Table 1 Perfusion values [ml/min/ml]

Case no.	Pathology	Normal mammary gland	Tumor		
			Invasive component	Intraductal component	LN
1	IDC	5–10	111	NA	150
2	DCIS	1–5	NA	42	
3	IDC with DS	5	95–106	80	
4	IDC with DS	10–20	194	NA	
5	IDC with DS	5	52	37–43	
6	IDC	10	48	NA	
7	DCIS	10–20	NA	140	

DS ductal spread, *NA* not applicable, *LN* lymph node

In general, the perfusion values tended to be slightly lower in the intraductal components than in invasive tumors.

Pathology findings and relationships with CT findings

In three patients with a pathologically demonstrated ductal spread, its extent was clearly depicted in the perfusion map images, parts of which could not be depicted by early-enhancement-phase CT. These images showed good agreement with the pathology findings (Figs. 1, 2, 3). In the four patients with localized tumors, including two with ductal carcinoma in situ, the tumor regions were the same in the perfusion map images, early-phase CT images, and pathology specimens. These results suggest that volume perfusion map imaging using a 256-row MSCT scanner can more precisely depict the extent of the ductal spread than conventional breast CT imaging.

Simulation for reduction of scan time points

The exposure dose was 43.7–45 mSv. To reduce the number of scan time points and the exposure dose, we calculated the variation rate of the TDC gradient to determine the perfusion value. The time points at 4, 6.5, 9.0, and 12.0 s were used as the start points of the TDC gradient, and those at 39.0, 44.0, and 49.0 s were used as the end points. The time point at 0 s was defined as the base value, and the data obtained at these eight points was used to calculate the TDC gradient. The variation rates from the original TDC gradients in each case were 0.7, 2.8, 1.65, 1.5, 2.3, 6.4, and 12.9%, respectively. The variation rates for evaluation of the intraductal component in cases three and four were 2.5 and 3.3%, respectively. The consistency of the graphs in patients showing a steady increase in the TDCs was ensured by performing position matching of the image data. However, in cases 6 and 7, which showed an irregular gradual increase in the TDCs, the variation rates were greater than 6%, which was probably attributable to shifting of the ROIs due to respiratory motion that could not be completely corrected. We speculate that breath-holding may reduce the variation rates, and the results suggest that comparable results could be obtained by performing scanning eight times with breath-holding.

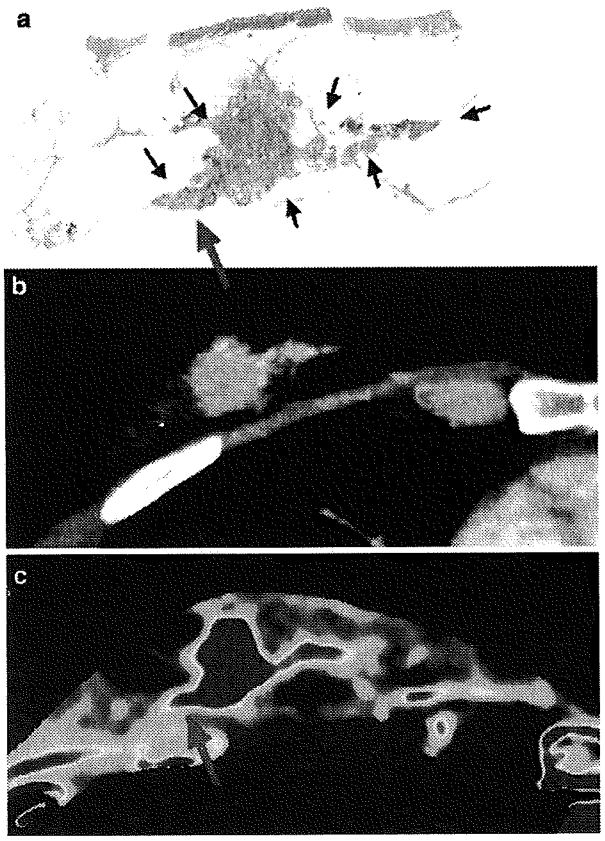

Fig. 1 A 44-year-old woman with invasive ductal carcinoma of the breast. **a** Panoramic view of the tumor in the resected tissues. **b** CT image in the early-enhancement phase. Tumor tissues extending to the left from the main tumor are not depicted. **c** Perfusion map image. The tumor tissues (including the tumor on the left side that could not be visualized in the early-enhancement phase) are clearly depicted (*pink arrow*), showing good agreement with the pathology findings

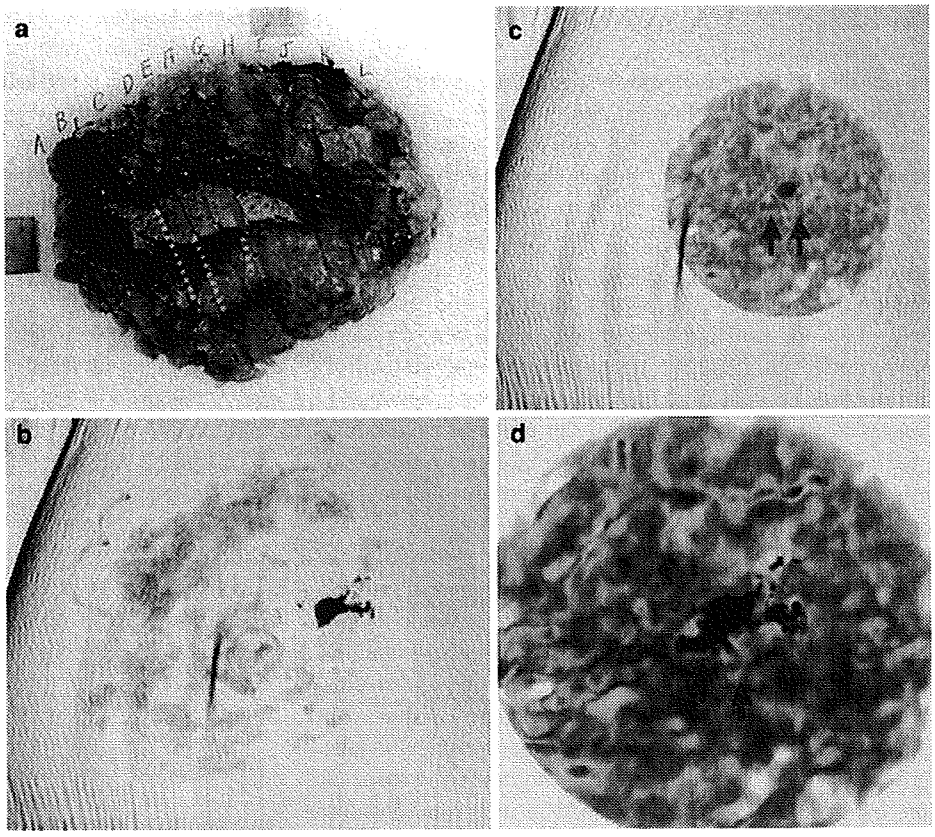

Fig. 2 A 60-year-old woman with invasive ductal carcinoma of the breast. **a** Image showing resected tissues only. *Pink lines* invasive ductal carcinoma. *Green lines* lesion with ductal spread. **b** Three-dimensional CT image in the early-enhancement phase. **c** Perfusion image. **d** Fusion image combining the images shown in **b** and **c**. The lesion with ductal spread located on the side toward the nipple from the main tumor could be depicted only in the perfusion map image (*pink arrows*)

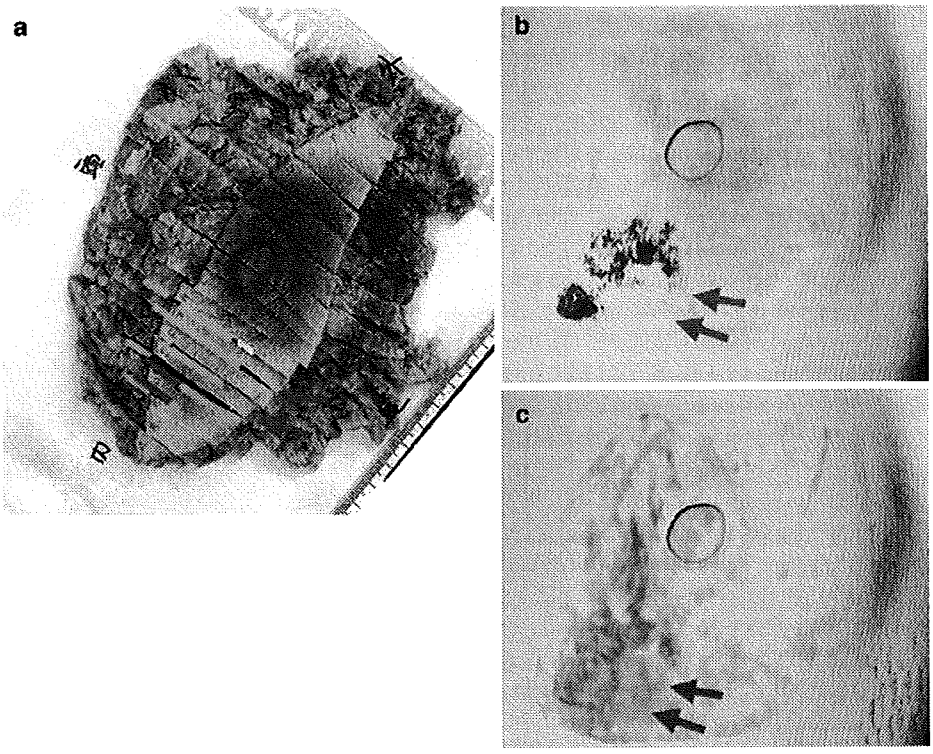

Fig. 3 A 45-year-old woman with invasive ductal carcinoma of the breast. **a** Image showing resected tissues only. *Red lines* tumor invasion. *Green lines* lesion with ductal spread. **b** Three-dimensional CT image in the early-enhancement phase (generated by the volume-rendering method). The intraductal component extending from the main tumor toward the nipple is depicted; the extent of tumor tissue on the right side of the tumor mass is not depicted (*pink arrows*). **c** Perfusion map image. The tumor tissues (including the ductal spread extending to the right of the main tumor that could not be depicted in the early-enhancement phase) are clearly depicted (*pink arrows*), showing good agreement with the pathology findings

Discussion

The results of the present study suggest that perfusion CT can depict the extent of breast cancer more precisely than conventional breast CT in the early-enhancement phase, especially in patients with ductal spread. Perfusion processing was performed for the data acquired by 256-row CT to obtain functional images. As a result, the tissue resolution was increased, making it possible to visualize small lesions, as shown in Figs. 1, 2, and 3. One reason for being able to visualize such small lesions was the integration effect of aggregate time. Then the total amount of information became big. These findings suggest that CT images can be improved not only in terms of spatial resolution by employing a larger number of detector rows, but also in terms of contrast levels by performing image processing, which may lead to higher sensitivity.

Precisely determining the extent of primary breast cancer before surgery is essential for achieving local control and an acceptable cosmetic result [9–11]. While breast MRI is known to be useful for assessing the extent of cancer, it requires both a dedicated breast coil and radiologists who are experts in breast imaging and familiar with the optimal imaging sequences and other technical details related to image interpretation [12]. Moreover, the shape of the breast in MRI images obtained in the prone position differs from that in the supine position during surgery. Breast CT images acquired in the supine position therefore provide more accurate information for surgical planning [1, 9]. Some studies have reported that MSCT images can be used to assess the extent of breast cancer with a high degree of accuracy [5–7, 13]. In another study, CT and MRI examinations were performed to assess the extent of cancer in the same patient, and the results showed that the MR images were superior or equal to the CT images in terms of sensitivity, that the CT images were superior in terms of specificity, and that the MR images and the CT images were equal in terms of diagnostic accuracy [14–16]. Recent improvements in MRI systems have led to an increase in the specificity of MRI images. Determining whether CT perfusion maps are superior to MR images for evaluating the extent of cancer is a subject for future research.

Only one recent report has described the usefulness of perfusion CT in patients with breast cancer [17]. The patients were examined using a 16-row MSCT scanner, and perfusion maps were obtained over a range of 8 mm. That study suggested the feasibility of breast cancer perfusion and reported differences in the perfusion values among histological subtypes.

One disadvantage of breast CT is radiation exposure. Scanning with the 256-row MSCT scanner was performed in patients at 16 time points from 0 to 54 s at 43.7–45 mSv. Simulation analysis suggested that the exposure dose could be reduced by half (to 20 mSv) if scanning were performed eight times with breath-holding. In addition, it is thought that the linear high-perfusion areas observed at the borders represented artifacts due to respiratory motion. It is therefore expected that clearer images could be obtained with breath-holding. At time points of 12.0 s or earlier and at 39.0 s or later, which were used in the simulation, if scanning were performed with breath-holding in the inspiratory phase, it might be possible to reduce the fluctuation in TDCs, permitting perfusion maps of equivalent level to be generated and images with fewer artifacts to be obtained. In the future, it should become possible to obtain clearer images with reduced exposure dose by taking these scan conditions into consideration.

This pilot study with 256-row MSCT is the world's first trial. We synchronized the timing for scan, injection speed, and perfusion analysis algorithm (maximum slope method), all of which have been used for liver cases. It remains a matter of discussion whether to evaluate an optimum model for outflow of contrast medium, injection speed, and scan timing.

Conclusion

The results of this pilot study suggest that volume perfusion images (as functional images) acquired using 256-row CT may be useful for depicting, with higher sensitivity, the extent of breast cancer. Further research is needed to validate these results.

Acknowledgments This work was supported in part by Health and Labour Sciences Research Grants for Third Term Comprehensive Control Research for Cancer.

References

1. Akashi-Tanaka S, Fukutomi T, Miyakawa K, Uchiyama N, Tsuda H. Diagnostic value of contrast-enhanced computed tomography for diagnosing the intraductal component of breast cancer. Breast Cancer Res Treat. 1998; 49:79–86.
2. Akashi-Tanaka S, Fukutomi T, Miyakawa K, Tsuda H. Diagnostic value of enhanced computed tomography in the detection of the widely spreading intraductal component of breast cancer: case reports. Breast Cancer. 1997; 4(1):29–32.
3. Akashi-Tanaka S, Fukutomi T, Miyakawa K, Nanasawa T, Matsuo K, Hasegawa T, et al. Contrast-enhanced computed tomography for diagnosing the intraductal component and small invasive foci of breast cancer. Breast Cancer. 2001; 8(1):10–15.
4. Akashi-Tanaka S, Fukutomi T, Sato N, Miyakawa K. The role of computed tomography in the selection of breast cancer treatment. Breast Cancer. 2003; 10(3):198–203.
5. Fujita T, Doihara H, Takabatake D, Takahashi H, Yoshitomi S, Ishibe Y, et al. Multidetector row computed tomography for diagnosing intraductal extension of breast carcinoma. J Surg Oncol. 2005; 91:10–6.

6. Uematsu T, Sano M, Homma K, Shiina M, Kobayashi S. Three-dimensional helical CT of the breast: accuracy for measuring extent of breast cancer candidates for breast conserving surgery. Breast Cancer Res Treat. 2001; 65:249–57.
7. Takase K, Furuta A, Harada N, Harada N, Takahashi T, Igarashi K, et al. Assessing the extent of breast cancer using multidetector row helical computed tomography. J Comput Assist Tomogr. 2006; 30:479–85.
8. Miles K. Measurement of tissue perfusion by dynamic computed tomography. Br J Radiol. 1991; 64:409–12.
9. Uematsu T, Sano M, Homma K, Sato N. Value of three-dimensional helical CT image-guided planning for made-to-order lumpectomy in breast cancer patients. Breast J. 2004; 10(1):33–7.
10. Esserman L, Hylton N, Yassa L, Barclay J, Frankel S, Sickles E. Utility of magnetic resonance imaging in the management of breast cancer: evidence for improved preoperative staging. J Clin Oncol. 1999; 17(1):110–9.
11. Newman LA, Kuerer HM. Advances in breast conserving therapy. J Clin Oncol. 2005; 23:1685–97.
12. NCCN Breast cancer panel members. Principles of dedicated breast MRI testing. NCCN Practice Guidelines in Oncology. vol. 2. 2008:30.
13. Inoue T, Tamaki Y, Hamada S, Yamamoto S, Sato Y, Tamura S, et al. Usefulness of three-dimensional multidetector-row CT images for preoperative evaluation of tumor extension in primary breast cancer patients. Breast Cancer Res Treat. 2005; 89(2):119–25.
14. Nakahara H, Namba K, Wakamatsu H, Watanabe R, Furusawa H, Shirouzu H, et al. Extension of breast cancer: comparison of CT and MRI. Radiat Med. 2002; 20(1):17–23.
15. Tozaki M, Uno H, Kobayashi T, Aiba K, Yoshida K, Takeyama H, et al. Histologic breast cancer extent after neoadjuvant chemotherapy: comparison with multidetector-row CT and dynamic MRI. Radiat Med. 2004; 22(4):246–53.
16. Shimauchi A, Yamada T, Sato A, Takase K, Usami S, Ishida T, et al. Comparison of MDCT and MRI for evaluating the intraductal component of breast cancer. Am J Roentgenol. 2006; 187(2):322–9.
17. Hirasawa H, Tsushima Y, Hirasawa S, Takei H, Taketomi-Takahashi A, Takano A, et al. Perfusion CT of breast carcinoma: arterial perfusion of nonscirrhous carcinoma was higher than that of scirrhous carcinoma. Acad Radiol. 2007; 14(5):547–52.

Image of the Month

A Case of Ductal Carcinoma In Situ of the Breast

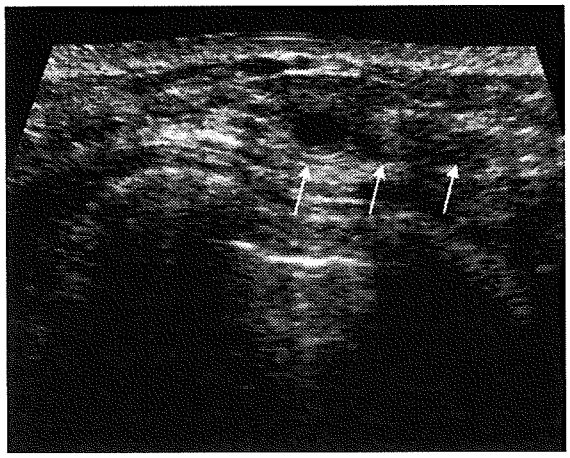

Figure 1.

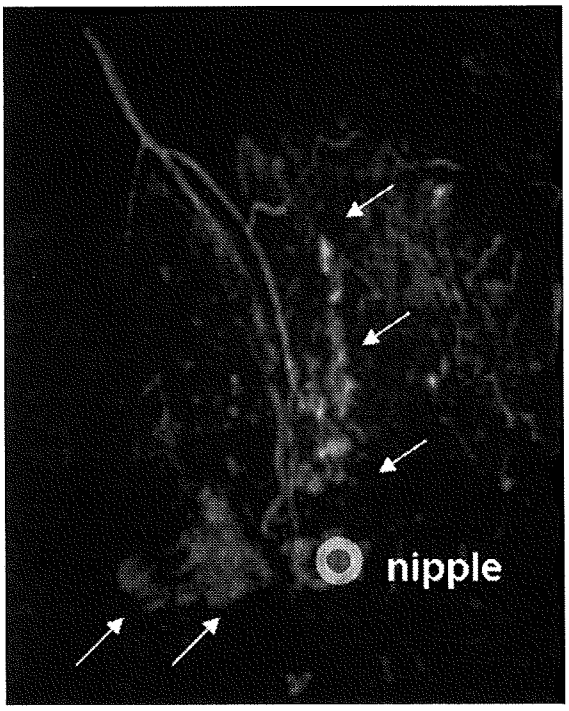

Figure 2.

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

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

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CASE REPORT

Primary small cell carcinoma of the breast

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Received: 19 December 2006 / Accepted: 3 March 2008 / Published online: 27 May 2008

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

Keywords Breast · Small cell carcinoma · Chemotherapy

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Introduction

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

Case report

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

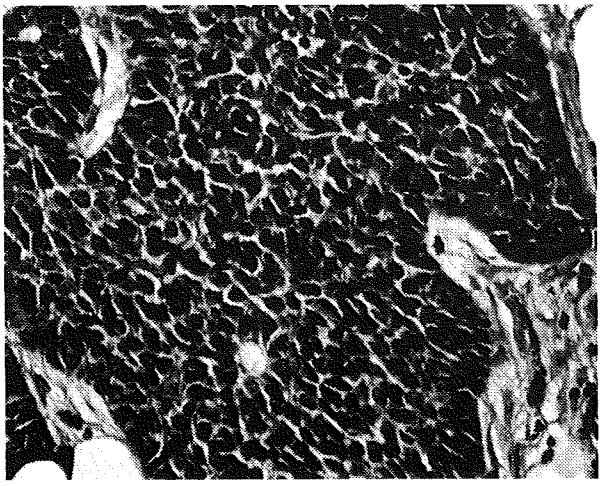

Fig. 1 Pathological findings (hematoxylin-eosin). Histopathological examination by hematoxylin and eosin staining showed that the neoplastic cells have scant cytoplasm and hyperchromatic nuclei. Some rosette-like structures are present in this nest

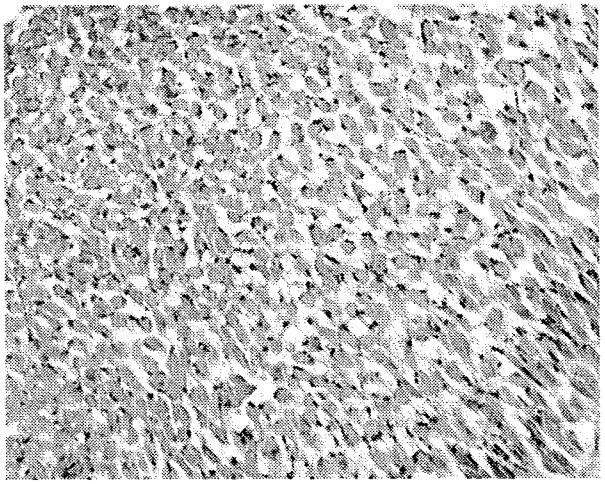

Fig. 2 Pathological findings (immunohistochemical staining for Grimelius). Grimelius staining was positive for narrow cytoplasm of neoplastic cells

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

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

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

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

Discussion

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

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

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

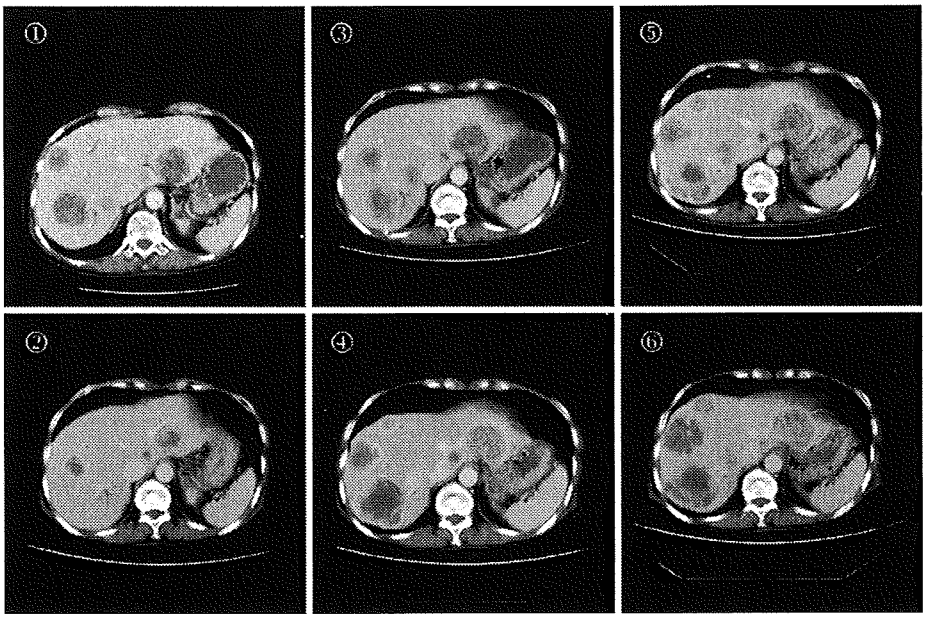

Fig. 3 Computed tomography (CT). Use of chemotherapy for hepatic metastasis and evaluation by CT. The patient was treated with a combination of carboplatin and CPT 11 for (1) to (2) period, and a partial response was achieved. The patient was treated with docetaxel and 5′-DFUR in periods (3) to (4),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

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

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

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

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

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

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

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

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

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

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

References

1. Adegbola T, Connolly CE, Mortimer G. Small cell neuroendocrine carcinoma of the breast: a report of three cases and review of the literature. J Clin Pathol. 2005;58:775–8.
2. Bigotti G, Coli A, Butti A, del Vecchio M, Tartaglione R, Massi G. Primary small cell neuroendocrine carcinoma of the breast. J Exp Clin Cancer Res. 2004;23:691–6.
3. Francois A, Chatikhine VA, Chevallier B, Ren GS, Berry M, Chevrier A, Delpech B. Neuroendocrine primary small cell carcinoma of the breast. Report of a case and review of the literature. Am J Clin Oncol. 1995;18:133–8.
4. Fukunaga M, Ushigome S. Small cell (oat cell) carcinoma of the breast. Pathol Int. 1998;48:744–8.
5. Hoang MP, Maitra A, Gazdar AF, Albores-Saavedra J. Primary mammary small-cell carcinoma: a molecular analysis of 2 cases. Hum Pathol. 2001;32:753–7.
6. Jundt G, Schulz A, Heitz PU, Osborn M. Small cell neuroendocrine (oat cell) carcinoma of the male breast. Immunocytochemical and ultrastructural investigations. Virchows Arch A Pathol Anat Histopathol. 1984;404:213–21.
7. Mariscal A, Balliu E, Diaz R, Casas JD, Gallart AM. Primary oat cell carcinoma of the breast: imaging features. AJR Am J Roentgenol. 2004;183:1169–71.
8. Papotti M, Gherardi G, Eusebi V, Pagani A, Bussolati G. Primary oat cell (neuroendocrine) carcinoma of the breast. Report of four cases. Virchows Arch A Pathol Anat Histopathol. 1992; 420:103–8.
9. Samli B, Celik S, Evrensel T, Orhan B, Tasdelen I. Primary neuroendocrine small cell carcinoma of the breast. Arch Pathol Lab Med. 2000;124:296–8.
10. Sebenik M, Nair SG, Hamati HF. Primary small cell anaplastic carcinoma of the breast diagnosed by fine needle aspiration cytology: a case report. Acta Cytol. 1998;42:1199–203.
11. Shin SJ, DeLellis RA, Ying L, Rosen PP. Small cell carcinoma of the breast: a clinicopathologic and immunohistochemical study of nine patients. Am J Surg Pathol. 2000;24:1231–38.
12. Sridhar P, Matey P, Aluwihare N. Primary carcinoma of breast with small-cell differentiation. Breast. 2004;13:149–51.
13. Stein ME, Gershuny A, Abdach L, Quigley MM. Primary small-cell carcinoma of the breast. Clin Oncol (R Coll Radiol). 2005;17:201–2.
14. Wade PM Jr, Mills SE, Read M, Cloud W, Lambert MJ 3rd, Smith RE. Small cell neuroendocrine (oat cell) carcinoma of the breast. Cancer. 1983;52:121–5.
15. Yamasaki T, Shimazaki H, Aida S, Tamai S, Tamaki K, Hiraide H, Mochizuki H, Matsubara O. Primary small cell (oat cell) carcinoma of the breast: report of a case and review of the literature. Pathol Int. 2000;50:914–8.
16. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connolly JL, et al. Prognostic factors in breast cancer. College of American pathologists consensus statement 1999. Arch Pathol Lab Med. 2000;124:966–78.
17. Remick SC, Hafez GR,Carbone PP. Extrapulmonary small cell carcinoma: a review of the literature with emphasis on therapy and outcome. Medicine. 1987;66:457–71.
18. Remick SC, Ruckdeschel JC. Extrapulmonary and pulmonary small cell carcinoma: tumor biology, therapy, and outcome. Med Pediatr Oncol. 1992;20:89–99.
19. Irfan C, Hakan K, Sernaz U, Kazim U, Ufuk U, Zafer K, Murat C, Mert S, Fusun T, Cem U. Extrapulmonary small-cell carcinoma compared with small-cell lung carcinoma. Am Cancer Soc. 2007;110:1068–76.
20. Brenner B, Tang LH, Klimstra DS. Small-cell carcinoma of the gastrointestinal tract: a review. J Clin Oncol. 2004;22:2730–9.
21. Galanis E, Frytak S, Lloyd RV. Extrapulmonary small cell carcinoma. Cancer. 1997;79:1729–36.
22. Kim JH, Lee SH, Park J, et al. Extrapulmonary small-cell carcinoma: a single-institution experience. Jpn J Clin Oncol. 2004;34:250–4.
23. Haider K, Shahid RK, Finch D, et al. Extrapulmonary small cell cancer: a Canadian province's experience. Cancer. 2006; 107:2262–9.
24. Sengoz M, Abacioglu U, Salepci T, et al. Extrapulmonary small cell carcinoma: multimodality treatment results. Tumori. 2003; 89:274–7.
25. Kurt E, Sezgin C, Evrensel T, et al. Therapy, outcome and analysis of c-kit expression in patients with extrapulmonary small cell carcinoma. Int J Clin Pract. 2005;59:537–43.
26. Van Der Gaast A, Verwey J, Prins E, et al. Chemotherapy as treatment of choice in extrapulmonary undifferentiated small cell carcinomas. Cancer. 1990;65:422–44.
27. Hidekazu K, Kazuo Y, Hiroshi M, Yutaka T. A case of primary small cell carcinoma of the breast. Breast Cancer. 2007;14:414–9.
28. Wenyong Z, Syed AH. Mammary small-cell carcinoma with dimorphic phenotype. The Breast J. 2007;13:529–30.
29. Shaco-Levy R, Dyomin V, Kachko L, Sion-Vardy N, Geffen DB, Koretz M. Small-cell carcinoma of the breast: case report. Eur J Gynaecol Oncol. 2007;28:142–4.

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

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

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

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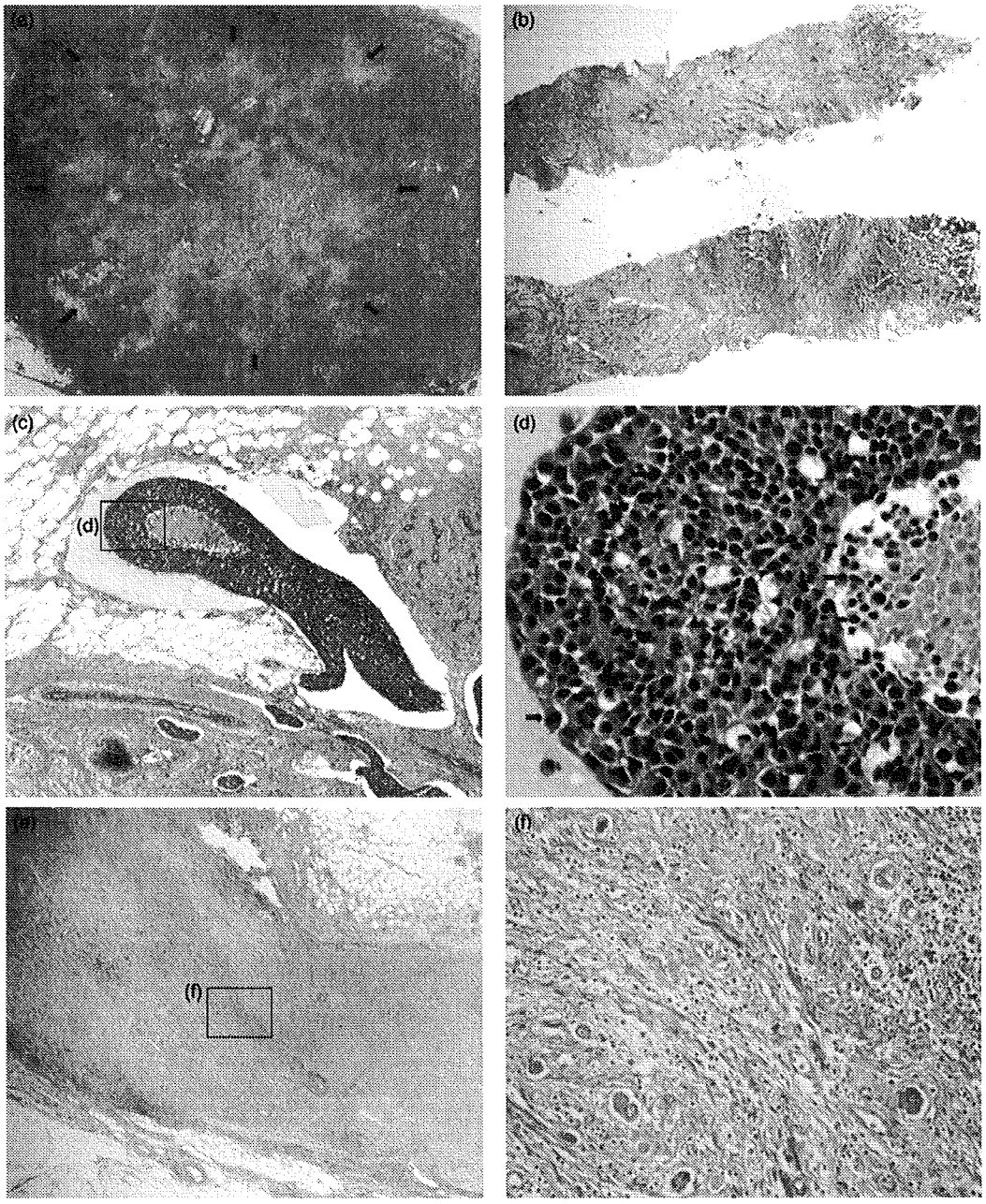

Fig. 1. Histological characteristics of fibrotic foci (FFs), lymph vessel tumor emboli, and nodal metastatic tumors. (a) An FF measuring 8.4 × 6.2 mm is visible within the tumor (arrows) in a surgical specimen. The FF has the appearance of a scar-like feature, and it is surrounded by invasive ductal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

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

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

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

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

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

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

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

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

Results

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

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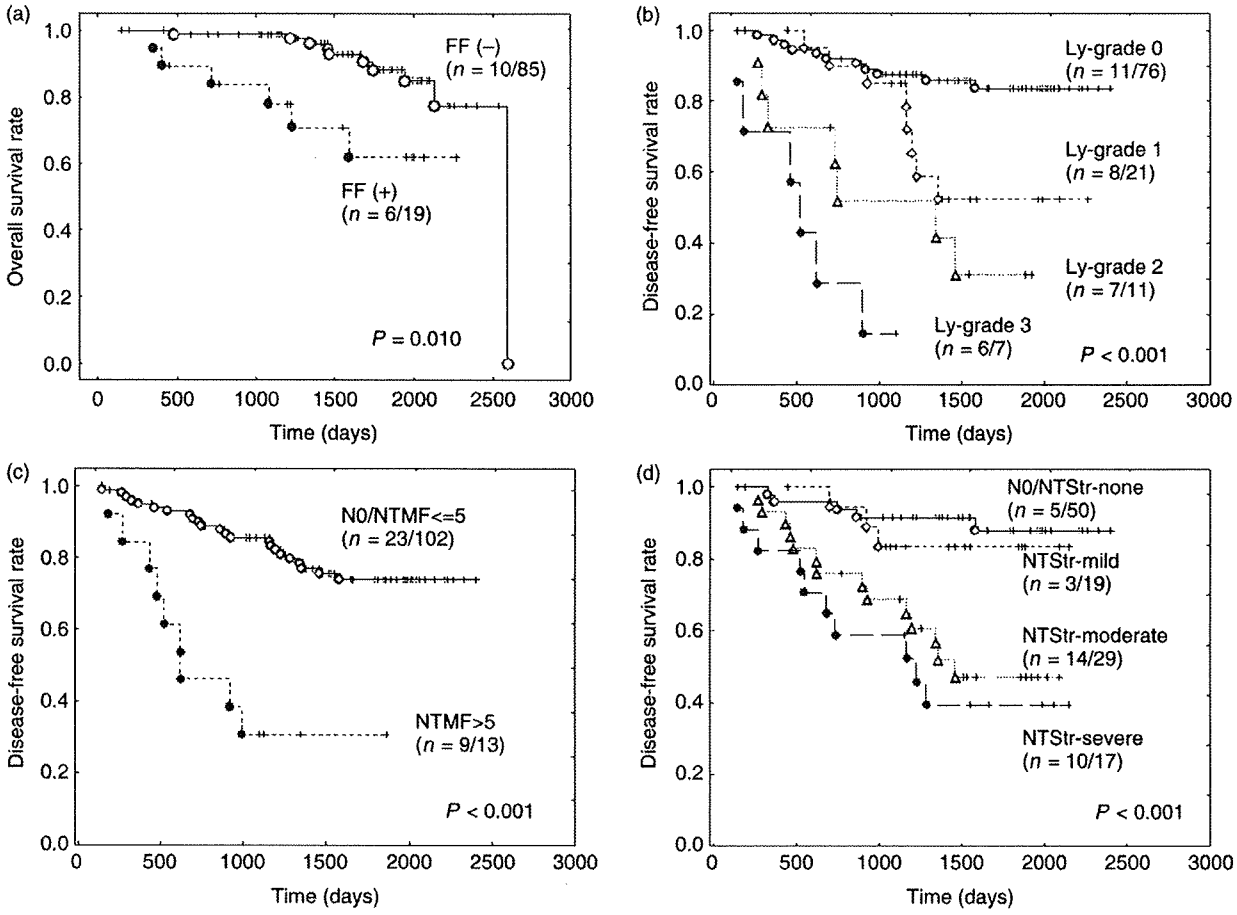

Fig. 2. (a–d) Overall survival curves and disease-free survival curves of invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy. (a) Patients with IDCs exhibiting fibrotic foci (FFs) assessed using biopsy specimens obtained before neoadjuvant chemotherapy have a significantly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

biopsy materials obtained before neoadjuvant chemotherapy exhibited blood vessel invasion.

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

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

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

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

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

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Table 3. *Continued*

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

Model 1

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

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

Model 2

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

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

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

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

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

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

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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

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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.