
Present	109	31 (28)		15 (14)	
UICC pN category					
N0	41	3 (7)	<0.001	2 (5)	0.032
N1	39	11 (28)		6 (15)	
N2	24	10 (42)		6 (25)	
N3	11	8 (73)		2 (18)	
Nodal metastatic tumor stroma					
N0/none	50	5 (10)	<0.001	4 (8)	0.009
Mild	19	3 (16)		1 (5)	
Moderate	29	14 (48)		6 (21)	
Severe	17	10 (59)		5 (29)	
Number of mitotic figures in nodal metastatic tumor (/1-high power field)					
N0/≤5	102	23 (23)	<0.001	12 (12)	0.002
>5	13	9 (69)		4 (31)	

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Table 3. *Continued*

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Histologic grade of nodal metastatic tumor					
N0	41	3 (7)	<0.001	2 (5)	0.022
1	10	5 (50)		1 (10)	
2	45	15 (33)		10 (22)	
3	19	9 (47)		3 (16)	
Nodes with extranodal blood vessel invasion					
N0	41	3 (7)	<0.001	2 (5)	0.004
Absent	45	13 (29)		5 (11)	
Present	29	16 (55)		9 (31)	
Nodes with extranodal invasion					
N0	41	3 (7)	<0.001	2 (5)	0.039
Absent	30	8 (27)		6 (20)	
Present	44	21 (48)		8 (18)	
UICC pTNM stage classification					
0	15	0	<0.001	0	0.014
I	1	0		0	
IIA	19	5 (26)		3 (16)	
IIB	30	4 (13)		3 (10)	
IIIA	23	9 (39)		4 (17)	
IIIB	16	6 (38)		4 (25)	
IIIC	11	8 (73)		2 (18)	
ER and PR status (n = 107)					
Negative	42	12 (29)	0.667	7 (17)	0.549
Positive	65	17 (26)		7 (11)	
HER2 status (n = 101)					
0 to 2	84	24 (29)	0.923	10 (11)	0.087
3	17	6 (35)		5 (29)	

ER and PR status negative, ER and PR both negative; ER and PR status positive, ER positive or PR positive, or both positive. N0, no nodal metastasis; N1, one to three nodal metastases; N2, four to nine nodal metastases; N3, 10 or more nodal metastases. ER, estrogen receptor; NIDC, non-invasive ductal carcinoma; pN, pathological regional lymph node; PR, progesterone receptor; UICC, International Union Against Cancer.

while clinical invasive tumor size and the UICC pN3 category were significantly associated with elevated HRs for tumor recurrence (Table 5, model 1). The presence of FF (assessed using biopsy materials) and the presence of nodes with extranodal invasion were significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 5, model 1). In model 2, the grading system for LVTEs (assessed using surgical materials), severe stroma in nodal metastatic tumors, and the presence of tumor necrosis (assessed using surgical materials) were significantly associated with elevated HRs for tumor recurrence in the multivariate analysis (Table 5). Grade 2 LVTEs (assessed using surgical materials) and five or more mitotic figures in nodal metastatic tumors were significantly associated with elevated HRs for tumor-related death in the multivariate analysis (Table 5).

Discussion

The results of this study clearly showed that a grading system for LVTEs (assessed using surgical materials) can be used to classify IDC patients with lymph vessel invasion who received neoadjuvant chemotherapy into low-risk, intermediate-risk, and high-risk groups; furthermore, this grading system for LVTEs was significantly associated with the HRs for tumor recurrence and tumor-related death in patients with IDC both overall and in patients with nodal metastasis, and the outcome predictive power of the grading system for LVTEs assessed using surgical materials was superior to that of the grading system for LVTE assessed using biopsy materials obtained before neoadjuvant chemotherapy. Although there have been many studies showing the prognostic usefulness of the presence of lymphatic invasion,[25–27] we previously demonstrated that the biological histological characteristics, especially mitotic figures and/or apoptotic figures, of tumor cells in lymph vessels are a more significant outcome predictor than the presence or absence of lymph vessel invasion or the number of lymph vessels that have been invaded.[28] We have also demonstrated that the location of lymph vessel invasion is an important outcome predictor for IDC patients,[18] but the result of this study clearly demonstrated that the grading system for LVTEs assessed using surgical materials is significantly superior to the location of lymph vessel invasion for accurately predicting the outcomes of IDC patients who have received neoadjuvant chemotherapy. Thus, this grading system for LVTEs assessed using surgical materials, but not biopsy materials, appears to be an excellent histological system for accurately predicting the outcome of IDC patients who do or do not receive neoadjuvant chemotherapy. Although we could not examine the outcome predictive power of the grading system for LVTEs in IDC patients without nodal metastasis in this study, we previously reported that this grading system for LVTEs assessed using surgical materials was a very important histological predictor of the prognosis of patients with IDC who did not receive neoadjuvant therapy independent of their nodal status.[11] Thus, the grading system for LVTE might be an important outcome predictor for IDC patients who have received neoadjuvant chemotherapy and do not have nodal metastasis, although the outcome predictive power of the grading system for LVTEs should be investigated in this patient

Table 4. Multivariate analyses for tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma (IDC) who received neoadjuvant chemotherapy

Factors	Tumor recurrence			Tumor-related death		
	HRs	95% CI	*P*-values	HRs	95% CI	*P*-values
Model 1						
Clinical invasive tumor size (mm) before neoadjuvant chemotherapy						
>20 to ≤50	Referent			Referent		
>50	2.2	1.1–4.4	0.034	–	–	
Grading system for lymph vessel tumor emboli assessed using biopsy materials obtained before neoadjuvant chemotherapy						
Grade 0	Referent			Referent		
Grade 1	–	–		0.7	0.03–14.2	0.796
Grade 2	–	–		5.9	1.3–27.9	0.025
Fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	–	–		6.2	1.9–19.6	0.002
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	2.9	1.1–8.0	0.034	1.2	0.2–9.1	0.868
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and ≤5	Referent			Referent		
>5	4.5	1.7–11.9	0.003	7.5	1.7–31.5	0.006
Nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and absent	Referent			Referent		
Present	4.8	2.3–10.6	<0.001	5.0	1.7–14.7	0.003
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and none	Referent			Referent		
Mild	0.7	0.1–5.3	0.719	1.1	0.04–28.3	0.967
Moderate	1.3	0.2–7.8	0.771	4.5	0.3–76.9	0.302
Severe	3.9	1.6–9.2	0.002	7.6	0.3–183.9	0.214
Model 2						
Grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy						
Grade 0	Referent			Referent		
Grade 1	3.2	1.2–8.6	0.020	0.2	0.01–3.8	0.302
Grade 2	9.5	3.3–27.3	<0.001	5.9	1.9–18.8	0.002
Grade 3	5.5	1.7–17.4	0.004	5.3	0.5–61.6	0.183
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	3.1	1.1–8.8	0.038	2.4	0.8–13.3	0.300
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and ≤5	Referent			Referent		
>5	3.7	1.2–11.7	0.027	12.6	3.2–48.5	<0.001
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and none	Referent			Referent		
Mild	0.4	0.05–3.1	0.366	0.2	0.01–6.9	0.395
Moderate	2.9	1.2–7.1	0.017	1.3	0.1–17.9	0.856
Severe	10.0	3.3–20.9	<0.001	3.5	1.1–10.8	0.034

–/–, not significant in univariate analysis; CI, confidence interval; HR, hazard rate; N0, no nodal metastasis.

population. Since the presently described grading system for LVTEs is based on assessments of mitotic figures and apoptotic figures in tumor cells located in lymph vessels, tumor cells with a high turnover rate in lymph vessels are more likely to be capable of spreading tumor nests throughout the lymph vessels than tumor cells with a low turnover rate. Thus, factors that accelerate the turnover rate of tumor cells in lymph vessels are probably very important for explaining the significant outcome of the predictive power of this grading system for LVTEs.

The histological characteristics of the nodal metastatic tumors were also significantly associated with tumor recurrence or tumor-related death in the patients with IDCs who received neoadjuvant chemotherapy in the current study. Among these histological characteristics, the degree of nodal tumor stroma and the number of mitotic figures in the nodal metastatic tumors were the most accurate predictors of outcome among the IDC patients who received neoadjuvant chemotherapy. We previously reported that severe tumor stroma and the number of mitotic figures in nodal metastatic tumors are significant predictors of outcome among IDC patients with nodal metastasis who did not receive neoadjuvant chemotherapy.[12,29] Thus, this study clearly confirmed that these two factors are also significant histological predictors of outcome among IDC patients with nodal metastasis who received neoadjuvant chemotherapy. We previously reported that the proliferative activity of tumor–stromal fibroblasts plays a very important role in nodal metastasis and distant organ metastasis by IDCs,[30,31] and that growth factors produced by tumor cells and tumor stromal cells play a very important role in tumor progression by IDC.[32] These findings strongly suggest that the tumor stroma plays a significant role in tumor progression in IDC. Furthermore, the gene expression profile and the protein expression profile of the tumor stroma have recently

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Table 5. Multivariate analyses for tumor recurrence and tumor-related death in lymph node–metastasis-positive invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	74	Present (n = 29)	HRs/95% CI P-values	Present (n = 14)	HRs/95% CI P-values
Model 1					
Clinical invasive tumor size (mm) before neoadjuvant chemotherapy					
>20 to ≤50	41	11 (27)	Referent	6 (15)	Referent
>50	33	18 (54)	2.7/1.2–5.7 0.013	8 (24)	–/–
Fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy					
Absent	60	22 (37)	Referent	8 (13)	Referent
Present	13	7 (54)	–/–	6 (46)	7.0/2.2–22.3 <0.001
UICC pN category					
N1	39	11 (28)	Referent	6 (15)	Referent
N2	24	10 (42)	2.3/0.6–8.0 0.211	6 (25)	–/–
N3	11	8 (73)	3.4/1.4–8.1 0.005	2 (18)	–/–
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant therapy					
≤5	61	20 (33)	Referent	10 (16)	Referent
>5	13	9 (69)	3.9/1.6–9.1 0.002	4 (31)	8.6/2.0–37.0 0.004
Nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy					
Absent	45	13 (29)	Referent	5 (11)	Referent
Present	29	16 (55)	2.4/0.7–7.7 0.143	9 (31)	5.3/1.5–18.3 0.007
Model 2					
Grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy					
Grade 0	39	9 (23)	Referent	6 (15)	Referent
Grade 1	19	8 (42)	2.7/1.0–7.4 0.047	2 (11)	1.2/0.7–7.0 0.872
Grade 2	9	6 (67)	8.5/2.6–27.8 <0.001	4 (44)	3.9/1.1–13.7 0.035
Grade 3	7	6 (86)	8.0/2.5–26.0 <0.001	2 (29)	3.4/0.6–19.2 0.172
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant therapy					
N0/none	9	2 (22)	Referent	2 (22)	Referent
Mild	19	3 (16)	0.8/0.1–6.9 0.826	1 (5)	–/–
Moderate	29	14 (48)	3.7/0.6–23.9 0.168	6 (21)	–/–
Severe	17	10 (59)	5.3/2.0–14.2 <0.001	5 (29)	–/–
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy					
Absent	54	18 (33)	Referent	9 (17)	Referent
Present	20	11 (55)	5.3/1.7–16.4 0.004	5 (25)	–/–
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy					
≤5	61	20 (33)	Referent	10 (16)	Referent
>5	13	9 (69)	2.0/0.4–9.5 0.376	4 (31)	6.1/1.6–22.9 0.008

–/–, not significant in univariate analysis; CI, confidence interval; HR, hazard rate; NIDC, non-invasive ductal carcinoma; pN, pathological regional lymph node; UICC, International Union Against Cancer.

Model 1

Tumor recurrence: adjusted for clinical invasive tumor size before neoadjuvant chemotherapy, tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy, nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, UICC pTNM-pN category assessed using surgical materials obtained after neoadjuvant chemotherapy, nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy, and histologic grade of primary invasive tumors assessed using surgical materials obtained after neoadjuvant chemotherapy.

Tumor-related death: adjusted for fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, and nodes with extranodal blood vessel invasion assessed using surgical materials obtained after neoadjuvant chemotherapy.

Model 2

Tumor recurrence: adjusted for grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy, tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy, nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, UICC pTNM-pN category assessed using surgical materials obtained after neoadjuvant chemotherapy, nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy, and histologic grade of nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy.

Tumor-related death: adjusted for grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, and nodes with extranodal blood vessel invasion assessed using surgical materials obtained after neoadjuvant chemotherapy.

been reported to play a very important roles in tumor progression in carcinoma,[33–35] and the interaction between tumor cells and stromal cells also plays a very important role in tumor progression in carcinoma.[36–38] Thus, tumor cell–stromal cell interactions probably heighten the malignant potential of nodal metastatic tumors with moderate to severe tumor stroma. Furthermore, in previous studies we and others have reported that a characteristic histological feature of the tumor stroma in primary invasive tumors, an FF, is a very useful prognostic histological tumor–stromal indicator for accurately predicting the outcome of IDC patients who did not receive neoadjuvant therapy;[16,17,39,40] the present study clearly demonstrated that the presence of FFs (assessed using biopsy materials obtained before neoadjuvant chemotherapy, but not using surgical materials obtained after neoadjuvant chemotherapy) was a significant tumor-death-related factor. Thus, tumor cell–stromal cell interactions in nodal metastatic tumors as well as in primary invasive tumors probably play very important roles in the progression of IDCs that have been treated with neoadjuvant chemotherapy, and in IDC patients who have received neoadjuvant chemotherapy, the outcome predictive power of FFs should be assessed using biopsy materials obtained before neoadjuvant chemotherapy.

The grading system for LVTEs assessed using surgical materials and the histological features of the nodal metastatic tumors mentioned above were superior to Fisher's classification or the classification of the JBCS for neoadjuvant chemotherapy for predicting the outcome of IDC patients who had received neoadjuvant chemotherapy in this study. The classification of the JBCS for neoadjuvant chemotherapy assesses the degree of fibrosis or the presence or absence of tumor necrosis in primary invasive tumors and tumors metastasizing to the lymph node, and a severe degree of fibrosis and the presence of tumor necrosis are considered as histological findings predicting a good response to neoadjuvant chemotherapy.[21] In the classification of JBCS for neoadjuvant chemotherapy, a complete response (grade 3) is regarded as necrosis or the disappearance of all tumor cells, with all carcinoma cells being replaced by granuloma-like and/or fibrous tissue. However, this study clearly demonstrated that the presence of tumor necrosis in primary invasive tumors and a moderate to severe degree of fibrosis in nodal metastatic tumors were important histological predictors of a poor prognosis among IDC patients who have received neoadjuvant chemotherapy. Therefore, determining whether the presence of tumor necrosis or the presence of tumor–stromal dense fibrosis in IDCs treated with neoadjuvant chemotherapy have truly been produced by neoadjuvant chemotherapy or not is of great importance, and the latter finding strongly suggests that the presence of tumor necrosis or the presence of tumor–stromal dense fibrosis may reflect biological tumor characteristics that are closely associated with a poor outcome among patients with IDCs. The tumor-related predictive ability of the presence of FF assessed using biopsy materials obtained before neoadjuvant chemotherapy was lost when the presence of FF was assessed using surgical materials obtained after neoadjuvant chemotherapy. This strongly suggests that FF-like stromal changes produced by neoadjuvant chemotherapy probably occurred in the IDCs treated with neoadjuvant chemotherapy, and the true FFs could not be differentiated from the FF-like stromal changes in IDCs. Thus, when the presence of tumor necrosis in primary invasive tumors or the presence of moderate to severe fibrosis in nodal metastatic tumors is observed during the pathological examination of IDCs treated with neoadjuvant therapy, the pathological assessment of the response to neoadjuvant chemotherapy should be carefully assessed as to whether the presence of tumor necrosis in primary invasive tumors or moderate to severe fibrosis in nodal metastatic tumors truly demonstrates a response to neoadjuvant chemotherapy. Although the outcome predictive power of FFs among patients with IDC was lost after neoadjuvant chemotherapy, the histological factors maintained their significant outcome predictive power among IDC patients who received neoadjuvant chemotherapy. Thus, pathologists carefully assess the response to neoadjuvant chemotherapy based on the presence of tumor necrosis in primary invasive tumors or the degree of fibrosis in nodal metastatic tumors, since pathologists might misjudge IDC patients who have received neoadjuvant chemotherapy and whose primary invasive tumors exhibited tumor necrosis or whose nodal metastatic tumors exhibited dense fibrosis as having attained a good response to neoadjuvant chemotherapy.

The results of this study clearly demonstrated that many histological factors of tumors assessed using biopsy materials, such as histologic grade and tumor necrosis, failed to show a significant association with tumor recurrence or tumor-related death. These findings strongly suggest that biopsy materials containing small amounts of primary invasive tumors do not accurately reflect the true biological malignant potential of IDCs. Thus, with the exception of evaluating the presence of FF, histological evaluations of the malignant potential of IDCs treated using neoadjuvant chemotherapy should be performed using surgical materials obtained after neoadjuvant chemotherapy.

In conclusion, this is the first study to clearly demonstrate that the presence of FF in biopsy materials obtained before neoadjuvant chemotherapy, the grading system for LVTEs in surgical materials obtained after neoadjuvant chemotherapy, and the histological characteristics of nodal metastatic tumors in surgical materials obtained after neoadjuvant chemotherapy were strongly associated with the outcome of IDC patients who received neoadjuvant chemotherapy. In the future, the following topics should be examined to clarify the tumor progression of IDCs treated with neoadjuvant chemotherapy based on the data in this study: (1) the functions of tumor cells in lymph vessels and nodal metastatic tumor cells should be determined; (2) the factors that accelerate the proliferative activity of tumor cells in lymph vessels or lymph nodes should be identified; and (3) the factors that accelerate tumor cell–stromal cell interactions in nodal metastatic tumors should be discerned.

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p53 expression in tumor stromal fibroblasts is associated with the outcome of patients with invasive ductal carcinoma of the breast

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The purpose of this study was to determine whether p53 protein expression in tumor stromal fibroblasts assessed immunohistochemically by the Allred score system is significantly associated with nodal metastasis by invasive ductal carcinoma (IDC), and significantly associated with the outcome of 1042 IDC patients according to adjuvant therapy status, UICC pTNM stage, and triple-negative IDC status, in multivariate analyses with well-known clinicopathological factors. The Allred scores for p53 expression in tumor stromal fibroblasts were significantly associated with the number of nodal metastases, and Allred scores of 4–8 for p53 in tumor stromal fibroblasts significantly increased the hazard rate for distant organ metastasis or for tumor death in the triple-negative IDC patients, and the UICC pTNM stage I, II, and III patients. The results indicated that p53 protein expression in tumor stromal fibroblasts is closely associated with the number of nodal metastases and the outcome of IDC patients. (*Cancer Sci* 2009; 100: 2101–2108)

It has recently been reported that the gene expression and protein expression profiles of the tumor stroma play very important roles in tumor progression in carcinoma,[1–3] and the interaction between tumor and stromal cells also plays a very important role in tumor progression by carcinoma.[4–6] We and others have already reported that a characteristic histological feature of tumor stroma, a fibrotic focus, is a very useful prognostic histological tumor stromal indicator for accurately predicting the outcome of patients with invasive ductal carcinoma (IDC),[7–10] and that growth factors produced by tumor cells and tumor stromal cells play a very important role in tumor progression by IDC.[11] In addition, proliferative activity of tumor stromal fibroblasts plays a very important role in nodal metastasis and distant organ metastasis by IDC.[12,13] These findings strongly suggest a significant role of the tumor stroma in tumor progression by IDC.

p53 is the most commonly mutated gene in human neoplasms,[14] and the p53 tumor suppressor protein is involved in the cell cycle, checkpoint control, repair of DNA damage, and apoptosis.[15,16] Also, besides their well-studied cell-autonomous role in cancer cells, mutations of the *p53* tumor suppressor gene have been described in stromal fibroblasts of breast and prostate carcinoma in humans and experimental animals.[17–20] A high frequency of *p53* mutations in tumor cells and the surrounding stroma has also reported,[17] and *p53* mutations in breast cancer stromal cells have been reported to be closely associated with nodal metastasis.[21] Based on the above findings, the *p53* status of tumor stromal fibroblasts may play a very important role in carcinoma progression by IDC.

The purpose of the present study was to determine whether p53 protein expression in tumor stromal fibroblasts is significantly associated with nodal metastasis by IDC, and significantly associated with the outcome of IDC patients with and without adjuvant therapy according to UICC pTNM stage, and triple-negative IDC status. The results indicated that p53 protein expression in tumor stromal fibroblasts is closely associated with the number of nodal metastases and the outcome of IDC patients.

Materials and Methods

Cases. The subjects of the present study were 1042 consecutive patients with IDC of the breast surgically treated at the National Cancer Center Hospital (Tsukiji, Tokyo) between January 2000 and December 2005. The IDC were diagnosed preoperatively by aspiration cytology, mammography, or ultrasonography. Clinical information was obtained from the patients' medical records after complete histological examination of all IDC. All patients were Japanese women, and they ranged in age from 23 to 77 years (median, 55 years). All had a solitary lesion; 497 patients were premenopausal, and 545 were postmenopausal. Partial mastectomy had been carried out in 462, and modified radical mastectomy in 580. Levels I and II axillary lymph node dissection had been carried out in all patients, and level III axillary lymph node dissection had been carried out in some of the IDC patients.

Of the 1042 patients who did not receive neoadjuvant therapy, 873 had received adjuvant therapy, which consisted of chemotherapy in 209 patients, endocrine therapy in 294 patients, and chemoendocrine therapy in 370 patients. The chemotherapy regimens used were anthracycline-based with or without taxane and non-anthracycline-based, and the endocrine therapy regimens consisted of tamoxifen with or without a gonadotropin-releasing hormone agonist, tamoxifen, with or without an aromatase inhibitor, an aromatase inhibitor alone, or a gonadotropin-releasing hormone agonist alone. There were no cases of inflammatory breast cancer in this series. All tumors were classified according to the pathological UICC-TNM (pTNM) classification.[22] The protocol of this study was reviewed by the institutional review board of the National Cancer Center (20-112), and all patients provided written informed consent.

For pathological examination, the surgically resected specimens were fixed in 10% formalin, and the size and gross appearance of the tumors were recorded. Their size was confirmed by

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comparison with tumor size on histological slides, and if there was more than one invasive focus, the size of the largest invasive focus was recorded as the invasive tumor size in this study.

Histological examination. Serial sections of each tumor area were cut from paraffin blocks. One section from each tumor was stained with hematoxylin and eosin (HE) and examined histologically to confirm the diagnosis, and another section was subjected to immunohistochemistry. The following 10 histological factors were evaluated: (1) invasive tumor size (≤20, 20–50, >50 mm); (2) histological grade (1–3);[23] (3) tumor necrosis (absent, present);[24] (4) fibrotic focus (FF) (absent, FF diameter ≤ 8 mm, FF diameter > 8 mm);[7,8] (5) lymphatic invasion (absent, present); (6) blood vessel invasion (absent, present); (8) adipose tissue invasion (absent, length ≤ 2 mm, length > 2 mm);[25] (9) skin invasion (absent, present); and (10) muscle invasion (absent, present).

Immunohistochemistry. Immunohistochemical staining for estrogen receptors (ER), progesterone receptors (PR), p53, and HER2 products was carried out with an autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA, USA). Antigen retrieval device for these antibodies and each specimen was immersed in citrate buffer and incubated at 121°C for 10 min. Immunoperoxidase staining was carried out using a labeled streptavidin–biotin (LSAB) staining kit (BioGenex) according to the manufacturer's instructions. The antibodies used were anti-ER mouse mAb (ER88; BioGenex), an anti-PR mAb (PR88; BioGenex), an anti-HER2 mAb (CB11; BioGnex), and a p53 mAb (DO7; Dako, Glostrup, Denmark). ER88, PR88, and CB11 were already diluted and DO7 was applied at 1:100 dilution. After immunostaining, the sections were counterstained with hematoxylin. Sections of IDC positive for ER, PR, HER2, and p53 were used each time as positive internal or external

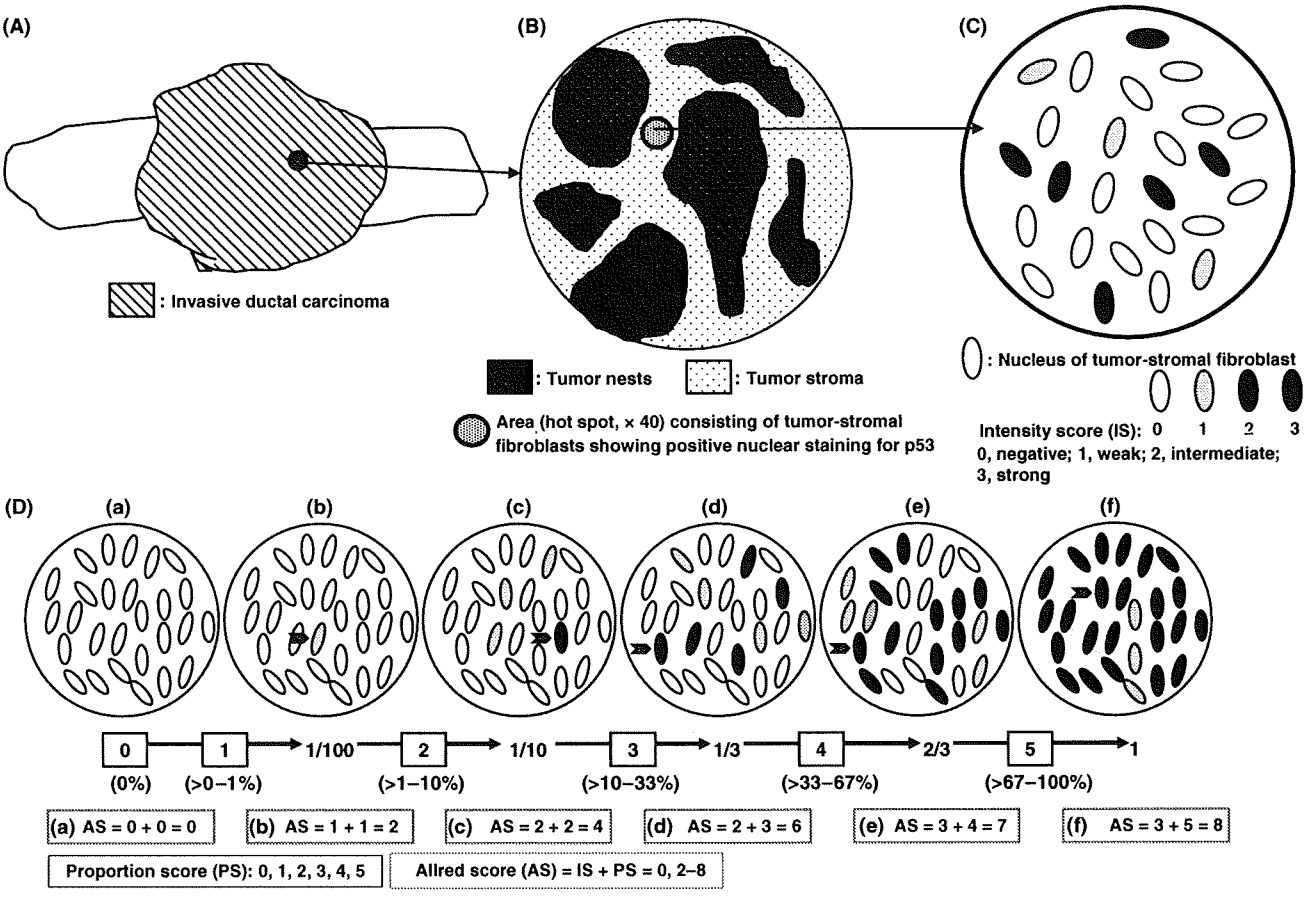

Fig. 1. Method for immunohistochemical assessment of tumor stromal fibroblasts in invasive ductal carcinoma (IDC). (A–D) First, sections on slides were examined for the presence or absence of p53 expression in tumor stromal fibroblasts in a medium-power field (×10 objective and ×10 ocular or ×20 objective and ×10 ocular), and areas in which tumor stromal fibroblasts showed p53 expression were found. Intensity score (IS) and proportion score (PS) were then assigned for p53 expression in tumor stromal fibroblasts in one high-power field in each medium-power field in which staining was found (×40 objective and ×10 ocular). The high-power field with the highest Allred score (IS + PS) for p53 expression was selected as the hot spot in the tumor. (C) Negative and positive tumor stromal fibroblasts for p53 expression were observed in the same high-power field, the hot spot in this tumor (×40 objective and ×10 ocular). Of the tumor stromal fibroblasts that showed positive nuclear staining for p53, two had a strong IS of 3 for p53, four had an IS of 2, and three had an IS of 1. The IS of tumor stromal fibroblasts for p53 expression in this case was 3. (D) PS for p53 expression. PS ranged from 0 to 5, and the highest PS was recorded as the PS of the case. The IS and PS for p53 expression in tumor stromal fibroblasts were then added to obtain a total score, the Allred score (AS), with total scores of 0 and 2–8. There was a hot spot in the tumor in each of the six IDC cases (a–f). The IDC case with hot spot a had an AS of 0, and the AS of the IDC case with hot spot b was 2. The IDC cases having hot spots c, d, e, and f had AS of 4, 6, 7, and 8, respectively. The IS for p53 in tumor stromal fibroblasts in each case were based on the tumor stromal fibroblasts with the highest IS for p53 expression (arrowheads).

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controls. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin.

Assessment of ER, PR, p53, and HER2 expression. Slides immunostained for ER, PR, and p53 in tumor cells were scored by the Allred scoring system as described previously.[26–30] Although the validity of the Allred scoring system for assessing expression of ER, PR, and p53 in tumor cells has been demonstrated,[26–30] the number of tumor stromal fibroblasts that express p53 in tumors is relatively small, and the distribution of tumor stromal fibroblasts expressing p53 is scattered even in IDC with tumor stromal fibroblasts having Allred scores of 4–8. We therefore modified the Allred scoring system to assess expression of p53 in tumor stromal fibroblasts by identifying the field with the highest proportion score (PS) and intensity score (IS) for p53 expression in the tumor area (hot spot) by scanning the entire tumor section stained for p53 at medium power (×10 objective and ×10 ocular) (Fig. 1A,B). The highest IS (0, none; 1, weak; 2, intermediate; 3, strong) for expression of p53, not the average IS in the original,[26–30] was assigned for tumor stromal fibroblasts (Figs 1C,D,2A–F), and the highest p53

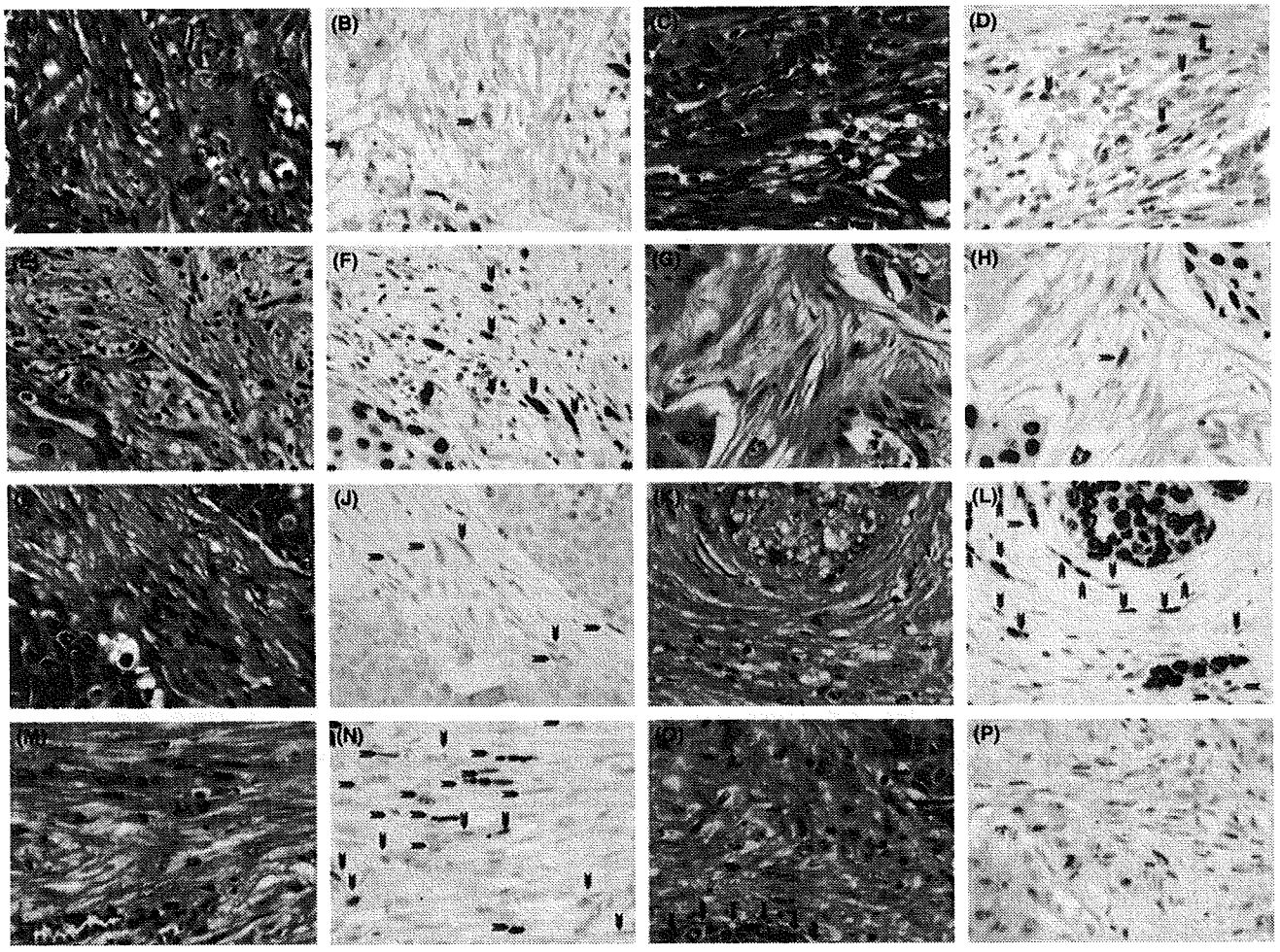


Fig. 2. (A,C,E,G,I,K,M,O) Histological features of tumor stromal fibroblasts, (B,D,F) intensity scores (IS), and (H,J,L,N,P) proportion scores (PS) for p53 expression in tumor stromal fibroblasts of invasive ductal carcinomas (IDC) in high-power fields (×40, hot spots). In general, tumor stromal fibroblasts had spindled acidophilic cytoplasm and oval nuclei, and were mixed with collagen fibers. Nucleoli of tumor stromal fibroblasts were inconspicuous. However, some tumor stromal fibroblasts exhibited epithelioid features, and had enlarged round to oval nuclei containing small nucleoli. Thus, the pathologist should confirm that cells showing p53 expression are tumor stromal fibroblasts or tumor cells not only by immunostaining, but also by hematoxylin–eosin staining. The tumor stroma contained tumor stromal fibroblasts with (A,B) an IS of 1 and (C,D) an IS of 2 for p53 expression (arrowheads). No tumor cells exhibited p53 expression (lower-right corner). (E,F) Tumor stromal fibroblasts with an IS of 3 for p53 expression were observed in the tumor stroma (arrowheads). Tumor cells with an IS of 3 or 2 for p53 expression are also observed (lower-left corner). (G,H) An Allred score (AS) of 3 for p53 expression in tumor stromal fibroblasts. One tumor stromal fibroblast in the high-power field had an IS of 2 for p53 expression (×40, hot spot, arrowhead). Tumor cells with an AS of 8 for p53 were also observed (lower-left corner and upper-right corner). (I,J) Tumor stromal fibroblasts with an IS of 1 or 2 for p53 expression were visible in the tumor stroma (arrowheads), and the PS of the tumor fibroblasts in this case was 2. Thus, the AS of the tumor stromal fibroblasts in the case was 4. Tumor cells were negative for p53 nuclear staining (upper-right corner and lower-left corner). (K,L) Tumor stromal fibroblasts with an IS of 3 or 2 for p53 expression were observed in the tumor stroma (arrowheads). The PS of the tumor stromal fibroblasts for p53 in this case was 3, and the AS of the tumor stromal fibroblasts for p53 expression in this case was 6. Tumor cells with an IS of 3 for p53 expression were also observed (upper-center and lower-right corner). (M,N) Tumor stromal fibroblasts had an IS of 3 or 2 for p53 expression (arrowheads), and the PS of the tumor stromal fibroblasts for p53 was 4. The AS of tumor stromal fibroblasts for p53 expression of this case is 7. No tumor cells are visible. (O and P) Many tumor stromal fibroblasts with an IS of 2 or 1 for p53 expression were observed in the tumor stroma between tumor cell nests (arrows), and their PS for p53 was 5. The AS of the tumor stromal fibroblasts for p53 expression in this case was 7.

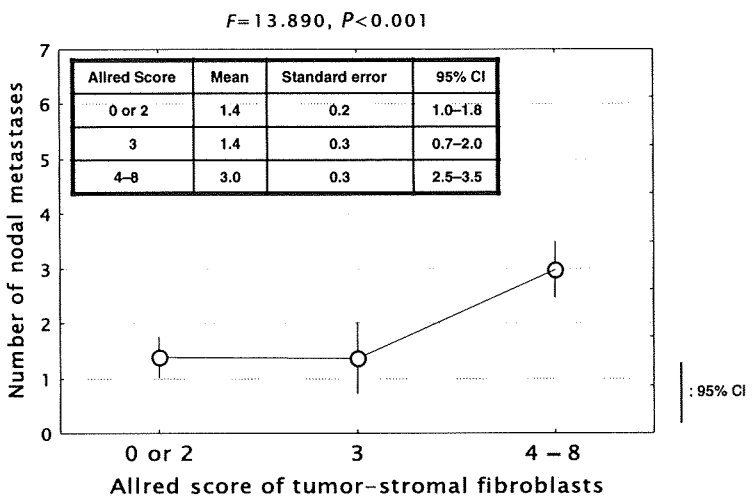
expression PS (0–5) was then to be evaluated in one high-power field (hot spot, ×40 objective and ×10 ocular) (Figs 1C,D,2G–P). The PS and IS of the tumor stromal fibroblasts were then added to obtain a total score, with possible total score of 0 and ranging from 2 to 8 (Figs 1D,2G–P). When examining tumor stromal fibroblasts for p53 staining, we always confirmed by HE stained specimen whether the cells that show positive staining for p53 is tumor-stromal fibroblasts or not. The HER2 status of the tumor cells was semiquantitatively scored on a 0–3 scale according to the level of HER2 protein expression.[31] Immunohistochemistry was used to score 1025 of the 1042 IDC for ER, PR, or HER2 expression and to score 1026 of them for p53 expression.

One author (TH) assessed all of the immunohistochemical parameters, and one of three other authors (HT, TS, or YS) identified the immunohistochemical parameters to confirm the IDC immunohistochemical characteristics recorded by TH. Discordant results were reevaluated jointly to reach a consensus. The histological and immunohistochemical examinations were carried out without knowledge of the patients' outcomes.

Patient outcome and statistical analysis. Survival was evaluated by follow up for a median period of 52 months (range, 18–102 months) until June 2008. Of the 1042 IDC patients, 924 patients were alive and well, 118 had developed tumor recurrence, and 29 had died of their disease, and an initial distant organ metastasis was observed in 85 of the 118 IDC patients with tumor recurrence. The measurements of tumor recurrence-free survival, initial distant organ metastasis-free survival, and overall survival started on the day of surgery. Tumor relapse was considered to have occurred whenever there was evidence of metastasis.

The Allred scores for ER, PR, and p53 expression in tumor cells and tumor stromal fibroblasts were classified into three categories according to the univariate analyses by the Cox proportional hazard regression model most significantly associated with tumor recurrence: (1) the Allred scores for ER in tumor cells were classified into the three categories 0 or 2, 3–6, and 7 or 8; (2) the Allred scores for PR in tumor cells were classified into the categories 0 or 2, 3–6, and 7 or 8; (3) the Allred scores for p53 in tumor cells were classified into the three categories 0 or 2 or 3, 4–6, and 7 or 8; and (4) the Allred scores for p53 in tumor stromal fibroblasts were classified into the three categories 0 or 2, 3, and 4–8. HER2 expression in tumor cells was classified into the three categories: 0 or 1, 2, and 3.

(A)



(B) Multiple regression analyses for the increase of number of nodal metastasis in invasive ductal carcinoma patients (n = 1021)

ß	Standard error	*P*-value
Invasive tumor size (<=20, >20 to <=50, and >50 mm)		
0.108	0.031	<0.001
Lymph vessel invasion		
0.199	0.003	<0.001
Skin invasion (absent and present)		
0.167	0.030	<0.001
p53 Allred scores in tumor-stromal fibroblasts (0 or 2, 3, and 4 to 8)		
0.091	0.030	0.002
Blood vessel invasion (absent and present)		
0.077	0.029	0.009
Histologic grade (1, 2, and 3)		
0.076	0.032	0.018
Progesterone receptor Allred scores in tumor cells (0 or 2, 3–6, and 7 or 8)		
–0.070	0.031	0.024

Fig. 3. Associations between (A) the number of nodal metastases and Allred scores for p53 in tumor stromal fibroblasts and (B) factors that were significantly associated with the number of nodal metastases in the multivariate analyses. (A) In invasive ductal carcinoma (IDC) patients, the increase in number of nodal metastases was significantly associated with the Allred scores for p53 in tumor stromal fibroblasts. (B) Multiple regression analysis revealed the factors that were significantly associated with the increase in number of nodal metastases in IDC patients. CI, confidence interval.

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