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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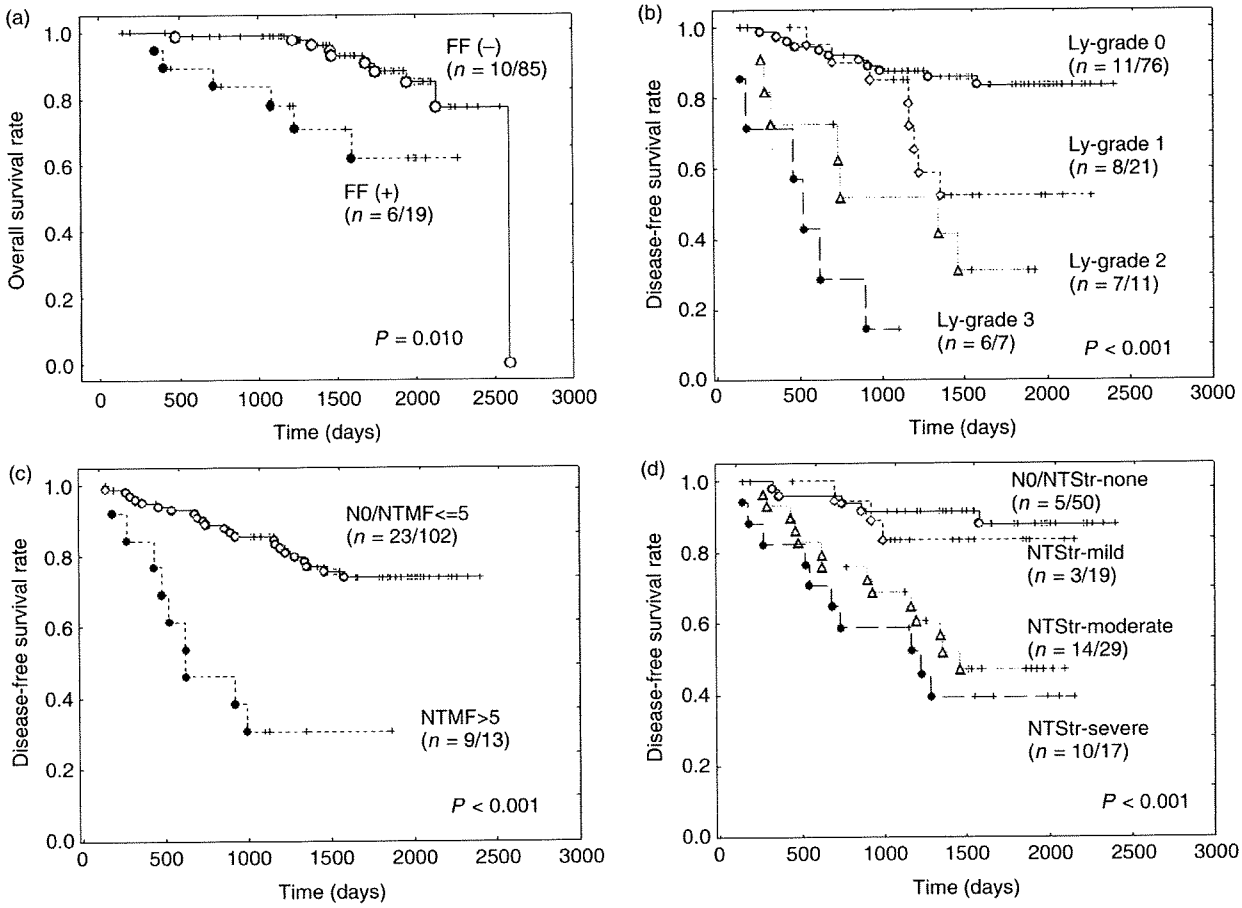

Fig. 2. (a–d) Overall survival curves and disease-free survival curves of invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy. (a) Patients with IDCs exhibiting fibrotic foci (FFs) assessed using biopsy specimens obtained before neoadjuvant chemotherapy have a significantly 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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References

1 Ragaz J, Baird R, Rebbeck P, Goldie A, Coldman A, Spinelli J. Preoperative adjuvant chemotherapy (neoadjuvant) for carcinoma of the breast: rationale and safety report. *Recent Results Cancer Res* 1985; **98**: 99–105.
2 Ragaz J. Preoperative (neoadjuvant) chemotherapy for breast cancer: outline of the British Columbia Trial. *Recent Results Cancer Res* 1986; **103**: 85–94.
3 Ferriere JP, Assier I, Cure H *et al.* Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol* 1998; **21**: 117–20.
4 Daidone MG, Silvestrini R, Luisi A *et al.* Changes in biological markers after primary chemotherapy for breast cancers. *Int J Cancer* 1995; **61**: 301–5.
5 Cavailles V, Gompel A, Portois MC *et al.* Comparative activity of pulsed or continuous estradiol exposure on gene expression and proliferation of normal and tumoral human breast cells. *J Mol Endocrinol* 2002; **28**: 165–75.
6 Koukourakis MI, Simopoulos C, Polychronidis A *et al.* The effect of trastuzumab/docatexel combination on breast cancer angiogenesis: dichotomus effect predictable by the HIFI alpha/VEGF pre-treatment status? *Anticancer Res* 2003; **23**: 1673–80.
7 Petit T, Borel C, Ghnassia JP *et al.* Chemotherapy response of breast cancer depends on HER-2 status and anthracycline dose intensity in the neoadjuvant setting. *Clin Cancer Res* 2001; **7**: 1577–81.
8 Chollet P, Amat S, Cure H *et al.* Prognostic significance of a complete

doi: 10.1111/j.1349-7006.2009.01264.x

pathological response after induction chemotherapy in operable breast cancer. *Br J Cancer* 2002; **86**: 1041–6.
9 Penault-Llorca F, Vincent-Salomon A. Roles of the pathologist in neoadjuvant chemotherapy: evaluation of response, prognostic and predictive factors. *Ann Pathol* 2003; **23**: 555–63.
10 Bollet MA, Sigal-Zafrani B, Gambotti L *et al.* Pathological response to preoperative concurrent chemo-radiotherapy for breast cancer: results of a phase II study. *Eur J Cancer* 2006; **42**: 2286–95.
11 Hasebe T, Yamauchi C, Iwasaki M *et al.* Grading system for lymph vessel tumor emboli for prediction of the outcome of invasive ductal carcinoma of the breast. *Hum Pathol* 2008; **39**: 427–36.
12 Hasebe T, Sasaki S, Imoto S *et al.* Histological characteristics of tumor in vessels and lymph nodes are significant predictors of progression of invasive ductal carcinoma of the breast: a prospective study. *Hum Pathol* 2004; **35**: 298–308.
13 Sobin LH, Wittekind CH, eds. *TNM Classification of Malignant Tumors*, Geneva: Wiley-Liss, 2002; 131–141.
14 Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957; **11**: 359–77.
15 Gilchrist KW, Gray R, Fowble B *et al.* Tumor necrosis is a prognostic predictor for early recurrence and death in lymph node-positive breast cancer: a 10-year follow-up study of 728 eastern cooperative oncology group patients. *J Clin Oncol* 1993; **11**: 1929–35.
16 Hasebe T, Tsuda H, Hirohashi S *et al.* Fibrotic focus in infiltrating ductal carcinoma of the breast: a significant histopathological prognostic parameter for predicting the long-term survival of the patients. *Breast Cancer Res Treat* 1998; **49**: 195–208.
17 Hasebe T, Sasaki S, Imoto S *et al.* Prognostic significance of fibrotic focus in invasive ductal carcinoma of the breast: a prospective observational study. *Mod Pathol* 2002; **15**: 502–16.
18 Yamauchi C, Hasebe T, Iwasaki M *et al.* Accurate assessment of lymph vessel tumor emboli in invasive ductal carcinoma of the breast according to tumor areas, and their prognostic significance. *Hum Pathol* 2007; **38**: 247–59.
19 Fisher B. Biological and clinical considerations regarding the use of surgery and chemotherapy in the treatment of primary breast cancer. *Cancer* 1977; **40**: 574–87.
20 Fisher B. Adjuvant chemotherapy in the primary management of breast cancer. *Med Clin North Am* 1977; **61**: 953–65.
21 Kurosumi M. Significance of histopathological evaluation in primary therapy for breast cancer – recent trends in primary modality with pathological complete response (pCR) as endpoint. *Breast Cancer* 2004; **11**: 139–47.
22 Wolff AC, Hammond ME, Schwartz JN *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131**: 18–43.
23 Cox DR. Regression models and life-tables. *J R Stat Soc* 1972; **34**: 187–220.
24 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
25 Lee AHS, Pinder SE, Macmillan RD *et al.* Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. *Eur J Cancer* 2006; **42**: 357–62.
26 El-Gohary YM, Metwally G, Saad RS *et al.* Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. *Am J Clin Pathol* 2008; **129**: 578–86.
27 Amaout-Alkarain A, Kahn HJ, Narod SA *et al.* Significance of lymph vessel invasion identified by the endothelial lymphatic marker D2-40 in node negative breast cancer. *Mod Pathol* 2007; **20**: 183–91.
28 Hasebe T, Sasaki S, Imoto S *et al.* Characteristics of tumors in lymph vessels play an important role in the tumor progression of invasive ductal carcinoma of the breast: a prospective study. *Mod Pathol* 2002; **15**: 904–13.
29 Hasebe T, Sasaki S, Imoto S *et al.* Significance of nodal metastatic tumor characteristics in nodal metastasis and prognosis of patients with invasive ductal carcinoma of the breast. *Cancer Sci* 2003; **94**: 181–7.
30 Hasebe T, Sasaki S, Imoto S *et al.* Proliferative activity of intratumoral fibroblasts is closely correlated with lymph node and distant organ metastases of invasive ductal carcinoma of the breast. *Am J Pathol* 2000; **156**: 1701–10.
31 Hasebe T, Sasaki S, Imoto S *et al.* Highly proliferative fibroblasts forming fibrotic focus govern metastasis of invasive ductal carcinoma of the breast. *Mod Pathol* 2001; **14**: 325–37.
32 Hasebe T, Imoto S, Ogura T *et al.* Significance of basic fibroblast growth factor and fibroblast growth factor receptor protein expression in the formation of fibrotic focus in invasive ductal carcinoma of the breast. *Jpn J Cancer Res* 1997; **88**: 877–85.
33 Finak G, Bertos N, Pepin F *et al.* Stromal gene expression predicts clinical outcome in breast cancer. *Nature Med* 2008; **14**: 518–27.
34 Singer CF, Gschwantler-Kaulich D, Fink-Retter A *et al.* Differential gene expression profile in breast cancer-derived stromal fibroblasts. *Breast Cancer Res Treat* 2008; **110**: 273–81.
35 Sheehan KM, Gulmann C, Eichler GS *et al.* Signal pathway profiling of epithelial and stromal compartments of colonic carcinoma reveals epithelial-mesenchymal transition. *Oncogene* 2008; **27**: 323–31.
36 Hasegawa M, Furuya M, Kasuya Y *et al.* CD151 dyanmicx in carcinoma-stroma interaction: integrin expression, adhesion strength and proteolytic activity. *Lab Invest* 2007; **87**: 882–92.
37 Loussouarn D, Campion L, Sagan C *et al.* Prognostic impact of syndecan-1 expression in invasive ductal carcinomas. *Br J Cancer* 2008; **98**: 1993–8.
38 Studebaker AW, Storci G, Werbeck JL *et al.* Fibroblasts isolated from common sites of breast cancer metastasis enhance cancer cell growth rates and invasiveness in an interleukin-6-dependent manner. *Cancer Res* 2008; **68**: 9087–95.
39 Baak JP, Colpaert CG, van Diest PJ *et al.* Multivariate prognostic evaluation of the mitotic activity index and fibrotic focus in node-negative invasive breast cancers. *Eur J Cancer* 2005; **41**: 2093–101.
40 Colpaert C, Vermeulen PB, van Beest P *et al.* Intratumoral hypoxia resulting in the presence of a fibrotic focus is an independent predictor of early distant relapse in lymph node-negative breast cancer patients. *Histopathology* 2001; **39**: 416–25.

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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

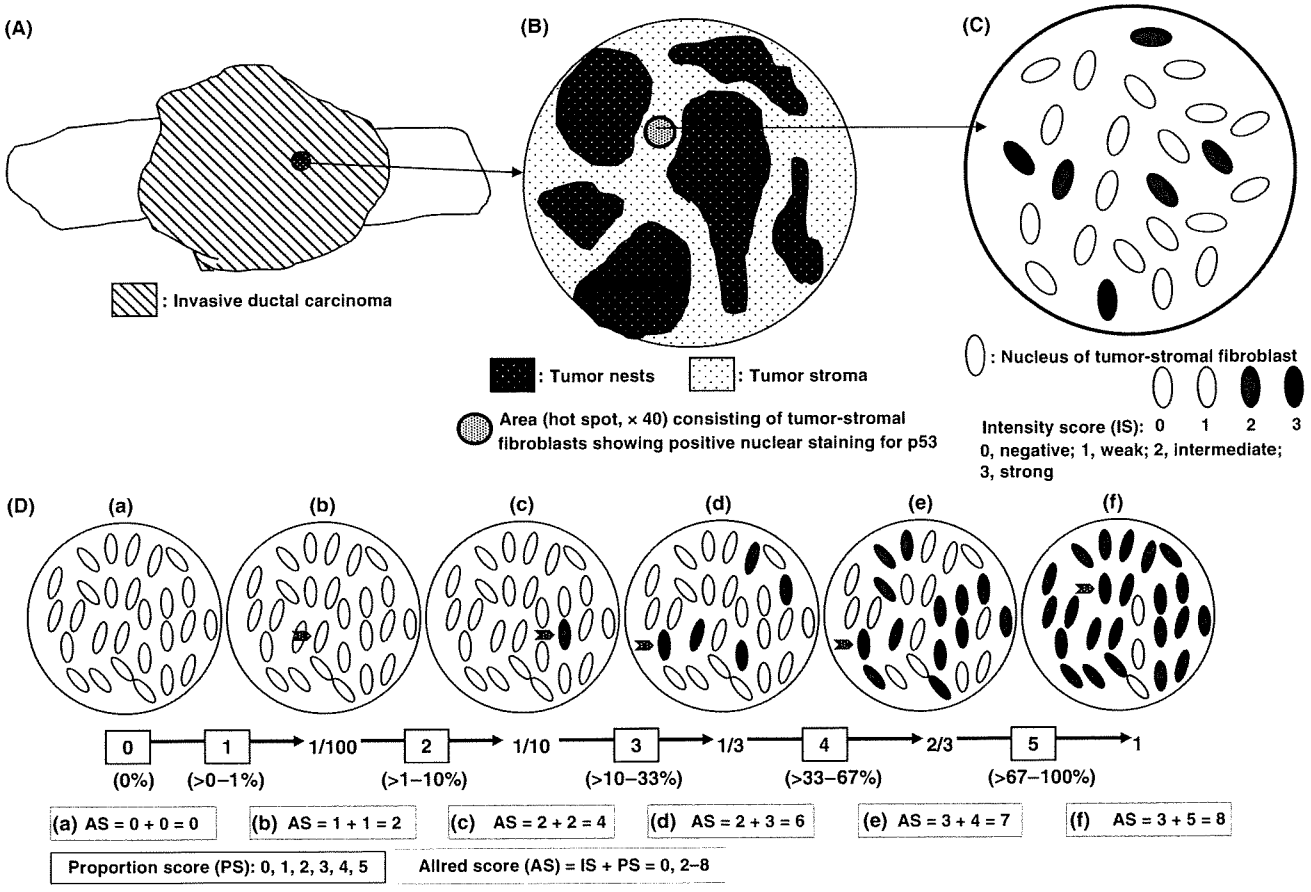

Fig. 1. Method for immunohistochemical assessment of tumor stromal fibroblasts in invasive ductal carcinoma (IDC). (A–D) First, sections on slides were examined for the presence or absence of p53 expression in tumor stromal fibroblasts in a medium-power field (×10 objective and ×10 ocular or ×20 objective 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

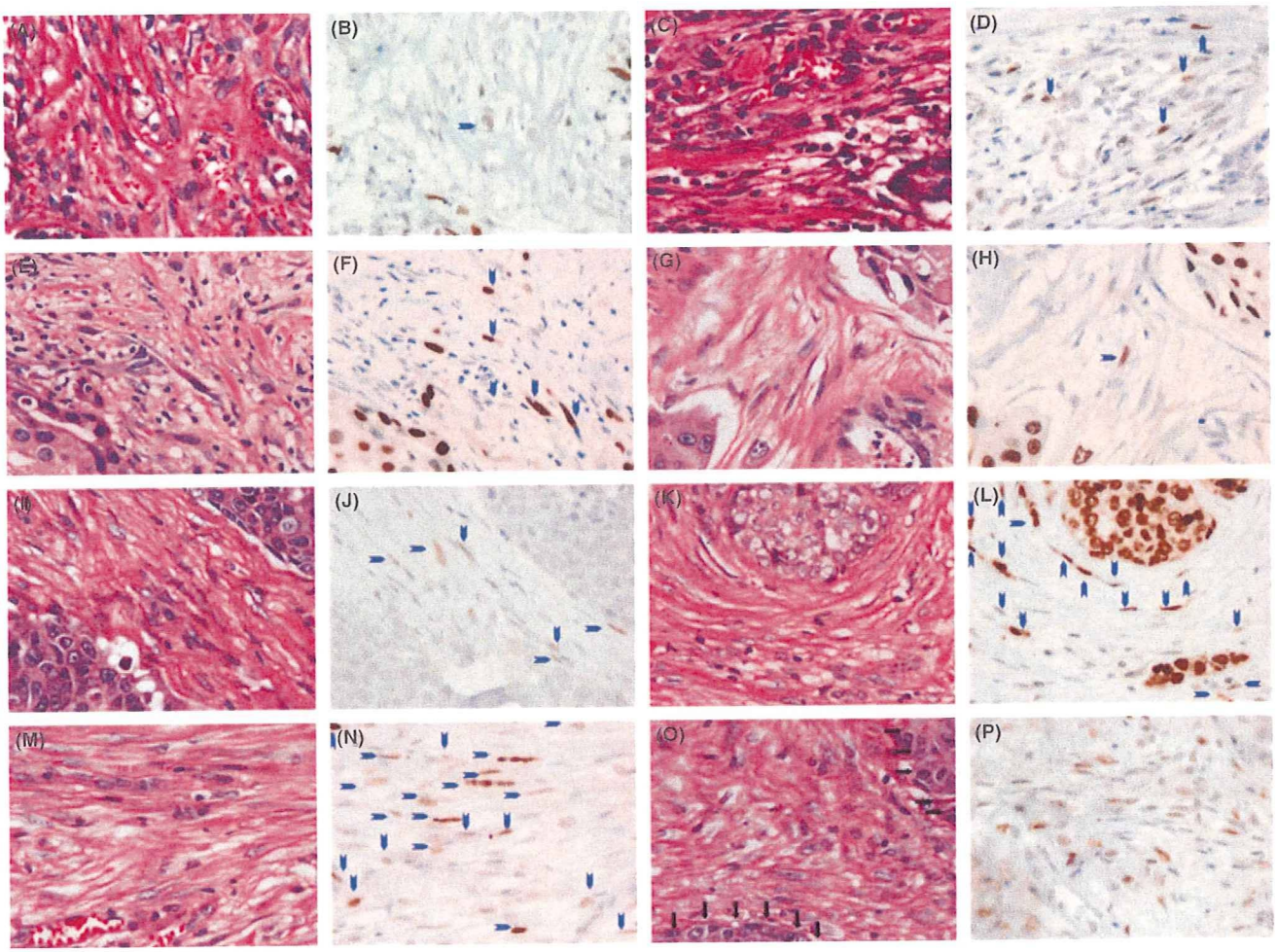


Fig. 2. (A,C,E,G,I,K,M,O) Histological features of tumor stromal fibroblasts, (B,D,F) intensity scores (IS), and (H,J,L,N,P) proportion scores (PS) for p53 expression in tumor stromal fibroblasts of invasive ductal carcinomas (IDC) in high-power fields (×40, hot spots). In general, tumor stromal fibroblasts 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.

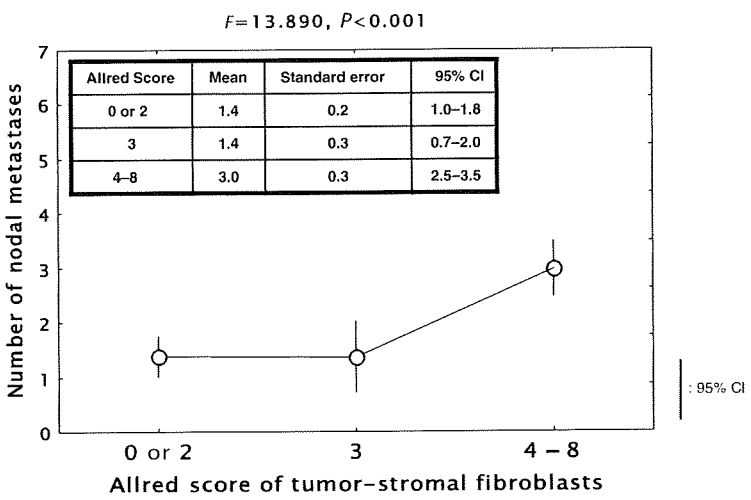
expression PS (0–5) was then to be evaluated in one high-power field (hot spot, ×40 objective and ×10 ocular) (Figs 1C,D,2G–P). The PS and IS of the tumor stromal fibroblasts were then added to obtain a total score, with possible total score of 0 and ranging from 2 to 8 (Figs 1D,2G–P). When examining 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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The 10 histological factors, and the Allred scores for ER, PR, and p53 in tumor cells, Allred scores for p53 in tumor stromal fibroblasts, categories of HER2 expression in tumor cells, and age (≤39 years and >39 years) were analyzed in association with nodal metastases and the outcome of the IDC patients. Univariate analysis associations with the number of nodal metastasess were carried out by ANOVA, and the factors significantly associated with the number of nodal metastases in the univariate analysis were then entered in a multiple regression analysis for multivariate analyses. Univariate analysis associations between the above factors and UICC pathological nodal status (N factor: N0, no nodal metastasis; N1, 1–3 nodal metastases; N2, 4–9 nodal metastases; and N3, 10 or more nodal metastasis) and the outcomes of the IDC patients were carried out using the Cox proportional hazard regression model. The factors significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses using the Cox proportional hazard regression model. The multivariate analyses were carried out separately in patients with and without adjuvant therapy according to UICC pTNM stage[22] and triple-negative IDC status. The case-wise and step-down method was applied until all of the remaining factors were significant at a *P*-value below 0.05. As there were fewer than 10 tumor deaths in the group of patients who did not receive adjuvant therapy, the group with UICC pTNM stage I disease, the group with UICC pTNM stage II disease, and the triple-negative-IDC group of patients, it was impossible to carry out multivariate analyses for tumor death in these groups. Survival curves were drawn by the Kaplan–Meier method.[32] All analyses were done with Statistica/Windows software (StatSoft, Tulsa, OK, USA).

Results

Analyses for the number of nodal metastases. Allred score for p53 in tumor stromal fibroblasts (Fig. 3A), Allred scores for ER, PR, and p53 in tumor cells, invasive tumor size, skin invasion, adipose tissue invasion, histological grade, fibrotic focus, lymph vessel invasion, and blood vessel invasion were significantly associated with the number of nodal metastases in the univariate analyses (data not shown). Invasive tumor size, lymph vessel invasion, skin invasion, p53 Allred score in tumor stromal fibroblasts, blood vessel invasion, histological grade, and Allred score for PR in tumor cells were significantly associated with the number of nodal metastases in the multivariate analyses (Fig. 3B).

Factors significantly associated with distant organ metastasis and tumor death. HER2 expression in tumor cells, Allred scores for p53 in tumor cells and in tumor stromal fibroblasts (Fig. 4A), Allred scores for ER in tumor cells, Allred scores for PR in tumor cells, invasive tumor size, histological grade, FF diameter, lymph vessel invasion, blood vessel invasion, UICC pN categories, and UICC pTNM stages were significantly associated with distant organ metastasis and tumor death in the univariate analyses (data not shown). Age was significantly associated with tumor recurrence in the univariate analyses, and skin invasion was significantly associated with tumor death in the univariate analyses (data not shown). Adjuvant therapy, muscle invasion, adipose tissue invasion, and tumor necrosis showed no significant association with tumor recurrence or tumor death in the univariate analyses (data not shown).

Among the patients who did not receive adjuvant therapy, only histological grade 3 significantly increased the hazard rate (HR) for distant organ metastasis in the multivariate analyses (data not shown).

In the UICC pTNM stage I patients who received adjuvant therapy, Allred scores of 4–8 for p53 in tumor stromal fibroblasts, histological grade 3, and FF diameter > 8 mm significantly increased the HR for distant organ metastasis in the multivariate analyses (Table 1).

In the UICC pTNM stage II patients who received adjuvant therapy, Allred scores of 4–8 for p53 tumor stromal fibroblasts, age ≤39 years, and FF diameter > 8 mm significantly increased the HR for distant organ metastasis in the multivariate analyses (Table 2).

Among the UICC pTNM stage III patients who received adjuvant therapy, p53 Allred scores of 4–8 in tumor stromal fibroblasts significantly increased the HR for distant organ metastasis and tumor death in the multivariate analyses (Table 3). FF diameter > 8 mm, the presence of blood vessel invasion, and category 3 HER2 expression in tumor cells significantly increased the HR for distant organ metastasis, and histological grade 3 significantly increased the HR for tumor death in the multivariate analyses (Table 3).

Among the triple-negative IDC patients, Allred scores of 4–8 for p53 in tumor stromal fibroblasts (Fig. 4B), UICC pN2 and pN3, and age ≤ 39 years significantly increased the HR for tumor recurrence in the multivariate analysis (Table 4).

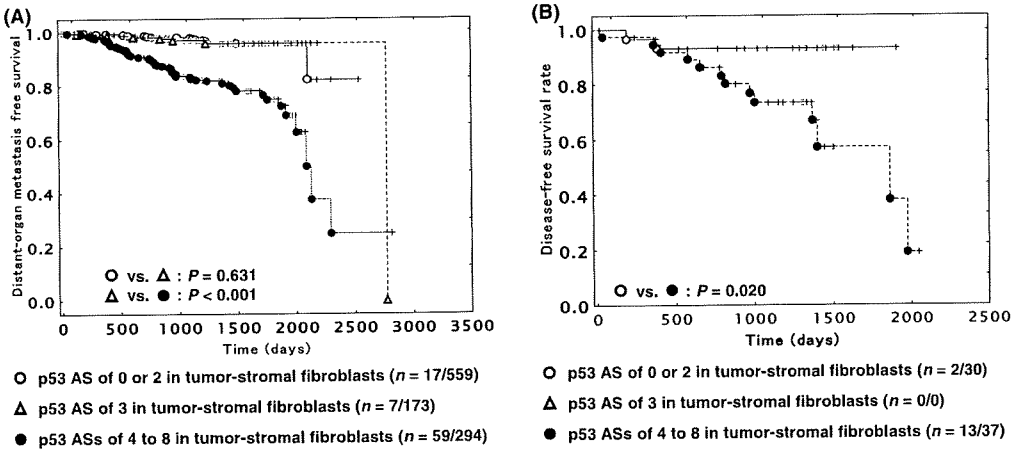

Fig. 4. (A) Distant organ metastasis-free survival curves of invasive ductal carcinoma (IDC) patients according to the Allred scores for p53 in their tumor stromal fibroblasts and (B) the disease-free survival curves of the triple-negative IDC patients. (A) Distant organ metastasis-free survival of IDC patients with Allred scores of 4–8 for p53 in tumor stromal fibroblasts is significantly shorter than that of IDC patients with other Allred scores for p53 in tumor stromal fibroblasts. (B) IDC patients with Allred scores of 4–8 for p53 in tumor stromal fibroblasts had a significantly shorter disease-free survival than IDC patients with Allred scores of 0 or 2 for p53.

Table 1. Multivariate analyses for distant organ metastasis in UICC pTNM stage I invasive ductal carcinoma patients who received adjuvant therapy (n = 247)

	Cases	DOMR (%)	HR	95% CI	*P*-value
Allred scores for p53 in tumor stromal fibroblasts					
0 or 2	148	2 (1)	Referent		
3	38	0	Referent		
4 to 8	61	9 (15)	32.2	3.4–306.1	0.003
Histological grade					
1	66	0	Referent		
2	123	2 (2)	Referent		
3	62	9 (15)	7.5	1.6–35.1	0.011
Fibrotic focus, diameter					
Absent	186	6 (3)	Referent		
≤8 mm	45	3 (7)	1.7	0.4–7.6	0.457
>8 mm	20	2 (10)	9.1	1.5–56.4	0.018

CI, confidence interval; DOMR, distant organ metastasis rate; HR, hazard rate.

Table 2. Multivariate analyses for distant organ metastasis in UICC pTNM stage II invasive ductal carcinoma patients who received adjuvant therapy (n = 435)

	Cases	DOMR (%)	HR	95% CI	*P*-value
Allred scores for p53 in tumor stromal fibroblasts					
0 or 2	226	8 (4)	Referent		
3	87	3 (4)	1.8	0.6–4.8	0.263
4 to 8	122	18 (15)	3.8	1.8–8.1	<0.001
Age (years)					
≤39	41	7 (17)	Referent		
>39	402	24 (6)	0.3	0.1–0.7	0.006
Fibrotic focus, diameter					
Absent	265	14 (5)	Referent		
≤8 mm	101	5 (5)	1.5	0.4–6.4	0.578
>8 mm	77	12 (16)	2.8	1.3–6.0	0.006

CI, confidence interval; DOMR, distant organ metastasis rate; HR, hazard rate.

Table 3. Multivariate analyses for distant organ metastasis, and tumor death in UICC pTNM stage III invasive ductal carcinoma patients who received adjuvant therapy (n = 185)

	Cases	DOMR (%)	HR	95% CI	*P*-value
Distant organ metastasis					
Allred scores for p53 in tumor stromal fibroblasts					
0 or 2	88	6 (7)	Referent		
3	22	2 (9)	0.64	0.1–4.1	0.639
4 to 8	74	25 (34)	6.2	2.7–13.8	<0.001
Fibrotic focus, diameter					
Absent	97	13 (13)	Referent		
≤8 mm	46	5 (11)	0.4	0.1–1.2	0.099
>8 mm	42	15 (36)	3.4	1.6–7.0	<0.001
Blood vessel invasion					
Absent	145	19 (13)	Referent		
Present	38	14 (37)	2.7	1.3–5.7	0.006
HER2 expression					
0 or 1	119	19 (16)	Referent		
2	38	4 (11)	0.7	0.2–2.3	0.588
3	27	10 (37)	2.4	1.1–5.3	0.023
Tumor death					
Allred scores for p53 in tumor-stromal fibroblasts					
0 or 2	88	1 (1)	Referent		
3	22	0	Referent		
4 to 8	74	16 (22)	18.1	2.4–139.5	0.005
Histological grade					
1	30	0	Referent		
2	76	3	Referent		
3	79	14 (18)	3.8	1.1–12.9	0.038

CI, confidence interval; DOMR, distant organ metastasis rate; HR, hazard rate; TDR, tumor death rate; –/–, not significant.

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Table 4. Clinicopathological factors significantly associated with tumor recurrence in triple-negative invasive ductal carcinoma patients in multivariate analyses (n = 74)

	Cases	TRR (%)	HR	95% CI	*P*-value
Allred scores for p53 in tumor-stromal fibroblasts					
0 or 2	30	2 (7)	Referent		
3	7	0	Referent		
4 to 8	37	13 (35)	12.0	2.3–62.0	0.003
UICC pN category					
pN0	41	3 (7)	Referent		
pN1	17	4 (24)	1.4	0.3–6.9	0.701
pN2	9	4 (44)	7.8	2.0–30.6	0.004
pN3	7	4 (57)	26.8	6.0–122.6	<0.001
Age (years)					
≤39	6	3 (50)	Referent		
>39	68	12 (18)	0.2	0.04–0.8	0.022

CI, confidence interval; HR, hazard rate; TRR, tumor recurrence rate; UICC pN0, no nodal metastasis; UICC pN1, one to three nodal metastases; UICC pN2, four to nine nodal metastases; UICC pN3, 10 or more nodal metastases. Triple-negative residual invasive ductal carcinoma means: (1) Allred scores for estrogen receptor of 0 or 2; (2) Allred score for progesterone receptor of 0 or 2; and (3) HER2 expression category of 0 or 1.

Discussion

Patocs *et al.*[21] showed a significant association between p53 mutations in tumor stroma and nodal metastasis in patients with sporadic breast cancer, and the results of the multivariate analyses in the present study clearly confirmed that p53 expression in tumor stromal fibroblasts, but not in tumor cells, is an important independent factor associated with the number of nodal metastases by IDC.

The present study also clearly demonstrated that Allred scores of 4–8 for p53 expression in tumor stromal fibroblasts significantly increased the HR for distant organ metastasis and tumor death in the IDC groups independent of UICC pTNM stage or triple negativity. Thus, Allred scores of 4–8 for p53 in tumor stromal fibroblasts can be concluded to be a very important factor for accurately predicting the outcome of IDC patients independent of UICC pTNM stage or triple negativity, and it can be concluded that the modified method that we used to assign Allred scores to p53 expression in tumor stromal fibroblasts should be used to accurately evaluate the malignant potential of IDC from the standpoint of the tumor stroma.

In the present study we did not test Allred scores for p53 expression for associations with the presence of *p53* gene abnormalities in the tumor stromal fibroblasts. Although *p53* mutations in tumor stromal fibroblasts are relatively common in primary breast cancer and other cancers and have a positive effect on cancer growth,[18–21,33] some studies show no *p53* mutations observed in the tumor stroma of breast cancer,[34,35] and the possibility of technical problems (e.g. PCR artifacts for *p53* gene abnormalities) is raised by Campbell *et al.*[36] Thus, although the mechanism responsible for increasing the malignant potential of IDC related to the expression of p53 in tumor stromal fibroblasts should be investigated from the standpoint of *p53* gene abnormalities, p53 immunoreactivity in tumor stromal fibroblasts may in fact reflect specific reactive changes within the stroma that are related to the outcome of patients with IDC.

The results of the present study clearly showed that FF diameter, age, blood vessel invasion, and UICC pN2 were good prognostic factors. In previous studies we found that FF diameter was a significant prognostic factor for IDC patients,[7–10,13,37] and the significant prognostic power of FF diameter in the IDC group was confirmed in the present study. Thus, one can conclude that FF diameter is an important histological outcome predictor for IDC patients, and biological characteristics of tumor stroma, e.g. tumor stromal fibroblasts expressing p53, FF, play a very important role in tumor progression of IDC of the breast.

In conclusion, this is the first study to clearly demonstrate that p53 expression by tumor stromal fibroblasts is strongly associated with the number of nodal metastases and the outcome of IDC patients. The modified Allred scoring system is very suitable for accurately assessing p53-expressing tumor stromal fibroblasts in IDC independent of adjuvant therapy, UICC pTNM stage, or the HR or HER2 status of the IDC. The p53 expression in tumor stromal fibroblasts will probably be a very important target for tumor gene therapy of IDC, although it will be necessary to confirm that the p53 Allred score also provides significant prognostic power for IDC patients in a prospective study.

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References

1 Finak G, Bertos N, Pepin F *et al.* Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 2008; **14**: 518–27.
2 Singer CF, Gschwantler-Kaulich D, Fink-Retter A *et al.* Differential gene expression profile in breast cancer-derived stromal fibroblasts. *Breast Cancer Res Treat* 2008; **110**: 273–81.
3 Sheehan KM, Gulmann C, Eichler GS *et al.* Signal pathway profiling of epithelial and stromal compartments of colonic carcinoma reveals epithelial–mesenchymal transition. *Oncogene* 2008; **27**: 323–31.
4 Hasegawa M, Furuya M, Kasuya Y *et al.* CD151 dyanmicx in carcinoma–stroma interaction: integrin expression, adhesion strength and proteolytic activity. *Lab Invest* 2007; **87**: 882–92.
5 Loussouarn D, Campion L, Sagan C *et al.* Prognostic impact of syndecan-1 expression in invasive ductal carcinomas. *Br J Cancer* 2008; **98**: 1993–8.
6 Studebaker AW, Storci G, Werbeck JL *et al.* Fibroblasts isolated from common sites of breast cancer metastasis enhance cancer cell growth rates and invasiveness in an interleukin-6-dependent manner. *Cancer Res* 2008; **68**: 9087–95.
7 Hasebe T, Tsuda H, Hirohashi S *et al.* Fibrotic focus in infiltrating ductal carcinoma of the breast: a significant histopathological prognostic parameter for predicting the long-term survival of the patients. *Breast Cancer Res Treat* 1998; **49**: 195–208.
8 Hasebe T, Sasaki S, Imoto S *et al.* Prognostic significance of fibrotic focus in invasive ductal carcinoma of the breast: a prospective observational study. *Mod Pathol* 2002; **15**: 502–16.

9 Colpaert C, Vermeulen PB, van Beest P *et al.* Intratumoral hypoxia resulting in the presence of a fibrotic focus is an independent predictor of early distant relapse in lymph node-negative breast cancer patients. *Histopathology* 2001; **39**: 416–25.
10 Baak JP, Colpaert CG, van Diest PJ *et al.* Multivariate prognostic evaluation of the mitotic activity index and fibrotic focus in node-negative invasive breast cancers. *Eur J Cancer* 2005; **41**: 2093–101.
11 Hasebe T, Imoto S, Ogura T *et al.* Significance of basic fibroblast growth factor and fibroblast growth factor receptor protein expression in the formation of fibrotic focus in invasive ductal carcinoma of the breast. *Jpn J Cancer Res* 1997; **88**: 877–85.
12 Hasebe T, Sasaki S, Imoto S *et al.* Proliferative activity of intratumoral fibroblasts is closely correlated with lymph node and distant organ metastases of invasive ductal carcinoma of the breast. *Am J Pathol* 2000; **156**: 1701–10.
13 Hasebe T, Sasaki S, Imoto S *et al.* Highly proliferative fibroblasts forming fibrotic focus govern metastasis of invasive ductal carcinoma of the breast. *Mod Pathol* 2001; **14**: 325–37.
14 Hainaut P, Hollstein M. p53 and human cancer: the first ten thousand mutations. *Adv Cancer Res* 2000; **77**: 81–137.
15 Hollstein M, Sidransky D, Vogelstein B *et al.* p53 mutations in human cancers. *Science* 1991; **253**: 49–53.
16 Kastan MB, Bartek J. Cell-cycle check-points and cancer. *Nature* 2004; **432**: 316–23.
17 Kurose K, Gilley S, Matsumoto PH *et al.* Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinoma. *Nat Genet* 2002; **32**: 355–7.
18 Hill R, Song Y, Cardiff RD *et al.* Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. *Cell* 2006; **123**: 1001–11.
19 Bierie B, Moses HL. Under pressure: stromal fibroblasts change their ways. *Cell* 2005; **123**: 985–7.
20 Moinfar F, Man YG, Arnould L *et al.* Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implication for tumorigenesis. *Cancer Res* 2000; **60**: 2562–6.
21 Patocs A, Zhang Li, Xu Y *et al.* Breast-cancer stromal cells with TP53 mutations and nodal metastases. *New Engl J Med* 2007; **357**: 2543–51.
22 Sobin LH, Wittekind C, eds. *TNM Classification of Malignant Tumors*, 6th edn. Geneva: Wiley-Liss, 2002.
23 Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957; **11**: 359–77.
24 Gilchrist KW, Gray R, Fowble B *et al.* Tumor necrosis is a prognostic predictor for early recurrence and death in lymph node-positive breast cancer: a 10-year follow-up study of 728 eastern cooperative oncology group patients. *J Clin Oncol* 1993; **11**: 1929–35.
25 Hasebe T, Imoto S, Sasaki S *et al.* A proposal for a new histological classification scheme for predicting short-term tumor recurrence and death in patients with invasive ductal carcinoma of the breast. *Jpn J Cancer Res* 1998; **89**: 1358–73.
26 Allred DC, Harvey JM, Berardo MD *et al.* Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998; **11**: 155–68.
27 Harvey JM, Clark GM, Osborne K *et al.* Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999; **17**: 1474–81.
28 Mohsin S, Weiss H, Havighurst T *et al.* Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol* 2004; **17**: 1545–54.
29 Badve SS, Baehner FL, Gray RP *et al.* Estrogen- and progesterone-receptor status in ECOG2197: comparison of immunohistochemistry by local and central laboratories and quantitative reverse transcription polymerase chain reaction by central laboratory. *J Clin Oncol* 2008; **26**: 2473–81.
30 Allred DC, Clark GM, Elledge R *et al.* Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 1993; **85**: 200–6.
31 Wolff AC, Hammond ME, Schwartz JN *et al.* American Scociety of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131**: 18–43.
32 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
33 Kiaris H, Chatzistamou I, Trimis G *et al.* Evidence for nonautonomous effect of p53 tumor suppressor in carcinogenesis. *Cancer Res* 2005; **65**: 1627–30.
34 Allinen M, Beroukhim R, Cai L *et al.* Molecular characterization of the tumor microenviroment in breast cancer. *Cancer Cell* 2004; **6**: 17–32.
35 Lebret SC, Newgreen DF, Thompson EW *et al.* Induction of epithelial to mesenchymal transition in PMC42-LA human breast carcinoma cells by carcinoma-associated fibroblast secreted factors. *Breast Cancer Res* 2007; **9**: R19.
36 Campbell IG, Qiu W, Polyak K *et al.* Breast-cancer stromal cells with *TP53* mutations. *New Engl J Med* 2008; **10**: 1634–5.
37 Hasebe T, Imoto S, Yokose T *et al.* Histopathologic factors significantly associated with initial organ-specific metastasis by invasive ductal carcinoma of the breast: a prospective study. *Hum Pathol* 2008; **39**: 681–93.

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Primary tumor resection improves the survival of younger patients with metastatic breast cancer

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Abstract. Current treatments for metastatic breast cancer (MBC) include palliation with chemotherapy and/or hormone therapy, neither of which has the effect of adequately improving survival. Local surgery to remove the primary breast tumor is performed to improve local control and prevent uncontrolled chest wall disease (UCD). From June 1962 to February 2007, 344 patients with MBC were treated at National Cancer Center Hospital. In our review of these cases, we evaluated the prognostic impact of local surgery and other clinicopathological features. One hundred and sixty patients (47%) underwent resection of primary breast tumor, while 184 (53%) patients were treated without surgery. Overall survival (OS) was prolonged in patients treated with surgery (p=0.049), younger patients (age <50, p=0.023), and patients with bone or soft tissue metastases (p=0.013). While surgery significantly improved OS in young patients (p=0.021), it did not increase OS in older patients (age >51, p=0.665) or patients with visceral metastasis (p=0.797). This study demonstrated that local surgery improved OS of patients with MBC; local surgery should therefore be considered, especially in young patients. Prospective studies are required to validate these findings and evaluate the impact of surgical intervention.

Introduction

Recently, breast cancer became the most common cancer in Japanese women; and its incidence continues to increase. The incidence of metastatic breast cancer (MBC), defined as a primary breast tumor with distant metastasis, is increasing, comprising ~3% of newly diagnosed breast cancers in Japan, which is similar to the 6% seen in the United States according to the Surveillance, Epidemiology, and End Results (SEER) data. Treatment for breast cancer has also been progressing rapidly. Surgical interventions have become significantly less invasive with the introduction of breast-conserving therapy and sentinel lymph node biopsy; systemic chemotherapeutic agents have become increasingly safe and effective. Although such treatments have made better control of MBC possible, the therapeutic guidelines for MBC have not changed. Palliative treatment remains standard care, utilizing systemic therapy with chemotherapeutic, hormonal, and biologic agents (1,2). Resection of the primary tumor was not considered curative treatment; it has been used solely as local therapy to prevent uncontrolled wall disease. Therefore, local surgery was performed relatively late in treatment and only if the primary tumor and metastases could not be reduced and controlled with systemic therapy.

The possibility that surgical procedures improve the survival of those patients has been reported retrospectively (3-6); this issue is still hotly debated at major breast conferences. The details of these studies, such as tumor sensitivity to systemic therapy and timing of surgery with respect to systemic treatment, were unclear. Improvements in primary systemic therapies have increased the numbers of MBC patients with resectable small primary tumors and controllable metastatic lesions after treatment. With all of these new developments, we need definitive guidelines for the treatment of these patients. In this study, we evaluate the efficacy of primary tumor resection at prolonging the overall survival of MBC patients and analyzed the relationship between response to surgery and clinicopathological features.

Patients and methods

Patients and treatments. Records of all patients with metastatic breast cancer (MBC) treated between June 1962 and February 2007 at the National Cancer Center Hospital (NCCH) was extracted from the database for inclusion in this retrospective study. Baseline information collected included patient demographics, tumor characteristics (size, node status, histological characteristics, estrogen and progesterone receptor status, and Her2/neu status), tumor site, number of metastases, type and timing of operative intervention, and use of hormonal therapy and chemotherapy. We classified patients into two categories based on the age when primary treatment began;

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younger patient was defined as <50 years old, while older included patients >51 years old. Sites of metastases were categorized as bone and/or soft tissue (bone, lymph nodes) or visceral (lungs, pleura, mediastinum, peritoneum, liver, and brain) metastases. All patients were treated with some form of systemic therapy, including chemotherapy and/or hormonal therapy.

In later years of the study (only after 2002), trastuzumab was administered to patients with tumors exhibiting HER2 overexpression. In the group who underwent surgery, surgical procedures included Halstead operation, modified radical mastectomy and breast conserving surgery with axillary dissection and simple mastectomy without axillary dissection. All primary breast tumors were removed completely. Time to surgery was calculated from the date in which primary treatment begun.

Metastatic involvement was determined by physical examination, biochemical analysis, and initial routine imaging procedures before or within one month of beginning primary treatment. Bone scans alone were not considered diagnostic of bone involvement. Abnormalities seen on bone scan were confirmed by radiography. Liver involvement was determined by computed tomography or ultrasound findings consistent with metastases. Pleural or peritoneal involvement was determined by positive cytology of effusion fluid and appropriate imaging studies. Cervical or contralateral lymph node involvement was classified as distant soft tissue metastases. Chest wall recurrence was excluded from soft tissue metastases.

Evaluation of pathological factors. Surgical specimens were sectioned at 7-10 mm for evaluation of the pathological response by pathologists. Expression levels of ER (1D5, Dako Cytomation), PgR (1A6, Novocastra), and HER2 (HercepTest®, Dako Cytomation) were examined by immunohistological staining. ER and PgR were classed as positive when >10% of cancer cell nuclei exhibited positive staining, regardless of intensity. HER2 was scored as follows: (0), negative for cells; (1+), slightly positive in >10% of cancer cells; (2+), moderately positive in >10% of cancer cells; and (3+), markedly positive in >10% of cancer cells. Immunohistochemistry (IHC) with scores of (2+) or (3+) were defined as HER2-positive.

Statistical analysis. Overall survival (OS) was calculated from the date upon which treatment was initiated to the date of death or last visit. Kaplan-Meier plots and log-rank test were used to assess differences in survival. All comparisons were two-tailed. Cox-proportional hazards models were fit for OS. $P<0.05$ were considered statistically significant.

Results

The medical records of 344 MBC patients treated at NCCH were reviewed in this study. Table I lists patient characteristics. The median age at initiation of primary treatment was 54 years (28-82). We evaluated 141 (41%) young patients <50 years of age and 203 (59%) older patients >51 years of age. Sixty-six (19%) patients were diagnosed between 1962-1980, 62 (18%) between 1981-1990, 96 (28%) between 1991-2000, and 120 (35%) between 2001-2007. Clinical tumor size at diagnosis was assessed as T1 in 21 (6%), T2 in 60 (17%), T3 in 53 (15%), and T4 in 208 (60%) patients. ER, PgR, and HER2 positivity was detected in 106 (31%), 87 (25%), and 84 (24%) patients, respectively. The ER/PgR and HER2 status of 137 (40%) and 149 (43%) patients, respectively,

Table I. Patient characteristics and Cox proportional hazard model for overall survival.

Parameters	No. of patients (%)	Hazard ratio (95% CI)
Age, median (range)	54 (28-82)	
≥51	203 (59)	1.00
≤50	141 (41)	0.87 (0.77-0.98)
Period of diagnosis		
1962-1980	66 (19)	1.00
1981-1990	62 (18)	0.87 (0.69-1.07)
1991-2000	96 (28)	0.95 (0.78-1.15)
2001-2007	120 (35)	0.85 (0.69-1.03)
Clinical T stage		
T1	23 (6)	1.00
T2	60 (17)	0.92 (0.65-1.41)
T3	53 (15)	1.01 (0.70-1.54)
T4	208 (60)	1.11 (0.82-1.63)
Estrogen receptor		
Positive	106 (31)	1.00
Negative	100 (29)	1.12 (0.93-1.33)
Unknown	138 (40)	1.25 (1.07-1.46)
Progesterone receptor		
Positive	87 (25)	1.00
Negative	120 (35)	1.10 (0.92-1.30)
Unknown	137 (40)	1.28 (1.10-1.50)
HER2		
Positive	84 (24)	1.00
Negative	111 (32)	0.96 (0.80-1.14)
Unknown	149 (43)	1.13 (0.96-1.31)
Site of metastases		
Bone/soft tissue	169 (49)	1.00
Visceral	175 (51)	1.16 (1.03-1.29)
Chemotherapy		
Yes	315 (88)	1.00
No	29 (12)	1.21 (0.96-1.48)
Hormone therapy		
Yes	172 (50)	1.00
No	146 (42)	0.90 (0.75-1.09)
Unknown	26 (8)	1.64 (1.22-2.13)
Local surgery		
No	184 (53)	1.00
Yes	160 (47)	0.89 (0.79-1.00)

were unknown. Bone and/or soft tissue and visceral metastases were present in 169 (49%) and 174 (51%) patients, respectively.

Three hundred and fifteen (88%) patients received chemotherapy, while 172 (50%) patients received hormonal therapy. Local surgery was performed for 160 (47%) patients. Surgical procedures included Halstead operation (n=101, 63%), modified radical mastectomy (n=34, 21%) and breast conserving surgery (n=4, 3%) with axillary dissection, and 21 patients (13%) underwent simple mastectomy without axillary dissection. All primary breast tumors were removed completely. One hundred and fifty (94%) of which underwent local surgery as primary therapy. The other patients underwent local surgery to avoid uncontrolled chest disease at late period of treatment when the primary tumors were regrowing. Local radiation after surgery was not used. There were patients without local surgery who underwent local radiation therapy.

Median follow-up time was 33 months (95% confidence interval, 29.2-38.0 months). We plotted overall survival on Kaplan-Meier curves of the patient cohort according to each parameter (Fig. 1). OS was significantly prolonged in patients receiving surgery [surgery vs. no surgery, median survival time (MST): 27 vs. 22 months, p=0.049], younger patients (younger vs. older, MST: 28 vs. 22 months, p=0.023), and patients with bone/soft tissue metastasis (bone/soft tissue vs. visceral, MST: 29 vs. 21 months, p=0.013). Hormonal therapy was also associated with improved OS (Fig. 1). Patients receiving hormonal therapy had a better prognosis than those who did not receive hormonal therapy. Chemotherapy was not associated with an improved OS. ER, PgR, and HER2 status, clinical tumor size, and period of diagnosis had no significant effects on OS (Table I).

The demographics and tumor characteristics of MBC patients treated with or without surgery are compared in Table II. Patients who underwent surgery tended to be younger (p=0.02) and were diagnosed earlier in the study period (p<0.0001) than patients who did not undergo surgery. Clinical tumor size did not differ between the two groups (p=0.39). Patients with bone/soft metastasis (p<0.0001) or those who received hormonal therapy (p=0.05) were more likely to undergo surgery. There was no significant factor to predict survival in multivariate analysis (data not shown).

Fig. 2 displays Kaplan-Meier curves describing the OS of patient cohorts who received local surgery or no surgery as classified according to age and site of metastases. Surgery was associated with a better prognosis in younger patients (surgery vs. no surgery, MST: 35 vs. 24 months, p=0.021). However, local surgery did not improve OS in older patients (p=0.665) and those with visceral metastases (p=0.797) and bone/soft tissue metastasis (p=0.095).

Discussion

The treatment of MBC has traditionally been palliative care with chemotherapy, hormonal therapy, and radiation therapy. According to the Hortobagyi algorithm (7), hormonal therapy is chosen as the first therapy for hormone receptor-positive MBC without visceral metastases. If MBC is hormone receptor-negative or resistant to hormone therapy, chemotherapy is used, but has the possibility of severely

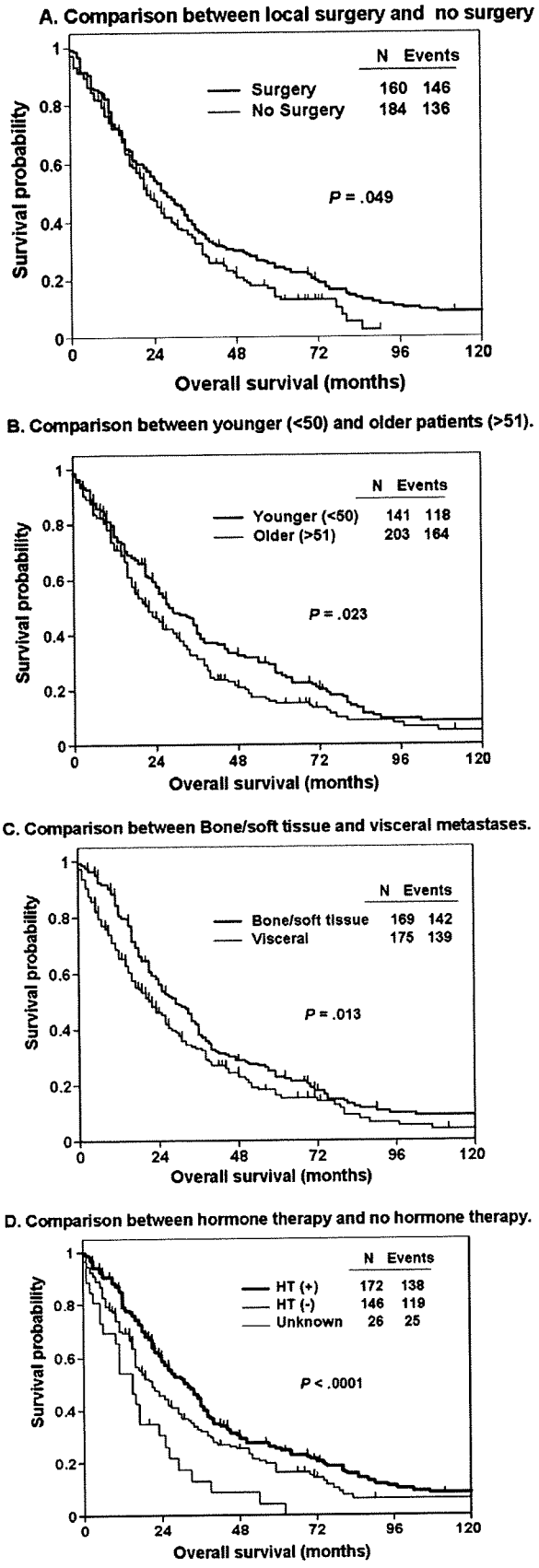

Figure 1. Kaplan-Meier curves of overall survival for MBC patients: (A) comparison of local surgery and no surgery; (B) comparison of younger (≤50) and older patients (≥51); (C) comparison of bone/soft tissue and visceral metastases; (D) comparison of hormone therapy and no hormone therapy.

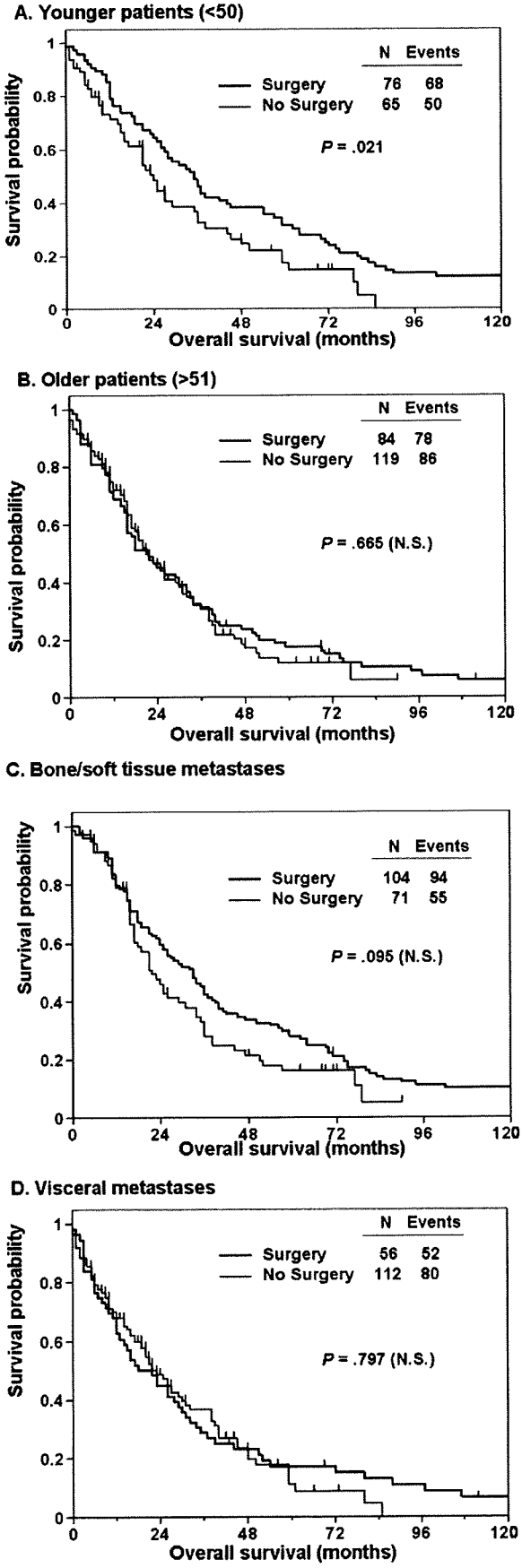

Figure 2. Kaplan-Meier curves of overall survival in the local surgery and no surgery groups: (A) younger patients (≤50); (B) older patients (≥51); (C) bone/soft tissue metastases; (D) visceral metastases.

Table II. Patient characteristics by surgery group.

Parameters	No. of pts (%) no surgery	Surgery	P-value
Age, median (range)			
<50	119 (59)	84 (41)	
>51	65 (46)	76 (54)	0.02
Period of diagnosis			
1962-1980	8 (12)	58 (88)	
1981-1990	8 (13)	54 (87)	
1991-2000	53 (55)	53 (45)	
2001-2007	115 (96)	5 (4)	<0.0001
Clinical T stage			
T1	12 (57)	9 (43)	
T2	29 (48)	31 (52)	
T3	23 (43)	30 (57)	
T4	119 (57)	89 (93)	0.39
Site of metastases			
Bone/soft tissue	67 (40)	102 (60)	
Visceral	116 (67)	58 (33)	<0.0001
Hormonal therapy			
Yes	84 (49)	88 (51)	
No	89 (61)	57 (39)	
Unknown	11 (42)	15 (58)	0.05

impacting quality of life. Current anti-tumor drugs, such as the anthracyclines and taxanes, are quite effective, as are molecularly targeted drugs such as trastuzumab. Using these drugs, the response rate of patients with locally advanced breast cancer was 80-90%; many primary breast cancers were reduced and resected in breast-conserving surgery (8,9). Other effective agent with fewer side effects, such as aromatase inhibitors and oral 5-fluorouracil, can prevent further disease progression, keeping patients stable and maintaining their quality of life for extended periods. Therefore, the control and/or reduction of both primary and metastatic lesions using systemic therapies has improved the living conditions of patients with MBC.

Surgery for breast cancer has also become safer and less invasive with the advent of improved surgical techniques and diagnosis, such as breast-conserving surgery and sentinel lymph node biopsy (10-12). These surgeries have few complications. However, several intensive chemotherapies have destructive high-grade and long-term side effects. Moreover, chemotherapy needs to be continued. According to the Hortobagyi algorithm, minimal surgery performed early in the treatment of MBC does not negatively impact quality of life. We need to evaluate prospectively the difference between local surgery and intensive chemotherapy. As studies have also demonstrated that local surgery for MBC avoids uncontrolled chest disease (13), local surgery for MBC should be discussed with patients as early as possible.