

Table 4 Parameters of the modified primary tumour/vessel tumour/nodal tumour classification for patients with invasive ductal carcinoma of the breast

Parameters	Scores
1. Fibrotic focus, diameter, in primary invasive tumours Absent/≤ 8 mm vs > 8 mm	0 vs 1
2. Nuclear feature of primary invasive ductal carcinomas Small/moderate vs marked	0 vs 1
3. Number of mitotic figures in primary invasive ductal carcinomas (/10 high-power fields) ≤ 19 vs > 19	0 vs 1
4. Grading system for lymph vessel tumour emboli Grades 0, 1, 2, and 3	0–3
5. Number of apoptotic figures in blood vessel tumour emboli Absent/≤ 2 vs > 2	0 vs 1
6. Grade of stromal fibrosis in metastatic mammary carcinoma to the lymph nodes n0/none/mild/moderate vs severe	0 vs 1
7. Maximum dimension of metastatic carcinoma to the lymph nodes (mm) n0/≤ 20 vs > 20	0 vs 1
8. Number of extranodal blood vessel tumour emboli n0/≤ 2 vs > 2	0 vs 1
9. Number of mitotic figures in metastatic carcinoma to the lymph nodes n0/≤ 5 vs > 5	0 vs 1
Total 0–11	

Abbreviation: no = no metastatic tumour.

Table 5 Tumour recurrence and death rates according to the modified primary tumour/vessel tumour/nodal tumour classification, the UICC pTNM stage classification, and the Nottingham Prognostic Index among all the patients with invasive ductal carcinoma (n = 1042)

Primary tumour/vessel tumour/nodal tumour classification					
Classes (scores)	Cases	TRR (%)	P-value	MR (%)	P-value
Class 0 (0)	349	11 (3)		2 (0.6)	
Class 1 (1/2)	466	66 (14)	<0.001	23 (5)	0.018
Class 2 (3/4)	151	56 (37)	0.005	26 (17)	0.002
Class 3 (5)	39	22 (56)	0.001	11 (28)	0.030
Class 4 (6/7)	29	21 (72)	0.390	19 (66)	0.505
Class 5 (8–11)	8	8 (100)	0.047	8 (100)	0.111
UICC pTNM stage classification					
Stage I (IA and IB)	352	26 (7)		9 (3)	
Stage II (IIA and IIB)	494	87 (18)	<0.001	34 (7)	0.004
Stage IIIA and IIIB	148	42 (28)	0.003	25 (17)	<0.001
Stage IIIC	48	29 (60)	<0.001	21 (44)	<0.001
Nottingham Prognostic Index					
Excellent prognostic group	130	1 (0.8)		0	
Good prognostic group	240	15 (6)	0.015	3 (1)	0.235
Moderate prognostic group I	252	38 (15)	0.002	10 (4)	0.069
Moderate prognostic group II	240	45 (19)	0.175	23 (10)	0.009
Poor prognostic group	118	48 (41)	<0.001	23 (19)	0.009
Very poor prognostic group	62	37 (60)	0.007	30 (48)	<0.001
Total	1042	169		67	

Abbreviations: TRR = tumour recurrence rate; MR = mortality rate.

Among the other classifications, the UICC pTNM stage classification showed significantly shorter crude disease-free survival and overall survival periods according to the increasing order of stages (Table 5). Among the three classifications, the Nottingham Prognostic Index clearly exhibited the lowest tumour recurrence rate in patients with a good prognosis (excellent prognostic group). The Nottingham Prognostic Index showed a significantly shorter crude disease-free survival period according to the increasing order of groups with the exception of moderate prognostic group II, but

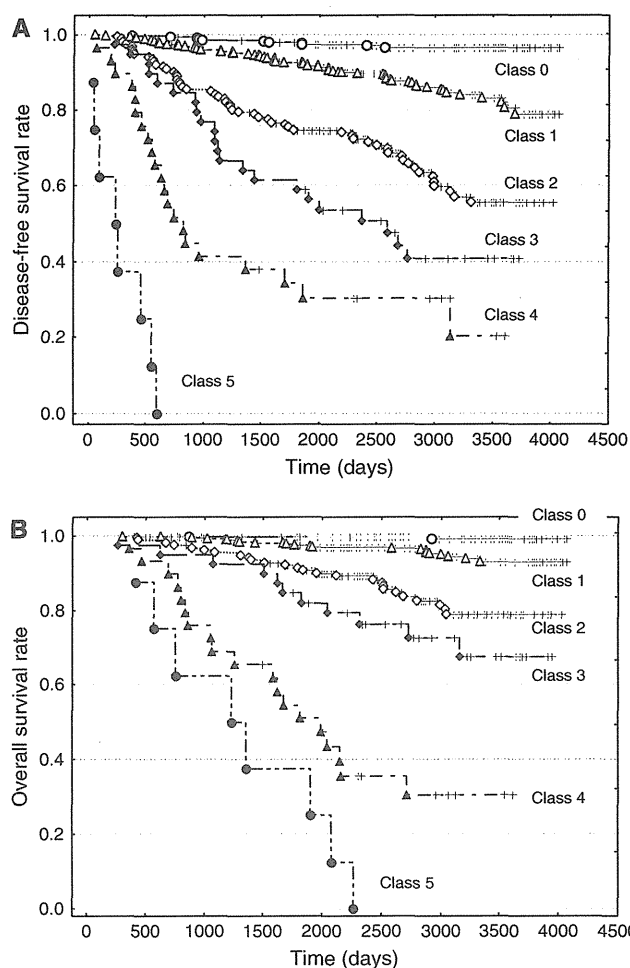


Figure 2 Disease-free survival curve and overall survival curve according to the modified PVN classification for all the patients in the present study (A and B). The disease-free survival curve (A) and the overall survival curve (B) for each class significantly decrease according to the increasing order of the classifications ($P < 0.001$).

significant differences in the overall survival periods were seen between the moderate prognostic group II and the poor prognostic group, and between the poor prognostic group and the very poor prognostic group out of the six groups (Table 5).

Comparison of the classifications

In model 1 multivariate analyses of all the patients, the modified PVN classification significantly increased the trend hazard ratios for tumour recurrence ($P < 0.001$) and tumour-related death ($P < 0.001$). Although the UICC pTNM classification showed a significant association with tumour recurrence ($P = 0.018$), it failed to show a significant association with tumour-related death ($P = 0.165$). HER2 category 3 had a significant association with tumour recurrence ($P = 0.033$). In model 2 multivariate analyses, the modified PVN classification significantly increased the trend hazard ratios for tumour recurrence ($P < 0.001$) and tumour-related death ($P < 0.001$). The Nottingham Prognostic Index also showed significant associations with tumour recurrence ($P = 0.003$) and tumour-related death ($P = 0.006$). HER2 category 3 failed to significantly increase the hazard ratio for tumour recurrence in model 2 multivariate analyses.

Table 6 Multivariate analyses for disease-free and overall survival for the modified primary tumour/vessel tumour/nodal tumour classification, the UICC pTNM stage classification, and the Nottingham Prognostic Index in patients with invasive ductal carcinoma according to nodal status or hormone receptor status

Classifications	Disease-free survival		Overall survival	
	Trend HR (95% CI)	Trend P-value	Trend HR (95% CI)	Trend P-value
<i>Patients with invasive ductal carcinoma without nodal metastasis (n = 592)</i>				
Model 2				
PVN (0–5)	2.1 (1.3–3.5)	0.003	3.4 (1.5–7.7)	0.004
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.4 (0.9–2.1)	0.065	1.3 (0.7–2.5)	0.449
<i>Patients with invasive ductal carcinoma with nodal metastasis (n = 450)</i>				
Model 1				
PVN (0–5)	2.2 (1.9–2.5)	<0.001	2.4 (1.9–2.9)	<0.001
pTNM (I, II, IIIA, IIIC)	1.2 (0.9–1.5)	0.180	1.2 (0.9–1.7)	0.232
Model 2				
PVN (0–5)	2.2 (1.8–2.6)	<0.001	2.1 (1.7–2.7)	<0.001
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.1 (0.9–1.4)	0.259	1.5 (1.1–2.0)	0.024
<i>Patients with invasive ductal carcinoma who were completely negative for hormone receptors (n = 125)</i>				
Model 1				
PVN (0–5)	2.3 (1.6–3.3)	<0.001	2.6 (1.7–4.3)	<0.001
pTNM (I, II, IIIA, IIIC)	1.3 (0.8–2.1)	0.344	1.3 (0.6–2.6)	0.548
Model 2				
PVN (0–5)	2.5 (1.7–3.6)	<0.001	2.4 (1.5–4.1)	<0.001
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.1 (0.7–1.6)	0.779	1.3 (0.7–2.5)	0.426
<i>Patients with invasive ductal carcinoma who were positive for one or two hormone receptors (n = 917)</i>				
Model 1				
PVN (0–5)	2.3 (1.9–2.6)	<0.001	2.4 (2.0–3.0)	<0.001
pTNM (I, II, IIIA, IIIC)	1.3 (1.0–1.6)	0.024	1.2 (0.9–1.6)	0.206
Model 2				
PVN (0–5)	2.0 (1.7–2.4)	<0.001	2.1 (1.6–2.7)	<0.001
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.3 (1.1–1.6)	0.002	1.4 (1.1–1.9)	0.013

Abbreviations: HR = hazard ratio; CI = confidence interval; PVN = modified primary tumour/vessel tumour/nodal tumour; NPI = Nottingham Prognostic Index; EPG = excellent prognostic group; GPG = good prognostic group; MPGI = moderate prognostic group I; MPGII = moderate prognostic group II; PPG = poor prognostic group; VPG = very poor prognostic group; pTNM = UICC pTNM; IIIA = UICC pTNM stages IIIA and IIIB.

In patients with invasive ductal carcinoma without nodal metastasis, the UICC pTNM classification failed to show a significant association with tumour recurrence or tumour-related death in univariate analyses (data not shown). In model 1 multivariate analyses, the modified PVN classification was significantly associated with tumour recurrence ($P < 0.001$) and tumour-related death ($P < 0.001$). In model 2 multivariate analyses, the modified PVN classification was significantly associated with tumour recurrence and tumour-related death, but the Nottingham Prognostic Index was not significantly associated with tumour recurrence or tumour-related death (Table 6).

In patients with invasive ductal carcinoma with nodal metastasis, the modified PVN classification showed significant associations with tumour recurrence and tumour-related death but the UICC pTNM classification did not show a significant association with tumour recurrence or tumour-related death in model 1 multivariate analyses (Table 6). In model 2 multivariate analyses, the modified PVN classification also showed significant associations with tumour recurrence and tumour-related death. The Nottingham Prognostic Index did not show a significant association with tumour recurrence, but a significant association with tumour-related death was observed (Table 6).

In patients with invasive ductal carcinoma who were completely negative for hormone receptors, only the modified PVN classification showed significantly increasing trend hazard ratios for tumour recurrence and tumour-related death in the multivariate analyses (Table 6).

In model 1 and 2 multivariate analyses of patients with invasive ductal carcinoma who were positive for one or two hormone receptors, the modified PVN classification exhibited significantly

increasing trend hazard ratios for tumour recurrence and tumour-related death (Table 6). The Nottingham Prognostic Index also showed significantly increasing trend hazard ratios for tumour recurrence and tumour-related death (Table 6). Although the UICC pTNM classification significantly increased the trend hazard ratio for tumour recurrence, it failed to significantly increase the trend hazard ratio for tumour-related death (Table 6). In model 1 and 2 multivariate analyses, the adjuvant therapy status significantly increased the trend hazard ratios for tumour-related death (model 1, $P = 0.007$; model 2, $P = 0.022$) but failed to significantly increase the trend hazard ratios for tumour recurrence (model 1, $P = 0.996$; model 2, $P = 0.597$).

In model 1 and 2 multivariate analyses of patients with invasive ductal carcinoma not treated with adjuvant therapy, the modified PVN classification significantly increased the hazard ratios for tumour recurrence (Table 7). The UICC pTNM classification and the Nottingham Prognostic Index failed to show significant associations with tumour recurrence (Table 7). HER2 category 3 significantly increased the trend hazard ratio for tumour recurrence in a model 1 multivariate analysis ($P = 0.048$) but failed to significantly increase the trend hazard ratio for tumour recurrence in a model 2 multivariate analysis ($P = 0.093$). As only five patients died as a result of their disease in this series, a multivariate analysis for tumour-related death could not be performed.

In model 1 and 2 multivariate analyses of patients with invasive ductal carcinoma treated with endocrine therapy, the modified PVN classification significantly increased the trend hazard ratios for tumour recurrence and tumour-related death (Table 7). The UICC pTNM classification and the Nottingham Prognostic Index

Table 7 Multivariate analyses for disease-free and overall survival for the modified primary tumour/vessel tumour/nodal tumour classification, the UICC pTNM stage classification, and the Nottingham Prognostic Index in patients with invasive ductal carcinoma according to adjuvant therapy status

Classifications	Disease-free survival		Overall survival	
	Trend HR (95% CI)	Trend (P-value)	Trend HR (95% CI)	Trend (P-value)
<i>Patients with invasive ductal carcinoma not treated with adjuvant therapy (n = 169)</i>				
Model 1				
PVN (0–5)	2.4 (1.4–4.1)	0.001	NA	
pTNM (I, II, IIIA, IIIB, IIIC)	1.2 (0.6–2.5)	0.653	NA	
Model 2				
PVN (0–5)	2.1 (1.2–3.7)	0.012	NA	
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.5 (0.8–2.4)	0.120	NA	
<i>Patients with invasive ductal carcinoma treated with endocrine therapy (n = 281)</i>				
Model 1				
PVN (0–5)	3.4 (2.5–4.8)	<0.001	5.6 (2.8–11.1)	<0.001
pTNM (I, II, IIIA, IIIB, IIIC)	1.3 (0.8–2.1)	0.291	0.5 (0.2–1.5)	0.205
Model 2				
PVN (0–5)	2.9 (1.9–4.5)	<0.001	4.7 (2.2–10.4)	<0.001
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.3 (0.9–1.8)	0.128	0.8 (0.4–1.8)	0.662
<i>Patients with invasive ductal carcinoma treated with chemoendocrine therapy (n = 375)</i>				
Model 1				
PVN (0–5)	2.0 (1.6–2.5)	<0.001	2.1 (1.5–3.0)	<0.001
pTNM (I, II, IIIA, IIIB, IIIC)	1.4 (0.9–1.9)	0.057	1.4 (0.9–2.3)	0.115
Model 2				
PVN (0–5)	1.7 (1.3–2.3)	<0.001	1.7 (1.1–2.7)	0.011
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.4 (1.1–1.8)	0.012	1.6 (1.1–2.5)	0.020
<i>Patients with invasive ductal carcinoma treated with chemotherapy (n = 217)</i>				
Model 1				
PVN (0–5)	2.1 (1.6–2.8)	<0.001	2.2 (1.7–2.8)	<0.001
pTNM (I, II, IIIA, IIIB, IIIC)	1.3 (0.9–2.0)	0.188	1.3 (0.9–1.8)	0.152
Model 2				
PVN (0–5)	2.3 (1.7–3.0)	<0.001	2.0 (1.5–2.7)	<0.001
NPI (EPG, GPG, MPGI, MPGII, PPG, VPG)	1.1 (0.8–1.5)	0.619	1.4 (0.9–2.1)	0.133

Abbreviations: HR = hazard ratio; CI = confidence interval; PVN = modified primary tumour/vessel tumour/nodal tumour; NPI = Nottingham Prognostic Index; EPG = excellent prognostic group; GPG = good prognostic group; MPGI = moderate prognostic group I; MPGII = moderate prognostic group II; PPG = poor prognostic group; VPG = very poor prognostic group; pTNM = UICC pTNM; IIIA = UICC pTNM stages IIIA and IIIB; NA = not available.

failed to show significant associations with tumour recurrence and tumour-related death (Table 7). In model 1 and 2 multivariate analyses, HER2 category 3 significantly increased the trend hazard ratios for tumour-related death (model 1 and model 2, $P < 0.001$) but failed to significantly increase the trend hazard ratios for tumour recurrence (model 1, $P = 0.082$; model 2, $P = 0.086$).

In model 1 and 2 multivariate analyses of patients with invasive ductal carcinoma treated with chemoendocrine therapy, the modified PVN classification significantly increased the hazard ratios for tumour recurrence and tumour-related death (Table 7). The UICC pTNM classification did not show significantly increasing trend hazard ratios for tumour recurrence and tumour-related death (Table 7). The Nottingham Prognostic Index significantly increased the trend hazard ratios for tumour recurrence and tumour-related death (Table 7).

In model 1 and 2 multivariate analyses of patients with invasive ductal carcinoma treated with chemotherapy, although the modified PVN classification significantly increased the trend hazard ratios for tumour recurrence and tumour-related death, the UICC pTNM classification and the Nottingham Prognostic Index failed to show significant associations with tumour recurrence or tumour-related death (Table 7).

DISCUSSION

We previously reported that the PVN classification can accurately predict the outcome of patients with invasive ductal carcinoma

in a manner that is independent of the nodal status or hormone receptor status (Hasebe *et al*, 2005), and the present study also clearly demonstrated that the modified PVN classification accurately predicted the outcome of patients with invasive ductal carcinoma in a manner that was independent of the nodal status, hormone receptor status, or adjuvant therapy status in a different group of patients with invasive ductal carcinoma. The clinical value of prognostic factors is particularly useful for the selection of different treatment regimens, especially adjuvant therapy in patients with invasive ductal carcinoma. One could argue that identifying patients with invasive ductal carcinoma who have a good prognosis and who do not require adjuvant therapy is of particular importance. The modified PVN classification was capable of classifying 815 (78%) out of 1042 patients as class 0 or 1, and patients belonging to these classes may be considered as good and moderately good prognostic groups, respectively. In contrast, patients belonging to class 2 or higher classes of the modified PVN classification may be considered as belonging to poor or very poor prognostic groups, respectively. In addition, the modified PVN classification had a superior outcome predictive power for the other two classifications in a manner that was independent of the adjuvant therapy status. Thus, the results of this study suggest that patients belonging to class 0 or 1 of the modified PVN classification can be spared adjuvant therapy, while patients belonging to class 2 or higher classes of the classification should be treated with adjuvant therapy in a manner that is independent of the nodal status or the hormone receptor status.

The factors included in the modified PVN classification were selected based on the precise analyses of this study using well-known clinicopathological factors, such as histologic grade, invasive tumour size, and nodal status. Among the nine factors in the modified PVN classification, seven of them were the histological factors that we proposed for primary invasive ductal carcinoma, carcinomas in vessels, and metastatic carcinoma to the lymph nodes (Hasebe *et al*, 1998, 2002a, 2003a, 2003b, 2004, 2008, 2010, 2011). This study clearly confirmed that these histological factors are important outcome predictors for different patient series of invasive ductal carcinoma of the breast. Among them, the outcome predictive power of the fibrotic focus among patients with invasive ductal carcinoma has also been confirmed by other investigators (Colpaert *et al*, 2001; Baak *et al*, 2005). Thus, these parameters are likely to be the most suitable parameters for accurately assessing the true biological malignant potential of invasive ductal carcinomas. In addition, we also confirmed the prognostic significance of the following factors that were previously reported by other investigators (Elston and Ellis, 1991) to be useful histological factors for predicting the outcome of patients with invasive ductal carcinomas: (1) the nuclear features of primary invasive ductal carcinoma and (2) the number of mitotic figures in primary invasive ductal carcinoma. Thus, the modified PVN classification appears to be better at accurately predicting the outcome of patients with invasive ductal carcinoma, compared with the other two classifications.

This study also strongly suggests that the tumour characteristics of invasive ductal carcinomas matter more than the quantity of tumour with regard to the accurate prediction of the outcome of patients with invasive ductal carcinoma. Both the UICC pTNM stage classification and the Nottingham Prognostic Index evaluate the malignant potential of invasive ductal carcinomas based on the invasive tumour size and the number of nodal metastases. These factors reflect the quantity of invasive ductal carcinoma cells. In contrast, almost all the factors in the modified PVN classification, exception of the maximum diameter of lymph node metastases, represent the tumour characteristics of invasive ductal carcinomas. In addition, we previously showed that mitotic figures and apoptotic figures in tumour cells of lymph vessel tumour emboli have significantly stronger outcome predictive powers than the number of lymph vessels that have been invaded (Hasebe *et al*,

2002b), and we devised a grading system for lymph vessel tumour emboli based on the presence of mitotic figures and apoptotic figures in the tumour cells of lymph vessel tumour emboli (Hasebe *et al*, 2008, 2010). As the modified PVN classification can evaluate the tumour characteristics of the invasive ductal carcinoma more precisely than the other two classifications, it appears to have a superior ability for accurately predicting patient outcome. Therefore, we concluded that the modified PVN classification is a useful prognostic histological classification available for predicting the outcome of patients with invasive ductal carcinoma of the breast.

We used the modified PVN classification for patients with invasive ductal carcinoma because our previous studies clearly demonstrated that the factors included in this classification were significant outcome predictors only for patients with invasive ductal carcinoma (Hasebe *et al*, 1998, 2002a, 2003a, 2003b, 2004, 2008, 2010, 2011). The UICC pTNM classification and the Nottingham Prognostic Index can be applied to all invasive breast carcinomas and may be superior to the modified PVN classification for predicting the outcome of overall patients with invasive carcinoma. Thus, we should confirm whether the modified PVN classification is also able to accurately predict the outcome of patients with non-ductal carcinomas of the breast in the future.

In conclusion, the current study clearly confirmed that the modified PVN classification is a useful histological classification for predicting the outcome of patients with invasive ductal carcinoma of the breast. Thus, pathologists should attempt to assess the true malignant potential of invasive ductal carcinomas using the criteria of the modified PVN classification.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) (C) (21590393) from the Japan Society for the Promotion of Science and was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (H21-006).

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Baak JP, Colpaert CG, van Diest PJ, Janssen E, van Diermen B, Albernaz E, Vermeulen PB, Van Marck EA (2005) Multivariate prognostic evaluation of the mitotic activity index and fibrotic focus in node-negative invasive breast cancers. *Eur J Cancer* 41: 2093–2101
- Blamey RW, Ellis IO, Pinder SE, Lee AHS, Macmillan RD, Morgan DAL, Robertson JFR, Mitchell MJ, Ball GR, Haybittle JL, Elston CW (2007) Survival of invasive breast cancer according to the Nottingham Prognostic Index in cases diagnosed in 1990–1999. *Eur J Cancer* 43: 1548–1555
- Colpaert C, Vermeulen PB, van Beest P, Goovaerts G, Weyler J, Van Dam P, Dirix L, Van Marck E (2001) 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* 39: 416–425
- Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 19: 403–410
- Gilchrist KW, Gray R, Fowble B, Fowble B, Tormey DC, Taylor SG (1993) 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* 11: 1929–1935
- Harvey JM, Clark GM, Osborne K, Allred DC (1999) 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* 17: 1474–1481
- Hasebe T, Iwasaki M, Akashi-Tanaka S, Hojo T, Shibata T, Sasajima Y, Tsuda H, Kinoshita T (2011) Prognostic significance of mitotic figures in metastatic mammary ductal carcinoma to the lymph nodes. *Hum Pathol*; e-pub ahead of print 17 June 2011
- Hasebe T, Okada N, Iwasaki M, Akashi-Tanaka S, Hojo T, Shibata T, Sasajima Y, Tsuda H, Kinoshita T (2010) Grading system for lymph vessel tumor emboli: significant outcome predictor for invasive ductal carcinoma of the breast. *Hum Pathol* 41: 706–715
- Hasebe T, Sasaki S, Imoto S, Mukai K, Yokose T, Ochiai A (2002a) Prognostic significance of fibrotic focus in invasive ductal carcinoma of the breast: a prospective observational study. *Mod Pathol* 15: 502–516
- Hasebe T, Sasaki S, Imoto S, Ochiai A (2003a) Histological characteristics of tumors in blood vessels play an important role in tumor progression of invasive ductal carcinoma of the breast. *Cancer Sci* 94: 158–165
- Hasebe T, Sasaki S, Imoto S, Ochiai A (2003b) Significance of nodal metastatic tumor characteristics in nodal metastasis and prognosis of patients with invasive ductal carcinoma of the breast. *Cancer Sci* 94: 181–187
- Hasebe T, Sasaki S, Imoto S, Ochiai A (2004) Histological characteristics of tumors in vessels and lymph nodes are significant parameter for predicting tumor progression of invasive ductal carcinoma of the breast: a prospective study. *Hum Pathol* 35: 298–308

- Hasebe T, Sasaki S, Imoto S, Ochiai A (2002b) 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* 15: 904–913
- Hasebe T, Sasaki S, Imoto S, Wada N, Ochiai A (2005) Primary tumor-vessel tumor-nodal tumor classification for patients with invasive ductal carcinoma of the breast. *Br J Cancer* 92: 847–856
- Hasebe T, Tsuda H, Hirohashi S, Shimosato Y, Tsubono Y, Yamamoto H, Mukai K (1998) 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* 49: 195–208
- Hasebe T, Yamauchi C, Iwasaki M, Ishii G, Wada N, Imoto S (2008) Grading system for lymph vessel tumor emboli for prediction of the outcome of invasive ductal carcinoma of the breast. *Hum Pathol* 39: 427–436
- Hasebe T, Okada N, Tamura N, Houjoh T, Akashi-Tanaka S, Tsuda H, Shibata T, Sasajima Y, Iwasaki M, Kinoshita T (2009) p53 expression in tumor stromal fibroblasts is associated with the outcome of patients with invasive ductal carcinoma of the breast. *Cancer Sci* 100: 2101–2108
- Mohsin S, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh le D, To TV, Qian Z, Love RR, Allred DC (2004) Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol* 17: 1545–1554
- Sobin LH, Gospodarowicz MK, Wittekind Ch (eds). (2009) *International Union Against Cancer TNM Classification of Malignant Tumours*, 7th edn, pp 181–193. Wiley-Liss: Geneva
- Sobin LH, Wittekind Ch (eds). (2002) *International Union Against Cancer TNM Classification of Malignant Tumors*, 6th edn, pp 131–141. Wiley-Liss: Geneva
- Sundquist M, Thorstenson S, Brudin L, Nordenskjold B (1999) Applying the Nottingham Prognostic Index to a Swedish breast cancer population. *Breast Cancer Res Treat* 53: 1–8
- Todd JH, Dowie C, Williams MR, Elston CW, Ellis O, Hinton CP, Blamey RW, Haybittle JL (1987) Confirmation of a prognostic index in primary breast cancer. *Br J Cancer* 56: 489–492
- Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF, American Society of Clinical Oncology/College of American Pathologists (2007) 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* 131: 18–43

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License.

Important Histologic Outcome Predictors for Patients With Invasive Ductal Carcinoma of the Breast

Takahiro Hasebe, MD, PhD,* Motoki Iwasaki, MD, PhD,† Sadako Akashi-Tanaka, MD, PhD,‡ Takashi Hojo, MD, PhD,‡ Tatsuhiko Shibata, MD, PhD,§ Takayuki Kinoshita, MD, PhD,‡ and Hitoshi Tsuda, MD, PhD||

Abstract: The pathologic diagnosis is regarded as the final diagnosis of a disease, and pathologic examination based on tumor histology is very important for the accurate assessment of the biological characteristics of tumors. The purpose of this study was to investigate the histologic factors that accurately predict patient outcome among 1042 patients with invasive ductal carcinoma of the breast. Both well-known histologic factors and our proposed histologic factors were examined according to several tumor statuses using multivariate analysis. This study clearly demonstrated that type 4 invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci are significant outcome predictors for lymph node-negative and lymph node-positive, the pathologic UICC-TNM stage II and III, luminal A-subtype, luminal B-subtype, and equivocal HER2 subtype invasive ductal carcinoma patients. Lymph vessel tumor embolus grades 2 and 3 were significant outcome predictors for lymph node-positive, UICC pTNM stages II and III, luminal A-subtype, and triple-negative invasive ductal carcinoma patients (except lymph vessel tumor embolus grade 2 in luminal A-subtype patients). More than 5 mitotic figures in metastatic carcinoma to the lymph nodes was a significant outcome predictor for lymph node-positive, UICC pTNM stage II, and luminal A-subtype invasive ductal carcinoma patients. A fibrotic focus diameter > 8 mm was a significant outcome predictor for UICC pTNM stages I and III invasive ductal carcinoma patients. These findings

strongly suggest that these histologic factors are very useful for accurately predicting the outcomes of patients with invasive ductal carcinoma of the breast.

Key Words: fibroblast, fibrotic focus, lymph vessel, lymph node, mitotic figure

(*Am J Surg Pathol* 2011;35:1484–1497)

Pathologic examination is performed in all hospitals worldwide, and the pathologic diagnosis is regarded as the final diagnosis of a disease. Thus, pathologic examination based on the histology of tumors obtained as biopsy or surgical specimens is very important for the accurate assessment of the biological characteristics of tumors. For patients with invasive ductal carcinoma of the breast, the invasive tumor size, histologic grade, and presence of vessel invasion or nodal metastasis are well-known histologic outcome predictors.^{2,5,7,9,21,22,25,29} We and other researchers have previously reported that the presence of a fibrotic focus is a very useful histologic tumor-stromal factor for accurately predicting the outcome of patients with invasive ductal carcinoma.^{3,6,12,13,14,23} In a different patient series, we also reported that the grading system for lymph vessel tumor emboli and the presence of > 5 number of mitotic figures in metastatic carcinoma to the lymph nodes are very useful histologic factors for accurately predicting the outcome of patients with invasive ductal carcinoma.^{15,16,18,20} Furthermore, we recently reported that the presence of atypical tumor-stromal fibroblasts in invasive ductal carcinomas with or without a fibrotic focus is a very important histologic outcome predictor for patients with invasive ductal carcinoma of the breast.¹⁹

The purpose of this study was to investigate which histologic factors, including factors that we have proposed, were most capable of accurately predicting the outcome of patients with invasive ductal carcinoma of the breast. The results of this study clearly demonstrated that the histologic factors proposed by us, such as the fibrotic focus diameter,^{13,14} the grading system for lymph vessel tumor emboli,^{16,18} the number of mitotic figures in metastatic carcinoma to the lymph nodes,^{15,20} and the types of invasive ductal carcinoma,¹⁹ are very useful histologic outcome predictors for invasive ductal carcinoma patients with several tumor statuses.

From the *Pathology Consultation Service, Clinical Trials and Practice Support Division, Center for Cancer Control and Information Services; †Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center; ‡Department of Breast Surgery, National Cancer Center Hospital; §Division of Cancer Genomics, National Cancer Center Research Institute; and ||Clinical Laboratory Division, National Cancer Center Hospital, Tsukiji, Chuo-ku, Tokyo.

Conflicts of Interest and Sources of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article. This study was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) (C) (21590393) from the Japan Society for the Promotion of Science and was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (H21-006).

Correspondence: Takahiro Hasebe, MD, PhD, Pathology Consultation Service, Clinical Trials and Practice Support Division, Center for Cancer Control and Information Services, National Cancer Center, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: thasebe@ncc.go.jp).

Copyright © 2011 by Lippincott Williams & Wilkins

METHODS

Cases

The participants of this study were 1042 consecutive patients with invasive ductal carcinoma of the breast who did not receive neoadjuvant therapy and were surgically treated at the National Cancer Center Hospital between January 2000 and December 2005 (almost the same case series as that used in our previous study^{18,19}). The invasive ductal carcinomas were diagnosed preoperatively using needle biopsy, aspiration cytology, mammography, or ultrasonography. All the patients were Japanese women, ranging in age from 23 to 72 years (median, 55 y). All patients had a solitary lesion; 498 patients were premenopausal, and 544 were postmenopausal. A partial mastectomy had been performed in 458 patients, and a modified radical mastectomy had been performed in 584 patients. Levels I and II axillary lymph node dissection was performed in all patients, and Level III axillary lymph node dissection had been performed in some of the patients.

Of the 1042 patients, 873 received adjuvant therapy, consisting of chemotherapy in 217 patients, endocrine therapy in 281 patients, and chemoendocrine therapy in 375 patients. The chemotherapy regimens used were anthracycline based with or without taxane and non-anthracycline based. 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. No cases of inflammatory breast cancer were included in this series. All the tumors were classified according to the pathologic UICC-TNM (pTNM) classification.²⁷ The protocol for this study (20 to 112) was reviewed by the Institutional Review Board of the National Cancer Center. For the pathologic examination, the surgically resected specimens were fixed in 10% formalin, and the size and gross appearance of the tumors were recorded. The tumor size was confirmed by comparison with the tumor size on the histologic slides.

Histologic Examination

Serial sections of each tumor area were cut from paraffin blocks. One section from each tumor was stained with hematoxylin and eosin and was examined histologically to confirm the diagnosis, and the other sections were subjected to immunohistochemistry. The following 7 well-known histologic factors were evaluated: (1) invasive tumor size (≤ 20 mm, > 20 to ≤ 50 mm, > 50 mm), (2) histologic grade (1, 2, 3),^{5,7} (3) tumor necrosis (absent, present),¹⁰ (4) blood vessel invasion (absent, present), (5) adipose tissue invasion (absent, present), (6) skin invasion (absent, present), and (7) muscle invasion (absent, present). In addition, the mitotic activity index was evaluated in primary invasive tumors.^{4,7,27} The mitotic activity index was evaluated at a high-power magnification in 10 consecutive neighboring fields of view in the

most cell-dense area and was determined based on the total number of mitotic structures counted in the 10 fields of views. We analyzed different prognostic thresholds as follows: (1) 0 to 9, 10 to 19, and 20 or higher; (2) 0 to 5, 6 to 10, and 10 or higher; (3) 0 to 2, 3 to 9, and 10 or higher; and (4) 0 to 9 and 10 or higher. Among these thresholds, as the first thresholds (0 to 9, 10 to 19, and 20 or higher) were the only thresholds to increase the hazard ratios for tumor recurrence ($P = 0.004$) and tumor-related death ($P = 0.022$) significantly, we selected the first thresholds for evaluating the mitotic activity index in this study. Next, the following 4 histologic factors that we proposed were evaluated: (1) fibrotic focus (absent, fibrotic focus diameter ≤ 8 mm, fibrotic focus diameter > 8 mm) (Fig. 1A, B),^{13,14} (2) grading system for lymph vessel tumor emboli (Fig. 1C–E),^{16,18} (3) number of mitotic figures in metastatic carcinoma to the lymph nodes (no nodal metastasis, ≤ 5 , > 5) (Fig. 2A, B),^{15,20} and (4) the type of invasive ductal carcinoma (types 1, 2, 3, and 4) (Fig. 2C–F).¹⁹ In brief,¹⁹ we examined the presence or absence of atypical tumor-stromal fibroblasts in the tumor stroma inside and outside of fibrotic foci in the invasive ductal carcinoma and classified the invasive ductal carcinomas into the following 4 types according to the presence or absence of fibrotic foci and the presence or absence of atypical tumor-stromal fibroblasts: (1) type 1 invasive ductal carcinoma not having fibrotic foci and atypical tumor-stromal fibroblasts; (2) type 2 invasive ductal carcinoma not having fibrotic foci but having atypical tumor-stromal fibroblasts; (3) type 3 invasive ductal carcinoma having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; and (4) type 4 invasive ductal carcinoma having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci. Types 2 and 4 invasive ductal carcinomas were then immunohistochemically studied using monoclonal antibodies to keratins (AE1/3) and α -smooth muscle actin (Fig. 2C, D, Fig. 2F) to confirm that the atypical tumor-stromal fibroblasts were not modified invasive tumor cells. In addition, some invasive ductal carcinomas contained large lymph vessel tumor emboli, and it was difficult to determine whether these components were true lymph vessel tumor emboli or a noninvasive ductal carcinoma component based on hematoxylin and eosin staining alone. We therefore performed immunohistochemical staining using D2-40 antibody (monoclonal mouse antibody, diluted 1:200; Signet, Dedham, MA) to confirm that the lymph vessel tumor emboli identified using hematoxylin and eosin staining were true tumor emboli (Fig. 1D).¹⁸

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

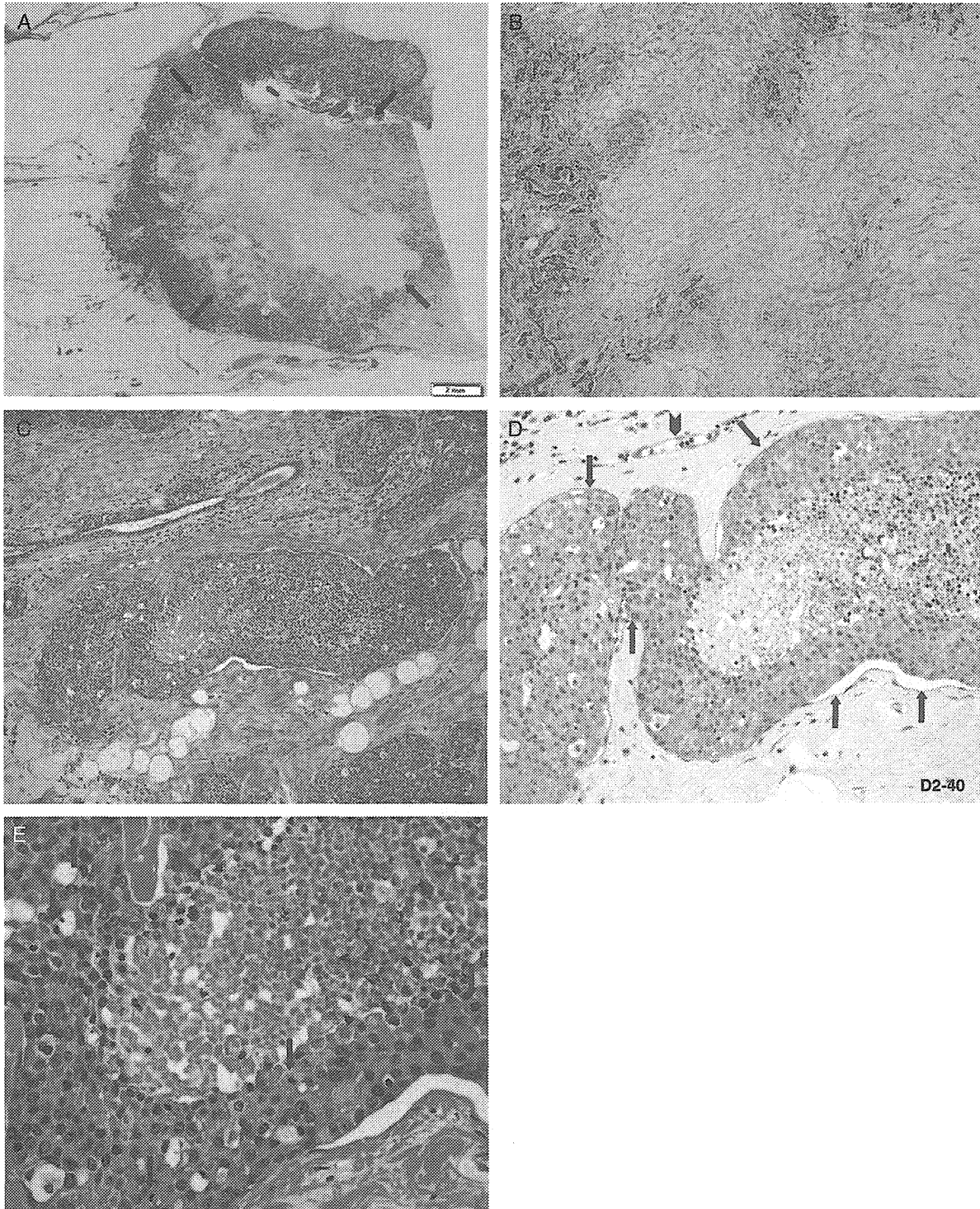


FIGURE 1. A and B, Invasive ductal carcinoma with a fibrotic focus. A, A fibrotic focus measuring 10.5×6.7 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus has a scar-like appearance and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists mainly of fibroblasts and collagen fibers arranged in a storiform pattern. C to E, Grade 3 lymph vessel tumor emboli. C, One very large lymph vessel tumor embolus located adjacent to one duct is present, and stroma-invasive carcinoma cell nests can be seen in the area surrounding the tumor embolus. D, The wall of the tumor lymph vessel containing the embolus is positive for D2-40 (arrows), and a small D2-40-negative artery is seen in the vicinity of the tumor embolus (arrowhead). E, Five mitotic tumor cells (arrows) and a nest consisting of many apoptotic tumor cells and apoptotic bodies are visible within the tumor embolus.

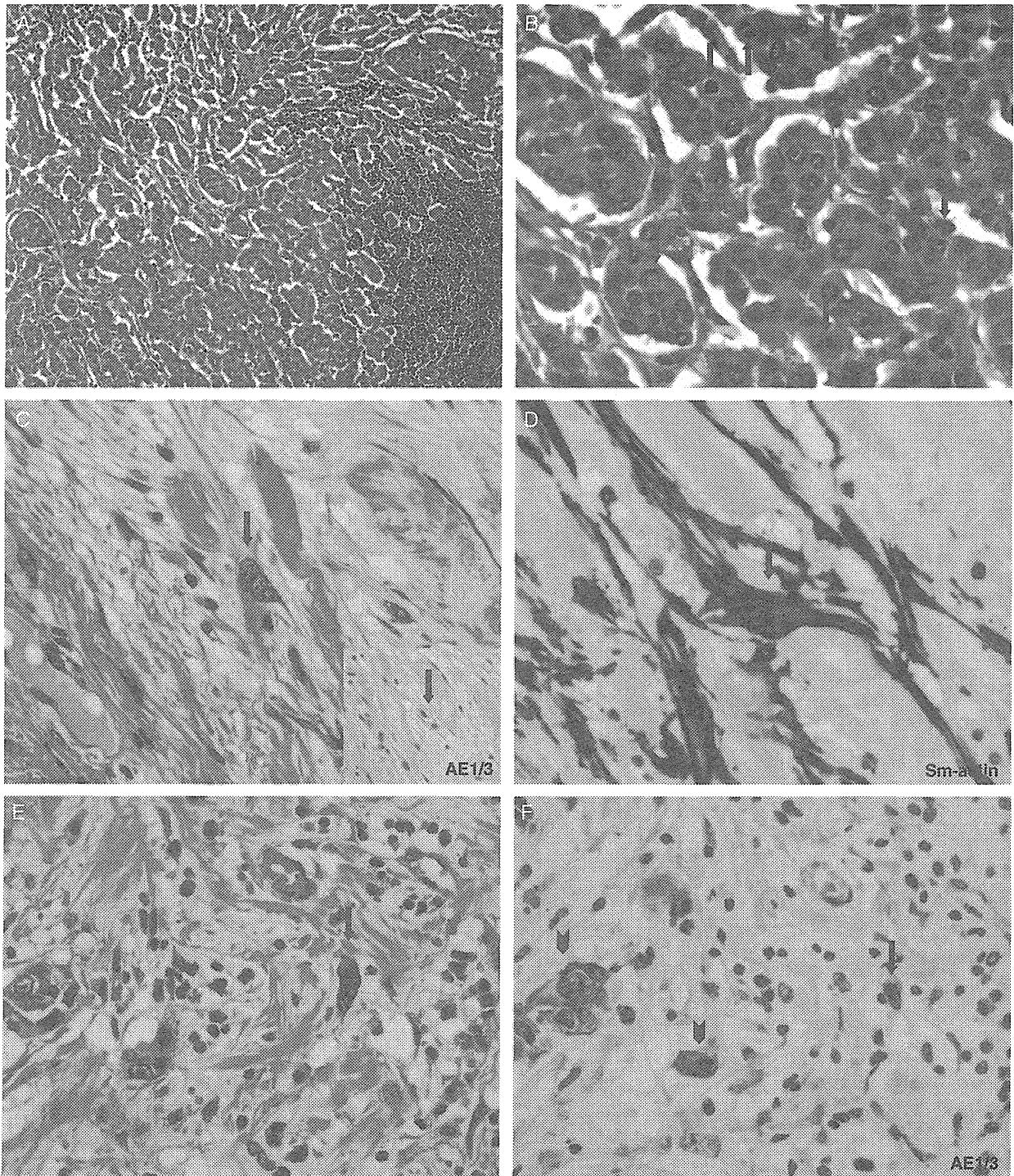


FIGURE 2. A and B, Metastasis of carcinoma to the lymph node. A, Metastatic carcinoma cells in the lymph node. B, Seven mitotic figures are visible in the tumor cells (arrows). C to F, Histologic features of atypical tumor-stromal fibroblasts. C, One atypical tumor-stromal fibroblast with a bizarre and convoluted large nucleus is visible (arrows), and the fibroblast is negative for keratins (AE1/3, arrow in the insert). D, Positive cytoplasmic staining for α -smooth muscle actin (arrow) in a fibroblast. E and F, One atypical tumor-stromal fibroblast with a large bizarre nucleus with obvious large nucleoli and coarsely granulated nuclear chromatin is visible in the vicinity of the tumor cells (arrow); the fibroblast exhibits negative staining for keratin (arrow), but tumor cells adjacent to the fibroblast are positive for keratins (AE1/3, arrowheads).

TABLE 1. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in All the Invasive Ductal Carcinoma Patients in this Series (n = 1042)

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Allred scores for progesterone receptors in tumor cells							
0 or 2	183	48 (26)	Referent	0.097	24 (13)	Referent	0.863
3 to 6	303	58 (19)	0.7 0.5-1.1		36 (12)	0.9 0.5-1.7	
7 or 8	556	78 (14)	0.6 0.4-0.9		29 (5)	0.5 0.3-0.8	
Blood vessel invasion							
Absent	891	139 (16)	Referent	0.010	62 (7)	Referent	0.004
Present	149	45 (30)	1.6 1.1-2.4		27 (18)	2.0 1.2-3.3	
Grading system for lymph vessel tumor emboli							
Grade 0	666	74 (11)	Referent	0.087	30 (5)	Referent	0.263
Grade 1	250	43 (17)	1.4 0.9-2.1		18 (7)	1.4 0.8-2.7	
Grade 2	97	46 (47)	3.0 1.9-4.7		24 (25)	3.2 1.9-5.2	
Grade 3	29	21 (72)	4.9 2.7-9.0	< 0.001	17 (59)	4.3 2.2-8.5	< 0.001
Fibrotic focus, diameter (mm)							
Absent	667	95 (14)	Referent	0.025	42 (6)	Referent	0.016
≤ 8	221	37 (17)	Referent		15 (7)	Referent	
> 8	154	52 (34)	1.7 1.1-2.6		32 (21)	1.9 1.1-7.9	
Histologic grade							
Grade 1	262	15 (5)	Referent	0.081	2 (0.7)	Referent	0.026
Grade 2	439	61 (14)	1.7 0.9-3.3		27 (6)	5.2 1.2-22.0	
Grade 3	341	108 (31)	2.4 1.1-5.4		60 (18)	5.7 1.3-24.4	
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	591	54 (9)	Referent	0.006	17 (3)	Referent	< 0.001
≤ 5	283	46 (16)	Referent		17 (6)	Referent	
> 5	165	84 (55)	1.9 1.2-3.0		55 (33)	3.8 2.3-6.3	
Types of invasive ductal carcinoma							
Type 1	627	78 (12)	Referent	0.008	34 (5)	Referent	0.126
Type 2	40	17 (43)	2.2 1.2-3.9		8 (20)	2.0 0.8-5.0	
Type 3	346	72 (21)	1.6 0.8-2.1		34 (10)	1.5 0.7-3.2	
Type 4	29	17 (59)	3.2 1.9-10.3	0.001	13 (45)	3.2 1.6-6.5	0.001

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis.

The antibodies used were the antiestrogen receptor mouse monoclonal antibody ER88 (BioGenex), the anti-progesterone receptor mouse monoclonal antibody PR88 (BioGenex), and the anti-HER2 mouse monoclonal antibody CB11 (BioGenex). ER88, PR88, and CB11 were previously diluted. After immunostaining, the sections were counterstained with hematoxylin. Sections of the invasive ductal carcinomas that were positive for estrogen receptor, progesterone receptor, and HER2 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin. Slides of the tumor cells immunostained for estrogen and progesterone receptors were scored using the Allred scoring system, as described previously.^{1,11,26} The Allred scores for estrogen and progesterone receptors

in the tumor cells were classified as follows¹⁷: (1) Allred score for estrogen receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8) and (2) Allred score for progesterone receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8). The HER2 status of the tumor cells was semiquantitatively scored on a scale of 0 to 3 according to the level of HER2 protein expression³⁰ and was classified into 3 categories: 0 or 1, 2 and 3.

The patients were classified into the following 4 subtypes according to their hormone receptor status and HER2 category^{8,24}: (1) luminal A subtype, comprised of estrogen receptor positive and/or progesterone receptor positive and HER2 category 0 or 1; (2) luminal B subtype, comprised of estrogen receptor positive and/or progesterone receptor positive and HER2 category 3; (3) HER2 subtype, comprised of estrogen receptor negative,

progesterone receptor negative, and HER2 category 3; and (4) triple negative subtype, comprised of estrogen receptor negative, progesterone receptor negative, and HER2 category 0 or 1. Invasive ductal carcinomas with an Allred score 0 or 2 for estrogen receptor and progesterone receptor were considered negative for estrogen receptor and progesterone receptor, respectively. As only HER2 samples scored as category 3 were considered positive,³⁰ a total of 182 patients with HER2 category 2 invasive ductal carcinoma were classified as equivocal HER2 subtype (without taking their hormone receptor status into account) in this study.

Patient Outcome and Statistical Analysis

Survival was evaluated using a median follow-up period of 98 months (range, 63 to 134 mo) until March 2011. Of the 1042 invasive ductal carcinoma patients, 858 patients were alive and well, 184 had developed tumor recurrences, and 89 had died of their disease. The tumor recurrence-free survival and overall survival periods were calculated using the time of surgery as the starting point. Tumor relapse was

considered to have occurred whenever evidence of distant-organ metastasis or local recurrence was found.

We analyzed the outcome predictive power of the 7 well-known histologic factors, the 4 histologic factors that we proposed (fibrotic focus, type of invasive ductal carcinoma, grading system for lymph vessel tumor emboli, No. of mitotic figures in metastatic carcinoma to the lymph nodes), the Allred scores for estrogen and progesterone receptors and the category of HER2 expression in the tumor cells, the use of adjuvant therapy (yes or no), patient age (< 39 y and > 39 y), and the UICC-pathologic nodal status²⁸ for tumor recurrence and tumor-related death in univariate analyses using the Cox proportional hazard regression model. The factors that were significantly associated with outcome in the univariate analyses were then entered together into a multivariate analysis. Univariate analysis and multivariate analysis were performed using the Cox proportional hazard regression model. The case-wise and step-down methods were applied until all the remaining factors were significant at a *P* value below 0.05. All the analyses were performed using Statistica/Windows software (StatSoft, Tulsa, OK).

TABLE 2. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in Invasive Ductal Carcinoma Patients With or Without Nodal Metastases

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	<i>P</i>	Cases (%)	HR 95% CI	<i>P</i>
Patients Without Nodal Metastasis (n = 591)							
Types of invasive ductal carcinoma							
Type 1	393	27 (7)	Referent		9 (2)	Referent	
Type 2	22	6 (27)	2.9	0.016	2 (9)	2.3	0.314
			1.2-6.9			0.5-11.0	
Type 3	163	15 (9)	0.6	0.341	4 (3)	0.5	0.414
			0.2-1.7			0.1-3.1	
Type 4	13	6 (46)	6.8	< 0.001	2 (15)	5.2	0.036
			2.8-16.3			1.1-24.7	
Patients With Nodal Metastases (n = 451)							
			Tumor Recurrence		Tumor-Related Death		
Blood vessel invasion							
Absent	364	93 (26)	Referent		48 (13)	Referent	
Present	87	37 (43)	2.0	< 0.001	24 (28)	2.0	0.025
			1.3-2.9			1.1-3.6	
Grading system for lymph vessel tumor emboli							
Grade 0	201	36 (18)	Referent		19 (10)	Referent	
Grade 1	139	34 (25)	1.6	0.072	14 (10)	1.5	0.319
			0.9-2.6			0.7-3.1	
Grade 2	83	40 (48)	3.3	< 0.001	23 (28)	2.7	0.005
			1.1-5.4			1.4-5.3	
Grade 3	28	20 (71)	5.2	< 0.001	16 (57)	4.0	< 0.001
			3.3-9.3			1.8-9.0	
No. mitotic figures in metastatic carcinoma to lymph nodes							
≤ 5	286	46 (16)	Referent		17 (6)	Referent	
> 5	165	84 (51)	3.0	< 0.001	55 (33)	3.3	< 0.001
			2.1-4.5			1.8-6.4	
Types of invasive ductal carcinoma							
Type 1	234	51 (22)	Referent		25 (11)	Referent	
Type 2	18	11 (61)	1.9	0.049	6 (33)	1.6	0.346
			1.0-3.4			0.6-4.5	
Type 3	183	57 (31)	1.9	0.089	30 (16)	1.0	0.932
			0.9-4.0			0.4-2.0	
Type 4	16	11 (69)	3.1	< 0.001	11 (69)	3.5	0.021
			1.6-6.0			1.2-10.1	

CI indicates confidence interval; HR, hazard ratio.

RESULTS

Among all the patients with invasive ductal carcinoma, the presence of blood vessel invasion, lymph vessel tumor embolus grades 2 and 3, a fibrotic focus diameter > 8 mm, histologic grade 3, > 5 mitotic figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 1). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence, and histologic grade 2 had a significantly higher hazard ratio for tumor-related death in a multivariate analysis (Table 1). An Allred score of 7 or 8 for progesterone receptor in the tumor cells had significantly lower hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 1).

Among patients with invasive ductal carcinoma without nodal metastasis, type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 2). A fibrotic focus > 8 mm ($P = 0.009$), histologic grades 2 ($P = 0.030$) and 3 ($P = 0.011$), type 2 invasive ductal carcinoma (Table 2), lymph vessel tumor embolus grade 2 or 3 ($P < 0.001$), and HER2 category 3 ($P = 0.028$) had significantly higher hazard ratios for tumor recurrence in a multivariate analysis. A mitotic

activity index of > 20 in primary invasive tumors had a significantly higher hazard ratio for tumor-related death in a multivariate analysis ($P = 0.011$).

Among patients with invasive ductal carcinoma with nodal metastases, the presence of blood vessel invasion, lymph vessel tumor grades 2 and 3, > 5 mitotic figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 2). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence in a multivariate analysis (Table 2).

Among patients with UICC pTNM stage I invasive ductal carcinoma, a fibrotic focus diameter > 8 mm (Fig. 3A, B) and histologic grade 3 had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). Histologic grade 2 (Table 3) and lymph vessel tumor embolus grade 2 or 3 had a significantly higher hazard ratio for tumor recurrence ($P < 0.001$), and an Allred score of 7 or 8 for estrogen receptors in tumor cells had a significantly lower hazard ratio for tumor recurrence ($P = 0.008$) in a multivariate analysis.

Among patients with UICC pTNM stage II invasive ductal carcinoma, the presence of blood vessel invasion, lymph vessel tumor embolus grades 2 and 3, > 5 mitotic

TABLE 3. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in UICC pTNM Stages I, II, and III Invasive Ductal Carcinoma Patients

	Cases	Tumor Recurrence			Tumor-Related Death		
		Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
UICC pTNM Stage I Patients (n = 363)							
Fibrotic focus, diameter (mm)							
Absent	273	18 (7)	Referent		5 (2)	Referent	
≤ 8	66	4 (6)	Referent		1 (2)	Referent	
> 8	24	6 (25)	3.1 1.2-7.9	0.020	4 (17)	6.9 1.5-20.6	0.009
Histologic grade							
Grade 1	128	1 (0.8)	Referent		0	Referent	
Grade 2	160	11 (7)	8.4 1.1-65.2	0.041	2 (1)	Referent	
Grade 3	75	16 (21)	14.6 1.9-113.2	0.010	8 (11)	7.6 1.5-37.9	0.014
UICC pTNM Stage II Patients (n = 487)							
Blood vessel invasion							
Absent	404	64 (16)	Referent		24 (6)	Referent	
Present	83	23 (28)	1.8 1.1-2.9	0.027	11 (13)	2.4 1.1-5.5	0.046
Grading system for lymph vessel tumor emboli							
Grade 0	296	35 (12)	Referent		10 (3)	Referent	
Grade 1	136	25 (18)	1.5 0.9-2.6	0.132	12 (9)	2.7 1.2-6.3	0.022
Grade 2	46	22 (48)	3.3 1.9-5.5	< 0.001	8 (17)	3.9 1.5-10.3	0.006
Grade 3	9	5 (56)	4.1 1.6-11.0	0.005	5 (56)	8.5 2.7-27.0	< 0.001
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	228	26 (11)	Referent		7 (3)	Referent	
≤ 5	184	27 (15)	Referent		8 (4)	Referent	
> 5	75	34 (45)	2.7 1.6-4.2	< 0.001	20 (27)	5.6 2.7-11.8	< 0.001

TABLE 3. (continued)

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Types of invasive ductal carcinoma							
Type 1	271	34 (13)	Referent		14 (5)	Referent	
Type 2	20	11 (55)	2.4	0.013	4 (20)	1.2	0.761
			1.2-4.7			0.4-4.2	
Type 3	184	35 (19)	1.3	0.391	13 (7)	0.8	0.695
			0.7-2.4			0.4-1.9	
Type 4	12	7 (58)	4.4	< 0.001	4 (33)	5.4	0.002
			1.9-10.3			1.8-9.5	
UICC pTNM Stage III Patients (n = 192)							
Fibrotic focus, diameter (mm)							
Absent	103	32 (31)	Referent		16 (27)	Referent	
≤8	47	16 (34)	Referent		8 (17)	Referent	
> 8	42	21 (50)	2.9	< 0.001	17 (41)	3.4	< 0.001
			1.6-5.0			1.7-6.6	
Grading system for lymph vessel tumor emboli							
Grade 0	79	19 (24)	Referent		13 (16)	Referent	
Grade 1	52	14 (27)	1.0	0.917	4 (8)	0.6	0.366
			0.5-2.3			0.2-1.9	
Grade 2	42	21 (50)	2.4	0.002	16 (38)	3.7	< 0.001
			1.4-4.3			1.8-7.4	
Grade 3	19	15 (79)	4.9	< 0.001	11 (58)	4.7	< 0.001
			2.5-9.8			2.0-10.9	
HER2 category							
0 or 1	126	41 (33)	Referent		24 (19)	Referent	
2	38	10 (26)	0.5	0.110	6 (16)	2.3	0.095
			0.2-1.2			0.8-5.9	
3	28	18 (64)	2.4	0.003	14 (50)	3.4	< 0.001
			1.4-4.3			1.7-6.7	
UICC pN category							
pN0	23	2 (9)	Referent		1 (4)	Referent	
pN1	36	8 (22)	1.5	0.617	6 (17)	2.7	0.411
			0.3-8.1			0.3-29.2	
pN2	85	30 (35)	1.6	0.539	16 (19)	2.5	0.435
			0.3-7.9			0.3-24.8	
pN3	48	29 (60)	2.1	0.004	21 (44)	1.9	0.037
			1.3-3.5			1.1-3.5	

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis; pN, pathologic regional lymph node; pN0, no nodal metastasis; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases.

figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence (Table 3), and lymph vessel tumor embolus grade 1 had a significantly higher hazard ratio for tumor-related death in a multivariate analysis (Table 3).

Among patients with UICC pTNM stage III invasive ductal carcinoma, a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, HER2 category 3, and the UICC pN3 category had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). A mitotic activity index of 20 or higher in the primary invasive tumors had a significantly higher hazard ratio for tumor recurrence in a multivariate analysis ($P = 0.005$).

Among patients with luminal A-subtype invasive ductal carcinoma, the presence of blood vessel invasion,

lymph vessel tumor embolus grade 3 (Fig. 3C, D), > 5 mitotic figures in metastatic carcinoma to the lymph nodes (Fig. 3E, F), type 4 invasive ductal carcinoma (Fig. 3G, H), and the UICC pN3 category had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel tumor embolus grade 2 (Table 4, Fig. 3C) and a fibrotic focus diameter > 8 mm ($P = 0.016$) had significantly higher hazard ratio for tumor recurrence, and the UICC pN1 category ($P = 0.019$) had a significantly higher hazard ratio for tumor-related death in a multivariate analysis.

Among patients with luminal B-subtype invasive ductal carcinoma, types 2 and 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Type 3 invasive ductal carcinoma (Table 4), lymph vessel tumor embolus grades 2 ($P = 0.030$) and 3 ($P < 0.001$), and the UICC pN3 category ($P < 0.001$) had significantly higher hazard ratio

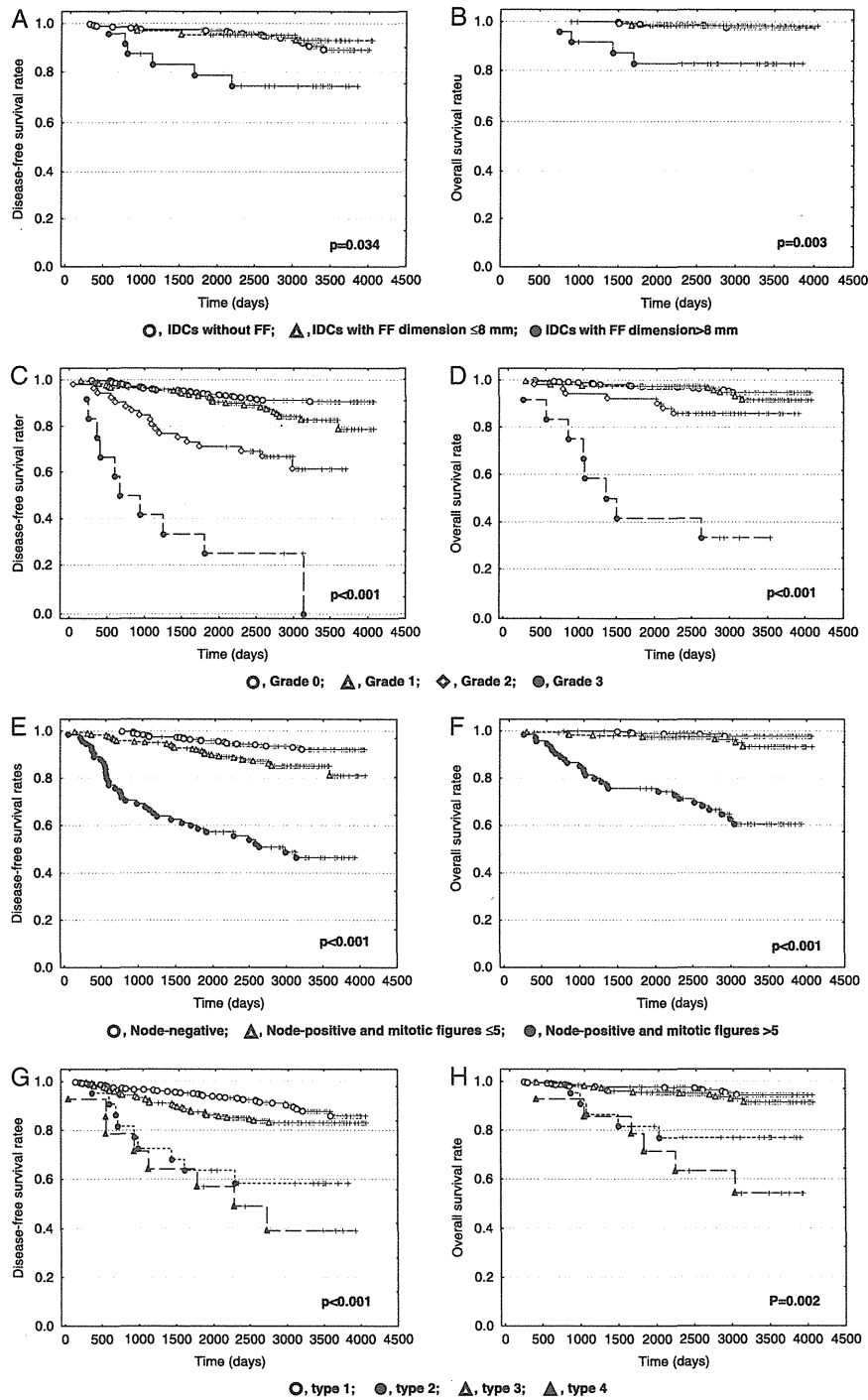


FIGURE 3. Disease-free survival curves and overall survival curves of invasive ductal carcinoma patients with the UICC pTNM stage I (A and B) and luminal-A subtype invasive ductal carcinoma patients (C–H). A and B, Patients with invasive ductal carcinoma with fibrotic foci dimension >8 mm have shorter disease-free and overall survival times than patients without fibrotic foci and those with fibrotic foci <8 mm. IDC, invasive ductal carcinoma; FF, fibrotic foci. C and D, The disease-free and overall survival curves decreased significantly according to the grade of lymph vessel tumor embolus. Grade, lymph vessel tumor embolus grade. E and F, The disease-free and overall survival curves decreased significantly according to the number of mitotic figures in metastatic carcinoma to the lymph nodes. Node negative, no nodal metastasis; node positive, nodal metastases; mitotic figures, number of mitotic figures in metastatic carcinoma to the lymph nodes. G and H, Patients with types 2, 3, and 4 invasive ductal carcinoma have shorter disease-free and overall survival times than patients with type 1 invasive ductal carcinoma.

TABLE 4. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in Patients With Invasive Ductal Carcinoma According to Biological Subtype

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Luminal A-subtype Patients (n = 658)							
Blood vessel invasion							
Absent	552	62 (11)	Referent		25 (5)	Referent	
Present	104	27 (26)	2.2 1.4-3.6	0.002	16 (15)	2.3 1.2-4.4	0.015
Grading system for lymph vessel tumor emboli							
Grade 0	425	36 (9)	Referent		17 (4)	Referent	
Grade 1	169	25 (15)	1.5 0.9-2.5	0.141	9 (5)	1.1 0.5-2.5	0.885
Grade 2	52	18 (35)	3.4 1.9-6.0	< 0.001	7 (14)	0.9 0.3-3.3	0.898
Grade 3	12	10 (83)	6.5 3.0-14.0	< 0.001	8 (66)	4.7 2.0-11.4	< 0.001
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	383	24 (6)	Referent		6 (2)	Referent	
≤5	200	27 (14)	Referent		8 (4)	Referent	
> 5	75	38 (51)	3.5 2.1-5.8	< 0.001	27 (36)	6.6 3.1-14.2	< 0.001
Types of invasive ductal carcinoma							
Type 1	409	38 (9)	Referent		16 (4)	Referent	
Type 2	22	9 (41)	1.9 0.8-4.3	0.152	5 (23)	1.6 0.4-5.7	0.470
Type 3	213	34 (16)	0.9 0.5-1.7	0.679	14 (7)	0.8 0.3-2.1	0.583
Type 4	14	8 (57)	2.4 1.0-5.6	0.045	6 (43)	3.9 1.5-9.9	0.005
UICC pN category							
pN0	383	24 (6)	Referent		6 (2)	Referent	
pN1	199	38 (19)	1.7 0.9-3.1	0.104	21 (11)	2.6 1.2-5.8	0.019
pN2	50	11 (22)	1.1 0.4-2.7	0.864	4 (8)	1.1 0.2-5.3	0.879
pN3	26	16 (62)	2.3 1.2-4.2	0.008	10 (39)	3.9 1.4-10.5	0.008
Luminal B-subtype Patients (n = 88)							
Types of invasive ductal carcinoma							
Type 1	51	8 (16)	Referent		3 (6)	Referent	
Type 2	6	4 (67)	8.6 2.4-30.7	0.001	3 (50)	21.2 2.5-184.7	0.006
Type 3	28	13 (46)	4.3 1.7-11.0	0.002	7 (25)	1.4 0.1-15.4	0.780
Type 4	3	2 (67)	13.2 2.5-68.2	0.002	2 (67)	150.9 1.1-2048.8	0.046
Equivocal HER2-subtype Patients (n = 182)							
Grading system for lymph vessel tumor emboli							
Grade 0	109	10 (9)	Referent		2 (2)	Referent	
Grade 1	49	10 (20)	1.7 0.6-4.5	0.299	5 (10)	7.7 1.4-42.9	0.020
Grade 2	18	10 (56)	6.8 2.1-22.6	0.002	5 (28)	14.3 2.6-77.9	0.002
Grade 3	6	2 (33)	3.7 0.9-16.1	0.079	2 (33)	16.8 2.2-127.6	0.006
Invasive tumor size (mm)							
≤20	84	6 (7)	Referent		2 (2)	Referent	
> 20-< 50	93	24 (26)	4.5 0.7-28.4	0.114	10 (11)	1.8 0.3-9.7	0.516
> 50	5	2 (40)	3.2 1.1-9.5	0.036	2 (40)	4.8 1.2-36.8	0.029
Types of invasive ductal carcinoma							
Type 1	95	13 (14)	Referent		6 (6)	Referent	
Type 2	6	2 (33)	2.7 0.5-15.7	0.265	0	Referent	
Type 3	75	13 (17)	0.8 0.4-2.0	0.697	6 (7)	1.3 0.4-4.5	0.692

TABLE 4. (continued)

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Type 4	6	4 (67)	11.9 3.1-44.8	< 0.001	2 (33)	7.6 1.5-38.0	0.013
Triple-negative Patients (n = 75)							
Grading system for lymph vessel tumor emboli							
Grade 0	51	6 (12)	Referent		2 (4)	Referent	
Grade 1	8	1 (13)	0.8 0.1-6.7	0.830	0	Referent	
Grade 2	11	10 (90)	19.1 3.6-101.2	< 0.001	7 (64)	24.1 4.7-122.8	< 0.001
Grade 3	5	4 (80)	65.3 5.9-722.6	< 0.001	3 (60)	32.6 5.2-209.0	< 0.001

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis; pN, pathologic regional lymph node; pN0, no nodal metastasis; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases.

for tumor recurrence, and the UICC pN2 category ($P = 0.019$), a fibrotic focus diameter 8 mm ($P < 0.001$), and an invasive tumor size > 50 mm ($P = 0.047$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis.

Among patients with HER2-subtype invasive ductal carcinoma (n = 39), the presence of blood vessel invasion ($P = 0.009$) and the UICC pN3 category ($P = 0.007$) had significantly higher hazard ratio for tumor recurrence in a multivariate analysis. As only 7 patients died as a result of their disease, a multivariate analysis for tumor-related death could not be performed in this patient series.

Among the patients with equivocal HER2 invasive ductal carcinoma, lymph vessel tumor embolus grade 2, invasive tumor size > 50 mm, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel embolus grades 1 and 3 (Table 4) and histologic grade 3 ($P = 0.010$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis. Among the patients with triple-negative invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3 had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4).

DISCUSSION

The histologic factors that significantly increased the hazard ratios for tumor recurrence or tumor-related death are shown in Table 5. Histologic factors that significantly increased hazard ratios for both tumor recurrence and tumor-related death were evaluated as A rank predictors, those that significantly increased the hazard ratio only for tumor-related death were evaluated as B rank predictors, those that significantly increased the hazard ratio only for tumor recurrence were evaluated as C rank predictors, and those that failed to significantly increase the hazard ratio for either tumor recurrence or tumor-related death were evaluated as D rank predictors.

The 20 histologic factors were then ranked in decreasing order of their contribution to the accurate prediction of tumor recurrence or tumor-related death according to various tumor statuses (Table 5). Among them, type 4 invasive ductal carcinoma was evaluated as A rank predictors in 7 of the tumor status classifications (A7), followed by lymph vessel tumor embolus grades 3 and 2 (A6), blood vessel invasion (A4), > 5 mitotic figures in metastatic carcinoma to the lymph nodes (A4), a fibrotic focus diameter > 8 mm (A3), histologic grade 3 (A2), UICC pN3 category (A2), invasive tumor size > 50 mm (A1), type 2 invasive ductal carcinoma (A1), HER2 category 3 (A1), and an Allred score of 7 or 8 for progesterone receptors in tumor cells (A1). These 12 histologic factors were also evaluated as B or C rank predictors in other tumor status classifications in which they were not identified as A rank predictors. Thus, these 12 histologic factors are likely to be very important histologic outcome predictors for patients with invasive ductal carcinoma of the breast.

Of note, only C rank predictors (blood vessel invasion and UICC pN3 category) were identified among these 12 histologic factors for patients with HER2-subtype invasive ductal carcinoma. This result may reflect the relatively small number of patients with HER2-subtype invasive ductal carcinoma in this study (39 patients, 4%), although this study included 1042 patients with invasive ductal carcinoma of the breast. Thus, a multi-institutional case study may be needed to clarify the optimal histologic predictors of outcome for patients with HER2-subtype invasive ductal carcinoma.

Although an absolute histologic predictor of outcome applicable to all patients with invasive ductal carcinoma was not identified in this study, appropriate histologic predictors of outcome were identified for various tumor statuses. We listed only the A rank predictors for each tumor status in Table 6. These factors are the core histologic predictors of outcome for patients with specific invasive ductal carcinoma tumor statuses

TABLE 5. Ranking of 18 Histologic Factors According to Outcome Predictive Power

	Rank	Overall (n = 1042)	N0 (n = 591)	N+ (n = 451)	Stage I (n = 363)	Stage II (n = 487)	Stage III (n = 192)	Luminal A (n = 658)	Luminal B (n = 88)	HER2 (n = 39)	eHER2 (n = 182)	TN (n = 75)
Type 4	A7; B0 C0; D4	A	A	A	D	A	D	A	A	D	A	D
Ly grade 3	A6; B1 C3; D1	A	C	A	C	A	A	A	C	D	B	A
Ly grade 2	A6; B0 C4; D1	A	C	A	C	A	A	C	C	D	A	A
BV invasion	A4; B0 C1; D6	A	D	A	D	A	D	A	D	C	D	D
> 5 MF, met ca to LN	A4; B0 C0; D6	A	NA	A	D	A	D	A	D	D	D	D
FF diameter > 8 mm	A3; B1 C2; D5	A	C	D	A	D	A	C	B	D	D	D
Histologic grade 3	A2; B1 C0; D7	A	C	D	A	D	D	D	D	D	B	D
pN3	A2; B0 C2; D4	D	NA	D	NA	NA	A	A	C	C	D	D
Tumor size > 50 mm	A1; B1 C0; D8	D	D	D	NA	D	D	D	B	D	A	D
Type 2	A1; B0 C4; D6	C	C	C	D	C	D	D	A	D	D	D
HER2 category 3	A1; B0 C2; D3	C	C	D	D	D	A	NA	NA	NA	NA	NA
PR Allred 7 or 8	A1; B0 C0; D6	A	D	D	D	D	D	NA	NA	NA	D	NA
Ly grade 1	A0; B2 C0; D9	D	D	D	D	B	D	D	D	D	B	D
MAI of > 20 in PIT	A0; B2 C0; D9	D	B	D	D	D	B	D	D	D	D	D
Histologic grade 2	A0; B1 C2; D8	B	C	D	C	D	D	D	D	D	D	D
pN2	A0; B1 C0; D7	D	NA	D	NA	NA	D	D	B	D	D	D
Skin invasion	A0; B1 C0; D8	B	D	D	NA	NA	D	D	D	D	D	D
pN1	A0; B1 C0; D9	D	NA	D	D	D	D	B	D	D	D	D
ER Allred 7 or 8	A0; B0 C1; D6	D	D	D	C	D	D	NA	NA	NA	D	NA
Type 3	A0; B0 C1; D10	D	D	D	D	D	D	D	C	D	D	D

A rank of A was given for factors significantly associated with tumor recurrence and tumor-related death in multivariate analyses; a rank of B was given for factors that were significantly associated with tumor-related death in a multivariate analysis; a rank of C was given for factors that were significantly associated with tumor recurrence in a multivariate analysis; a rank of D was given for factors that were not associated with either tumor recurrence or tumor-related death in multivariate analyses or in univariate analyses; Overall, all the patients in this study; N0, patients without nodal metastasis; N+, patients with nodal metastasis; Stages I, II, and III, UICC pTNM stages I, II and III, respectively; Luminal A, luminal-A subtype invasive ductal carcinoma patients; Luminal B, luminal-B subtype invasive ductal carcinoma patients; HER2, HER2-subtype invasive ductal carcinoma patients; eHER2, equivocal HER2 subtype; TN, triple-negative invasive ductal carcinoma patients; Type 4, type 4 invasive ductal carcinoma; Ly grade, grading system for lymph vessel tumor emboli; Ly grade 3, grade 3 lymph vessel tumor emboli; Ly grade 2, grade 2 lymph vessel tumor emboli; BV, blood vessel; MF, mitotic figures; met, metastatic; ca, carcinoma; LN, lymph nodes; FF, fibrotic focus; pN3, UICC pN3 category; Tumor size, primary invasive tumor size; Type 2, type 2 invasive ductal carcinoma; PR, progesterone receptor; Allred, Allred score; Ly grade 1, grade 1 lymph vessel tumor emboli; MAI, mitotic activity index; PIT, primary invasive tumor; pN2, UICC pN2 category; pN1, UICC pN1 category; ER, estrogen receptor; Type 3, type 3 invasive ductal carcinoma; NA, not available.

and may enable pathologists or clinicians to predict the outcomes of many patients with invasive ductal carcinoma accurately. Among these factors, a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, types 2 and 4 invasive ductal carcinoma, and > 5 mitotic figures in metastatic carcinoma to the lymph nodes were histologic factors that we proposed.^{12-16,18-20} Thus, many readers may believe that the reliabilities of these factors as outcome predictors are inferior to those of well-known histologic factors, such as the presence of blood vessel invasion, histologic grade 3, HER2

category 3, and UICC pN3 category. However, outcome predictive power of a fibrotic focus among patients with invasive ductal carcinoma without nodal metastasis or patients with early invasive ductal carcinoma has also been confirmed by other investigators.^{3,6,23} We have also confirmed the outcome predictive powers of the grading system for lymph vessel tumor emboli and the presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes in different invasive ductal carcinoma patient groups.^{15,16,18,20} Thus, the proposed histologic factors seem to be very useful as predictors of outcome

TABLE 6. Best histologic Factors for Predicting Outcome Among Patients With Invasive Ductal Carcinoma According to Tumor status

Lymph Node Status	
Lymph node-negative invasive ductal carcinoma Type 4 invasive ductal carcinoma	
Lymph node-positive invasive ductal carcinoma Blood vessel invasion > 5 mitotic figures in metastatic carcinoma to lymph nodes	Lymph vessel tumor embolus grades 2 and 3 Type 4 invasive ductal carcinoma
UICC pTNM stage I invasive ductal carcinoma Fibrotic foci diameter > 8 mm	UICC pTNM stage Histologic grade 3
UICC pTNM stage II invasive ductal carcinoma Blood vessel invasion > 5 mitotic figures in metastatic carcinoma to lymph nodes	Lymph vessel tumor embolus grades 2 and 3 Type 4 invasive ductal carcinoma
UICC pTNM stage III invasive ductal carcinoma Fibrotic foci diameter > 8 mm Lymph vessel tumor embolus grades 2 and 3 and UICC pN3	HER2 category 3 Type 4 invasive ductal carcinoma
Luminal A invasive ductal carcinoma Blood vessel invasion > 5 mitotic figures in metastatic carcinoma to lymph nodes and UICC pN3	Carcinoma subtype Lymph vessel tumor embolus grade 3 Type 4 invasive ductal carcinoma
Luminal B invasive ductal carcinoma Types 2 and 4 invasive ductal carcinoma	
Equivocal HER2 invasive ductal carcinoma Invasive tumor size > 50 mm Type 4 invasive ductal carcinoma	Lymph vessel tumor embolus grade 2
Triple-negative invasive ductal carcinoma Lymph vessel tumor embolus grades 2 and 3	

among patients with invasive ductal carcinoma of the breast.

This study clearly demonstrated that the outcome predictive power of the invasive tumor size is inferior to that of a fibrotic focus, blood vessel invasion, the grading system for lymph vessel tumor emboli, histologic grade, or the type of invasive ductal carcinoma. The outcome predictive power of the number of nodal metastases was also inferior to the number of mitotic figures in metastatic carcinoma to the lymph nodes in this study. The number of nodal metastases and the invasive tumor size reflect the quantity of invasive ductal carcinoma cells, whereas the presence of a fibrotic focus, blood vessel invasion, grading system for lymph vessel tumor emboli, histologic grade, type of invasive ductal carcinoma, and the number of mitotic figures in metastatic carcinoma to the lymph nodes reflect the tumor characteristics of invasive ductal carcinomas. Furthermore, we observed that 1 UICC stage I patient with 1 micrometastasis with > 5 mitotic figures died of her diseases in this study (data not shown). Thus, histologic factors reflecting tumor characteristics are most likely superior to histologic factors reflecting tumor quantity as outcome predictors.

In conclusion, this study clearly demonstrated that our proposed histologic factors, such as type 4 invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3, presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes, and a fibrotic focus diameter > 8 mm, are very important histologic factors for accurately predicting the outcomes of patients with

invasive ductal carcinoma. Combined pathologic examinations based on these histologic factors and A-ranked, well-known histologic factors (blood vessel invasion, histologic grade, HER2 category, and UICC pN category) would most likely enable pathologists to assess the true malignant potential of invasive ductal carcinomas of the breast accurately.

REFERENCES

- 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;85:200–206.
- Andersson Y, Frisell J, Sylvan M, et al. Breast cancer survival in relation to the metastatic tumor burden in axillary lymph nodes. *J Clin Oncol.* 2010;28:2868–2873.
- 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;41:2093–2101.
- Baak JP, van Dieast PJ, Voorhorst F, et al. The prognostic value of proliferation in lymph-node-negative breast cancer patients is age dependent. *Eur J Cancer.* 2007;43:527–535.
- Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer.* 1957;11:359–377.
- Colpaert C, Vermeulen PB, Jeuris W, et al. Early distant relapse in “node-negative” breast cancer patients is not predicted by occult axillary lymph node metastases, but by the features of the primary tumour. *J Pathol.* 2001;193:442–449.
- Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology.* 1991;19:403–410.
- Fernandes RCM, Bevilacqua JLB, Soares IC, et al. Coordinated expression ER, PR and HER2 define different prognostic subtypes

- among poorly differentiated breast carcinomas. *Histopathology*. 2009;55:346–352.
9. Gabos Z, Thoms J, Ghosh S, et al. The association between biological subtype and locoregional recurrence in newly diagnosed breast cancer. *Breast Cancer Res Treat*. 2010;124:187–194.
 10. 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;11:1929–1935.
 11. 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;17:1474–1481.
 12. Hasebe T, Tsuda H, Tsubono Y, et al. Fibrotic focus in invasive ductal carcinoma of the breast: a histopathological prognostic parameter for tumor recurrence and tumor death within three years after the initial operation. *Jpn J Cancer Res*. 1998;88:590–599.
 13. 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;15:502–516.
 14. 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.
 15. 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.
 16. 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;39:427–436.
 17. Hasebe T, Okada N, Tamura N, et al. p53 expression in tumor-stromal fibroblasts is closely associated with the outcome of patients with invasive ductal carcinoma. *Cancer Sci*. 2009;100:2101–2108.
 18. Hasebe T, Okada N, Iwasaki M, et al. Grading system for lymph vessel tumor emboli: significant outcome predictor for invasive ductal carcinoma of the breast. *Hum Pathol*. 2010;41:706–715.
 19. Hasebe T, Iwasaki M, Akashi-Tanaka S, et al. Atypical tumor-stromal fibroblasts in invasive ductal carcinoma of the breast. *Am J Surg Pathol*. 2011;35:325–336.
 20. Hasebe T, Iwasaki M, Akashi-Tanaka S, et al. Prognostic significance of mitotic figures in metastatic mammary ductal carcinoma to the lymph nodes. *Hum Pathol*. 2011 (In press).
 21. Jatoi I, Hilsenbeck SG, Clark GM, et al. Significance of axillary lymph node metastasis in primary breast cancer. *J Clin Oncol*. 1999;17:2334–2340.
 22. Lauria R, Perrone F, Carlomagno C, et al. The prognostic value of lymphatic and blood vessel invasion in operable breast cancer. *Cancer*. 1995;76:1772–1778.
 23. Maiorano E, Regan MM, Viale G, et al. Prognostic and predictive impact of central necrosis and fibrosis in early breast cancer: results from two international breast cancer study group randomized trials of chemoendocrine adjuvant therapy. *Breast Cancer Res Treat*. 2010;121:211–218.
 24. Marotti JD, Collins LC, Hu R, et al. Estrogen receptor- β expression in invasive breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. *Mod Pathol*. 2010;23:197–204.
 25. Miyashita M, Ishida T, Ishida K, et al. Histopathological subclassification of triple negative breast cancer using prognostic scoring system: five variables as candidates. *Virchows Arch*. 2010;458:65–72.
 26. Mohsin S, Weiss H, Havighurst T, et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol*. 2004;17:1545–1554.
 27. Skaland I, van Diest PJ, Janssen EAM, et al. Prognostic differences of World Health Organization-assessed mitotic activity index and mitotic impression by quick scanning in invasive ductal breast cancer patients younger than 55 years. *Hum Pathol*. 2008;39:584–590.
 28. Sobin LH, Gospodarowicz MK, Wittekind Ch. International Union Against Cancer TNM classification of malignant tumours. In: Sobin LH, Gospodarowicz MK, Wittekind Ch. eds. 7th eds. *Classification of Malignant Tumours*. Geneva: Wiley-Liss; 2009:181–193.
 29. Weigand RA, Isenberg WM, Russo J, et al. Blood vessel invasion and axillary lymph node involvement as prognostic indicators for human breast cancer. *Cancer*. 1982;50:962–969.
 30. 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.

Atypical Tumor-stromal Fibroblasts in Invasive Ductal Carcinoma of the Breast

Takahiro Hasebe, MD, PhD,* Motoki Iwasaki, MD, PhD,† Sadako Akashi-Tanaka, MD, PhD,‡
Takashi Hojo, MD, PhD,‡ Tatsuhiko Shibata, MD, PhD,§ Yuko Sasajima, MD, PhD,||
Takayuki Kinoshita, MD, PhD,‡ and Hitoshi Tsuda, MD, PhD||

Abstract: Tumor-stromal fibroblasts have recently been reported to play important roles in the tumor progression of cancer in various organs. The purpose of this study was to investigate whether any characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of 1042 patients with invasive ductal carcinoma of the breast. We observed a small number of tumor-stromal fibroblasts with characteristic nuclear features existing inside and outside of fibrotic foci and named them atypical tumor-stromal fibroblasts. We then classified invasive ductal carcinomas into 4 types (1, 2, 3, and 4) according to the absence or presence of fibrotic foci and the absence or presence of atypical tumor-stromal fibroblasts. We then analyzed the outcome predictive powers of these types of invasive ductal carcinomas using multivariate analyses that included well-known clinicopathologic factors. The multivariate analyses showed that type 4 invasive ductal carcinomas with fibrotic foci and atypical tumor-stromal fibroblasts had significantly higher hazard ratios for tumor recurrence and tumor-related death, independent of the nodal status and histologic grade, and the type 2 invasive ductal carcinomas without fibrotic foci but with atypical tumor-stromal fibroblasts had a significant higher hazard ratio for tumor recurrence among patients with invasive ductal carcinoma with nodal metastasis and those with histologic grade 3 disease. The results of this study clearly indicated that the presence of atypical tumor-stromal fibroblasts, especially in fibrotic foci, is significantly associated with

tumor recurrence and tumor-related death of patients with invasive ductal carcinoma of the breast.

Key Words: fibroblast, fibrotic focus, p53, tumor cell-stromal cell interaction, breast

(*Am J Surg Pathol* 2011;35:325–336)

Tumor-stromal fibroblasts, or the so-called cancer-associated fibroblasts, have recently been reported to play important roles in the tumor progression of cancer in various organs.^{9,10,21,23,25,28,30} Among tumor-stromal fibroblasts, tumor-stromal fibroblasts that form fibrotic foci have a more significant power for the accurate prediction of the outcome of patients with invasive ductal carcinoma than tumor-stromal fibroblasts that do not form fibrotic foci.¹⁰ A fibrotic focus is a characteristic histologic feature of tumor stroma with scar-like features or a radiating fibrosclerotic core that is surrounded by invasive ductal carcinoma cells.^{8,10,11} A fibrotic focus is composed of a mixture of fibroblasts and various amounts of collagen fibers, with the fibroblasts and collagen fibers composing the fibrotic focus exhibiting a storiform arrangement. We and other researchers have already reported that a fibrotic focus is a very useful histologic tumor-stromal indicator for accurately predicting the outcome of patients with invasive ductal carcinoma.^{3,8,10,11,26} In addition, we recently showed that p53 expression in tumor-stromal fibroblasts was a very important outcome predictor for patients with invasive ductal carcinoma who had or had not received neoadjuvant therapy.^{14,17} Among tumor-stromal fibroblasts expressing p53, the tumor-stromal fibroblasts that also formed fibrotic foci apparently played a very important role in tumor progression in invasive ductal carcinoma of the breast.¹⁵

The purpose of this study was to investigate whether characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of patients with invasive ductal carcinoma. This is because no other studies earlier have investigated the histologic features of tumor-stromal fibroblasts and their association with the outcome of patients with invasive ductal carcinoma of the breast. The results of this study clearly indicated that characteristic histologic features of the nuclei in

From the *Clinical Trials and Practice Support Division, Pathology Consultation Service, Center for Cancer Control and Information Services; †Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center; ‡Department of Breast Surgery; ||Clinical Laboratory Division, National Cancer Center Hospital; and §Cancer Genomics Project, National Cancer Center Research Institute, Chuo-ku, Tokyo.

Supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) (C) (21590393) from the Japan Society for the Promotion of Science and was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (H21-006).

The authors declare no conflict of interest.

Correspondence: Takahiro Hasebe, MD, PhD, Clinical Trials and Practice Support Division, Pathology Consultation Service, Center for Cancer Control and Information Services, National Cancer Center, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: thasebe@ncc.go.jp).

Copyright © 2011 by Lippincott Williams & Wilkins