Tumor cells in lymph vessels and lymph nodes closely associated with nodal metastasis by invasive ductal carcinoma of the breast

Takahiro Hasebe,[1] Satoshi Sasaki,[2] Shigeru Imoto[3] and Atsushi Ochiai[1, 4]

[1]Pathology Division, [2]Epidemiology and Biostatistics Division, National Cancer Center Research Institute East, and [3]Department of Surgery (SI), National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577

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No studies have ever precisely investigated the mechanism of nodal metastasis based on the histological characteristics of tumor cells in lymph vessels and lymph nodes. The purpose of this study was to investigate whether the histological characteristics of tumor cells in lymph vessels and lymph nodes of 393 patients with invasive ductal carcinoma (IDC) were significantly associated with increased nodal metastasis compared with well known histological characteristics of their primary-invasive tumor cells. Multivariate analyses showed that having a single nodal metastasis was closely dependent on primary-invasive tumor size or distance of lymph vessel tumor emboli from the margin of the primary-invasive tumor ($P<0.05$) and that having 2 or more nodal metastases was significantly associated with the histological characteristics of the nodal metastatic tumors independently of the size of the primary-invasive tumor, and the number of nodes with extra-nodal invasion (ENI) significantly increased the relative risk (RR) of 4 or more nodal metastases in IDCs ≤20 mm and >20 to ≤50 mm in size ($P<0.05$). In IDCs >50 mm in size, number of lymph vessels invaded, severe fibrosis of the stroma of extra-nodal invasive tumors, and distance of ENI from the node significantly increased the RR of 10 or more nodal metastases in the multivariate analysis ($P<0.05$). The results of this study strongly suggest that the histological characteristics of tumor cells in lymph nodes and lymph vessels play an important role in nodal metastasis in IDCs of the breast. (Cancer Sci 2003; 94: 508–514)

Nodal metastases are formed by tumor cells within primary tumors invading lymph vessels and establishing metastases when they arrive at lymph nodes. Although several studies have shown that the number of lymph vessels invaded plays an important role in lymph node metastasis[1–3] and that several factors are closely associated with lymph node metastasis,[4–6] these studies investigated which factors were significantly associated with nodal metastasis based only on the characteristics of the primary-invasive tumors. Recently we have demonstrated that the histological characteristics of tumor cells in lymph vessels play a very important role in the establishment of nodal metastasis of invasive ductal carcinoma (IDC) of the breast.[7] However, no studies have been conducted to identify the histological characteristics of tumor cells in lymph nodes, lymph vessels, or primary-invasive tumors that play an important role in the increased nodal metastases of IDC of the breast.

The purpose of this study was to identify the histological characteristics of primary-invasive tumors, of tumors in lymph vessels, and of tumors in lymph nodes that are significantly associated with increased nodal metastases based on tumor histology in order to clarify the mechanism of nodal metastasis in IDC of the breast. We found that the histological characteristics of the tumor cells in the lymph nodes had a more significant effect on nodal metastases in IDCs of the breast than those of tumor cells in lymph vessels, not to mention those of the primary-invasive tumors.

Materials and Methods

Cases. This was based on study of 393 consecutive cases of IDC of the breast surgically treated between July 1992 and November 1998 at the National Cancer Center Hospital East. Clinical information was obtained from the patients' medical records. All patients were Japanese women, and they ranged in age from 28 to 78 years (mean, 51 years). All had a solitary lesion. There were 210 pre-menopausal patients, and 183 post-menopausal patients. Partial mastectomy was performed in 55, modified radical mastectomy in 314, and standard radical mastectomy in 24. Axillary lymph node dissection of levels I, II, ±III was carried out in all patients, and the number of nodes dissected per patient ranged from 9 to 58, with a median number of 22. Lymph node metastasis was present in 205 patients. None of the patients had received radiotherapy or chemotherapy before surgery, and 290 patients received adjuvant therapy. There were no cases of inflammatory breast cancer in this series. All tumors were classified according to the pathological TNM (pTNM) classification.[8]

Before pathological examination, the surgically resected specimens were fixed in 10% formalin overnight at 4°C. The size and gross appearance of the tumors were recorded, and the former was confirmed by comparison with tumor size on histological slides. Entire tumor areas were prepared for histological sections, and the sections were processed routinely and embedded in paraffin.

Histological examination of primary-invasive tumor cells. Serial sections of each tumor were cut from the paraffin blocks, and one section from each patient was stained with hematoxylin and eosin and examined pathologically to confirm the diagnosis.

The primary-invasive tumors were examined for the following parameters: 1) invasive tumor size (mm), 2) structural features (papillary, cribriform, solid, strand), 3) nuclear atypia (mild, moderate, severe), 4) number of mitotic and apoptotic figures, 5) tumor necrosis (absent, present),[9] 6) fibrotic focus (FF) dimension (mm),[10, 11] and 7) distance and width of adipose tissue invasion (mm). The number of tumor cell mitotic figures was counted in 10 high-power fields at the advanced edge of the tumor, and the field containing the greatest number of tumor cell mitotic figures was selected for comparison with the number of mitotic figures in the tumors in lymph vessels and lymph nodes. Similarly, the one of the 10 fields examined that contained the greatest number of tumor cell apoptotic figures was selected.[12]

Microscopic examination of tumor cells in lymph vessels. In accordance with the criteria used to identify lymph vessels,[7, 13] in this study we defined tumor cell nests in vessels lined by endothelium with no supporting smooth muscle or elastica as "lymph

[4]To whom correspondence should be addressed. E-mail: aochiai@east.ncc.go.jp

vessel invasion by tumor cells" (Fig. 1). We evaluated lymph vessel invasion at or beyond the border between the stroma of surrounding tumor area and invasive tumor area to differentiate lymph vessel invasion from artifactual spaces that often form around nests of tumor cells within an invasive carcinoma.

The following parameters of tumor cell nests in lymph vessels were examined in every case of IDC: 1) number of lymph vessels invaded (equal to number of lymph vessel tumor emboli (LVTE)), 2) maximum dimension of tumor emboli (mm), 3) maximum distance of tumor emboli from the margin of the primary-invasive tumor (mm), 4) structural features (papillary, cribriform, solid, strand), 5) nuclear atypia (mild, moderate, severe), and 6) number of mitotic and apoptotic figures. The structural features, nuclear atypia, and numbers of mitotic and apoptotic figures in each IDC tumor were assessed in same way as in the primary-invasive tumor. The maximum dimension of the tumor embolus in each lymph vessel and the maximum distance between each lymph vessel tumor embolus and the margin of the primary-invasive tumor were measured with a microscope equipped with a ×10 eyepiece containing a graticule.

Microscopic examination of nodal metastatic tumors in the lymph nodes. The following histological parameters were examined: 1) maximum dimension of nodal metastatic tumors, 2) numbers of lymph nodes with extra-nodal invasion (Fig. 2A), 3) maximum distance (mm) of extra-nodal invasion from node (Fig. 3), 4) maximum width (mm) of extra-nodal invasion (Fig. 3), 5) number of extra-nodal blood vessel tumor emboli (Fig. 2B), 6) maximum dimension (mm) of extra-nodal blood vessel tumor emboli, 7) maximum distance (mm) of extra-nodal blood vessel tumor emboli from nodes (Fig. 3), 8) numbers of mitotic and apoptotic figures in tumors in the lymph node, 9) numbers of mitotic and apoptotic figures in extra-nodal blood vessel tumors (Fig. 2C), 10) nuclear atypia of nodal tumors (mild, moderate, severe), 11) growth features of intra- and extra-nodal tumors (papillary, cribriform, solid, strand), and 12) grade of stromal fibrosis of intra- and extra-nodal tumors (none, mild, moderate, severe) (Fig. 2, D, E and F).

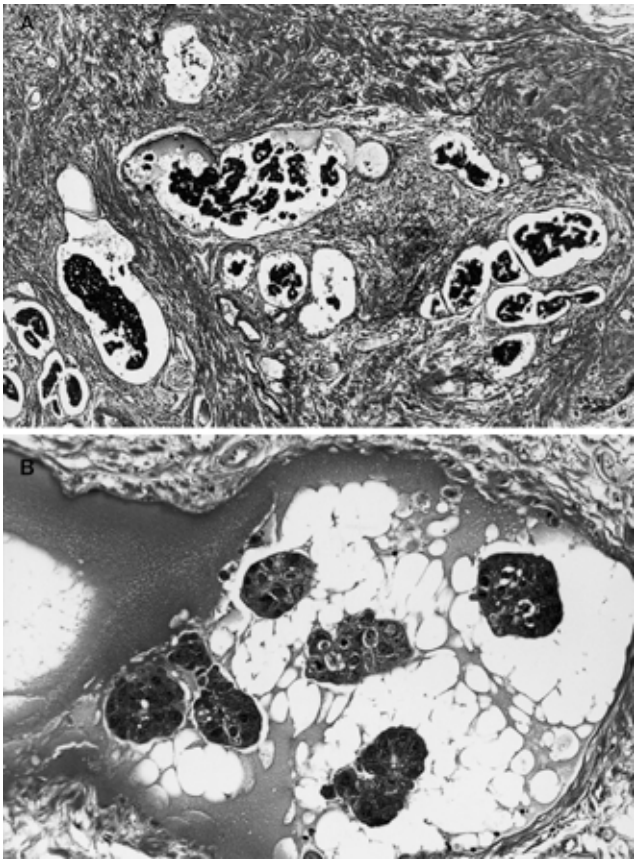

Fig. 1. Lymphatic vessel invasion by invasive ductal carcinoma (IDC) cells. (A) Several lymph vessel tumor emboli are seen. (B) The spaces around the clump of tumor cells are lined with endothelium and filled with lymph fluid.

Lymph nodes were evaluated for metastasis by examining single HE-stained sections of whole lymph nodes that had been processed routinely and embedded in paraffin. Extra-nodal invasion was defined as extension of tumor cells through the capsule of at least one lymph node into the perinodal adipose tissue. The presence of tumor cells within the capsule or in perinodal vessels was not considered extra-nodal invasion. In this study, we found the perinodal adipose tissue of all lymph nodes dissected contained blood vessels with a smooth-muscle-supported endothelial lining, and since it was very easy to assess tumor emboli in these blood vessels, we decided to evaluate its significance in nodal metastasis. The distance and width of extra-nodal invasion, and the diameter and distance of extra-nodal blood vessel tumor emboli were measured with a microscope equipped with a 10× eyepiece containing a graticule. If several foci of extra-nodal invasion were observed in one nodal metastatic tumor, the greatest distance and width of each extra-nodal invasion were considered as the distance and width of the extra-nodal invasion, respectively. Nuclear atypia, structural atypia, numbers of mitotic and apoptotic figures, and grade of stromal fibrosis of intra- and extra-nodal metastatic tumors were evaluated in the same manner as for primary-invasive tumors and tumor cell nests in lymph vessels.

One of the authors (T.H.) assessed all characteristics of the primary-invasive tumors and tumors in lymph vessels and lymph nodes, and another author (A.O.) identified the characteristics of IDCs to confirm the tumor cell characteristics in the tumors examined by T.H. Whenever there was a discrepancy, these two authors re-examined the slides to reach a consensus.

Statistical analysis. The IDCs were classified according to pTNM classification[8] into tumors ≤20 mm in size, >20 to ≤50 mm in size, and >50 mm in size, and the frequency of each class of nodal metastasis increased significantly with tumor size ($P<0.001$) (Table 1). Among the ≤20 mm IDCs there were 11 cases in the 4 to 9 nodal metastasis class, and among the >50 mm IDCs there were 13 cases in the 1 to 9 nodal metastasis class. Since the numbers in each class in the ≤20 mm and >50 mm IDCs were small, cases belonging to these nodal metastasis classes in each tumor size class were combined in the current study.

Among the IDCs ≤20 mm, >20 to ≤50 mm, and >50 mm in size, the histological characteristics of the primary-invasive tumors, of tumor cell nests in lymph vessels, and of nodal metastatic tumors that were significantly different between the nodal classes were examined by means of univariate analyses using the Mann-Whitney test. Then, to identify the characteristics most significantly associated with each nodal class, the histological characteristics of these tumors that were significantly different in the univariate analyses were entered into the logistic regression multivariate analysis[14] by the step-down method until all remaining characteristics were significant at a P value below 0.05.

Among the IDCs associated with nodal metastasis, the histological characteristics of nodal tumors that were most significantly associated with the presence of extra-nodal invasion were assessed by means of the Mann-Whitney test, and the histological characteristics of IDCs ≤20 mm, >20 to ≤50 mm, and >50 mm in sizes significantly associated with the presence of extra-nodal invasion were entered into the logistic regression multivariate analyses using the step-down method.

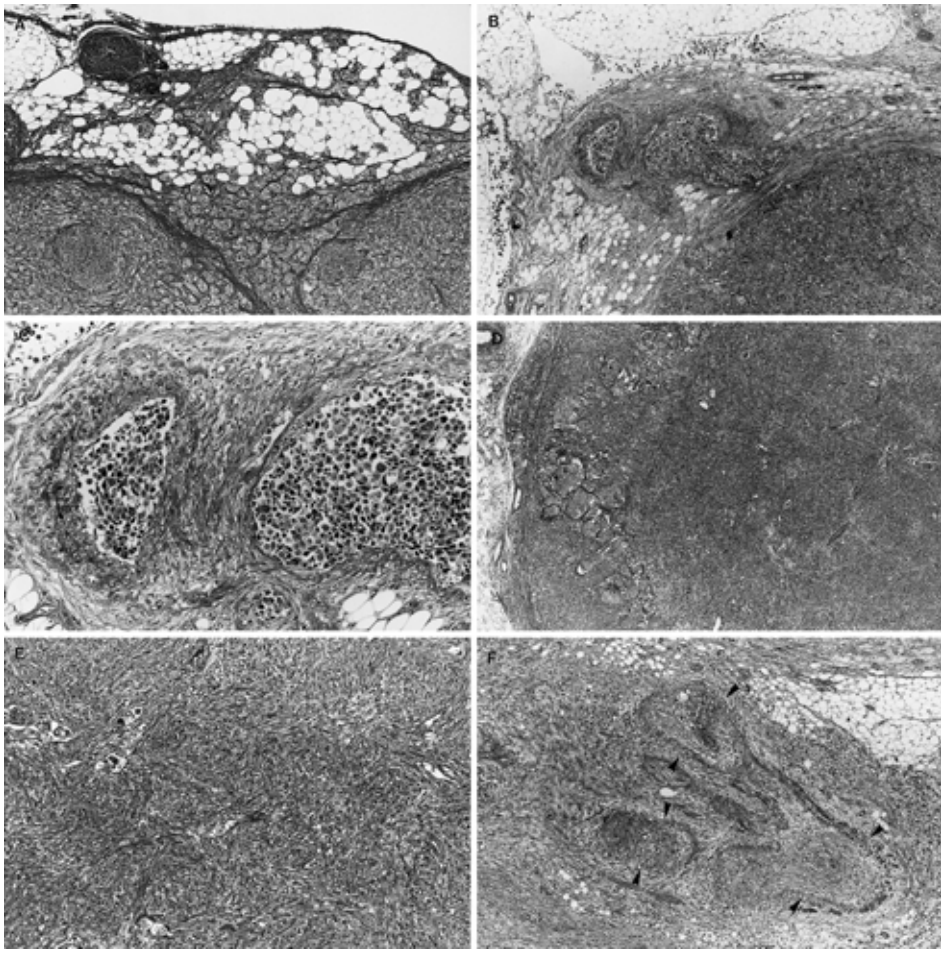

Fig. 2. Histological features of nodal metastatic tumors. (A) The tumor cells invade through the capsule of the lymph node, and extend into perinodal adipose tissue. (B) A large tumor embolus is observed in an extra-nodal blood vessel. (C) A tumor cell nest in an extra-nodal blood vessel lined by endothelium and supported by smooth muscle contains several apoptotic tumor cells. (D) Nodal tumors show severe fibrosis of the stroma. (E) Tumor cells grow in a strand surrounded by an abundant fibrous stroma. (F) Tumor cells invade through the nodal capsule with an abundant fibrous stroma, and invade extra-nodal blood vessels (arrowheads).

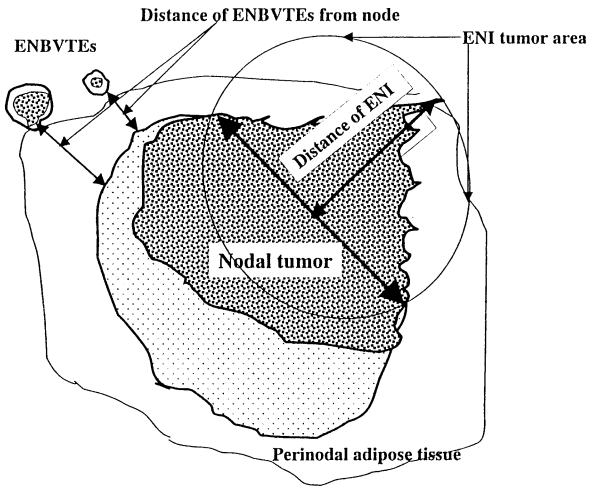

Fig. 3. Schematic drawing of the method of measuring the distances of extra-nodal invasion and extra-nodal blood vessel tumor emboli from lymph nodes. ENBVTE, extra-nodal blood vessel tumor emboli; ENI, extra-nodal invasion.

Table 1. Number of IDCs in each nodal class according to primary tumor size

Nodal class	No. of patients (%) Tumor size (mm)			*P* value
	≤20	>20 to ≤50	>50	
N0	100 (65)	83 (43)	5 (12)	
N1	22 (14)	25 (13)	2 (5)	
N2/3	22 (14)	26 (13)	7 (17)	
N4 to 9	9 (6)	32 (16)	4 (10)	
N>9	2 (1)	30 (15)	24 (56)	<0.001
Total	155	196	42	

IDC, invasive ductal carcinoma; N0, no nodal metastasis; N1, 1 nodal metastasis; N2/3, 2 or 3 nodal metastases; N4 to N9, 4 to 9 nodal metastases; N>9, 10 or more nodal metastases.

In the analyses, mild, moderate, and severe nuclear atypia of the primary-invasive tumors, of the lymph vessel tumor emboli, and of the nodal metastatic tumors was scored as 0, 1, and 2, respectively, and papillary, cribriform, solid, and strand features were scored as 0, 1, 2, and 3, respectively. Mild, moderate, and severe stromal fibrosis of primary-invasive tumors and nodal tumors were scored as 0, 1, and 2, respectively. Other histological characteristics of the three tumor components, e.g., size of

Table 2. Multivariate analyses for histological characteristics significantly different in each nodal class of IDCs ≤20 mm in size (*n*=155)

Characteristics	Multivariate RR/95% CI/*P* value
N0 (*n*=100) vs. N1 IDCs (*n*=22)	
Distance (mm) of lymph vessel tumor emboli	1.23/1.00–1.53/0.049
N1 (*n*=22) vs. N2/3 IDCs (*n*=22)	
Nodal tumor size (mm)	1.25/1.04–1.52/0.028
N2/3 (*n*=22) vs. N>3 IDCs (*n*=11)	
Number of nodes with extra-nodal invasion	3.25/1.29–8.18/0.018

IDC, invasive ductal carcinoma; RR, relative risk; CI, confidence interval; N0, IDCs without nodal metastasis; N1 IDCs, IDCs with 1 nodal metastasis; N2/3 IDCs, IDCs with 2 or 3 nodal metastases; N>3 IDCs, IDCs with 4 or more nodal metastases. Multivariate analyses were performed by the logistic regression model.

Table 3. Multivariate analyses for histological characteristics significantly different in each nodal class of IDCs >20 to ≤50 mm in size (*n*=196)

Characteristics	Multivariate RR/95% CI/*P* value
N0 (*n*=83) vs. N1 IDCs (*n*=25)	
Width (mm) of adipose tissue invasion	1.07/1.00–1.16/0.037
Primary invasive tumor size (mm)	1.07/1.00–1.15/0.045
N1 (*n*=25) vs. N2/3 IDCs (*n*=26)	
Nodal tumor stroma	2.29/1.03–5.02/0.044
N2/3 (*n*=26) vs. N4–9 IDCs (*n*=32)	
Number of nodes with extra-nodal invasion	2.85/1.56–5.25/0.001
N4–9 (*n*=32) vs. N>9 IDCs (*n*=33)	
Number of nodes with extra-nodal invasion	2.12/1.35–3.32/0.002
Number of apoptotic figures in ENBVTE	10.80/1.53–75.22/0.019

IDC, invasive ductal carcinoma; RR, relative risk; CI, confidence interval; N0, IDCs without nodal metastasis; N1 IDCs, IDCs with 1 nodal metastasis; N2/3 IDCs, IDCs with 2 or 3 nodal metastases; N4–9 IDCs, IDCs with 4 to 9 nodal metastases; N>9 IDCs, IDCs with 10 or more nodal metastases; ENBVTE, extra-nodal blood vessel tumor emboli. Multivariate analyses were performed by the logistic regression model.

the primary-invasive tumor size, numbers of lymph vessels invaded, and distance or width of extra-nodal invasion, were recorded and evaluated as actual numbers. All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK).

Results

Histological characteristics that significantly differed between nodal classes of IDCs ≤20 mm in size. The distance of tumor emboli in lymph vessels was the only histological characteristic associated with significantly increased relative risk (RR) of 1 nodal metastasis in the multivariate analysis (Table 2, Fig. 4A).

Nodal tumor size significantly increased the RR of 2 or 3 nodal metastases (Fig. 4B), and number of nodes with extra-nodal invasion significantly increased the RR of 4 or more nodal metastases in the multivariate analyses (Fig. 4C).

Histological characteristics that significantly differed between nodal classes of IDCs >20 to ≤50 mm in size. Width of adipose tissue invasion by primary-invasive tumors, and primary-invasive tumor size significantly increased the RRs of 1 nodal metastasis in the multivariate analysis (Table 3).

Only nodal tumor stroma significantly increased the RR of 2 or 3 nodal metastases (Fig. 4D) and only number of nodes with extra-nodal invasion significantly increased the RR of 4 to 9 nodal metastases (Fig. 4E) in the multivariate analyses.

As for 10 or more nodal metastases, number of nodes with extra-nodal invasion and number of apoptotic figures in extra-nodal blood vessel tumor emboli significantly increased the RRs of 10 or more nodal metastases in the multivariate analysis (Fig. 4, E and F).

Histological characteristics that significantly differed between the nodal classes of IDCs >50 mm in size. Number of apoptotic figures in the primary-invasive tumors was the only parameter in the univariate analysis that significantly differed between IDCs without nodal metastasis and with 1 to 9 nodal metastases (P=0.018).

Stroma of extra-nodal invasive tumors, distance of extra-nodal invasion, and number of lymph vessel tumor emboli significantly increased the RRs of 10 or more nodal metastases in the multivariate analysis (Table 4, Fig. 4, G, H and I).

Histological characteristics significantly associated with the presence of nodes with extra-nodal invasion. Among IDCs ≤20 mm in size, nodal tumor stroma and structural features of nodal tumors significantly increased the RRs of the presence of nodes with extra-nodal invasion in the multivariate analysis (Table 5).

In IDCs >20 to ≤50 mm and those >50 mm in size, only nodal tumor stroma significantly increased the RRs of the presence of nodes with extra-nodal invasion in the multivariate analysis.

Table 4. Multivariate analyses for histological characteristics significantly different in each nodal class of IDCs >50 mm in size (*n*=42)

Characteristics	Multivariate RR/95% CI/*P* value
N1–9 (*n*=13) vs. N>9 IDCs (*n*=24)	
Stroma of tumors in extra-nodal invasion	1.62/1.30–2.00/<0.001
Distance (mm) of extra-nodal invasion	1.45/1.17–1.80/0.003
Number of lymph vessels invaded by tumor cells	1.35/1.12–1.66/0.006

IDC, invasive ductal carcinoma; RR, relative risk; CI, confidence interval; N0, IDCs without nodal metastasis; N1–9 IDCs, IDCs with 1 to 9 nodal metastases; N>9 IDCs, IDCs with 10 or more nodal metastases. Multivariate analyses were performed by the logistic regression model.

Table 5. Multivariate analyses for histological characteristics significantly associated with the presence of ENI in lymph nodes according to tumor size of IDCs

Characteristics	Multivariate RR/95% CI/*P* value
IDCs, ≤20 mm in size (*n*=55)	
ENI– (*n*=27) vs. ENI+ (*n*=28)	
Nodal tumor stroma	3.35/1.59–7.06/0.002
Structural features of nodal tumors	5.47/1.13–27.05/0.038
IDCs, >20 to ≤50 mm in size (*n*=113)	
ENI– (*n*=39) vs. ENI+ (*n*=74)	
Nodal tumor stroma	3.94/2.00–7.01/<0.001
IDCs, >50 mm in size (*n*=37)	
ENI– (*n*=4) vs. ENI+ (*n*=33)	
Nodal tumor stroma	4.76/1.00–22.61/0.048

IDCs, invasive ductal carcinoma; RR, relative risk; CI, confidence interval; ENI, extra-nodal invasion. Multivariate analyses were performed by the logistic regression model.

Discussion

The current study clearly demonstrated that 1 nodal metastasis was closely dependent on the histological characteristics of the

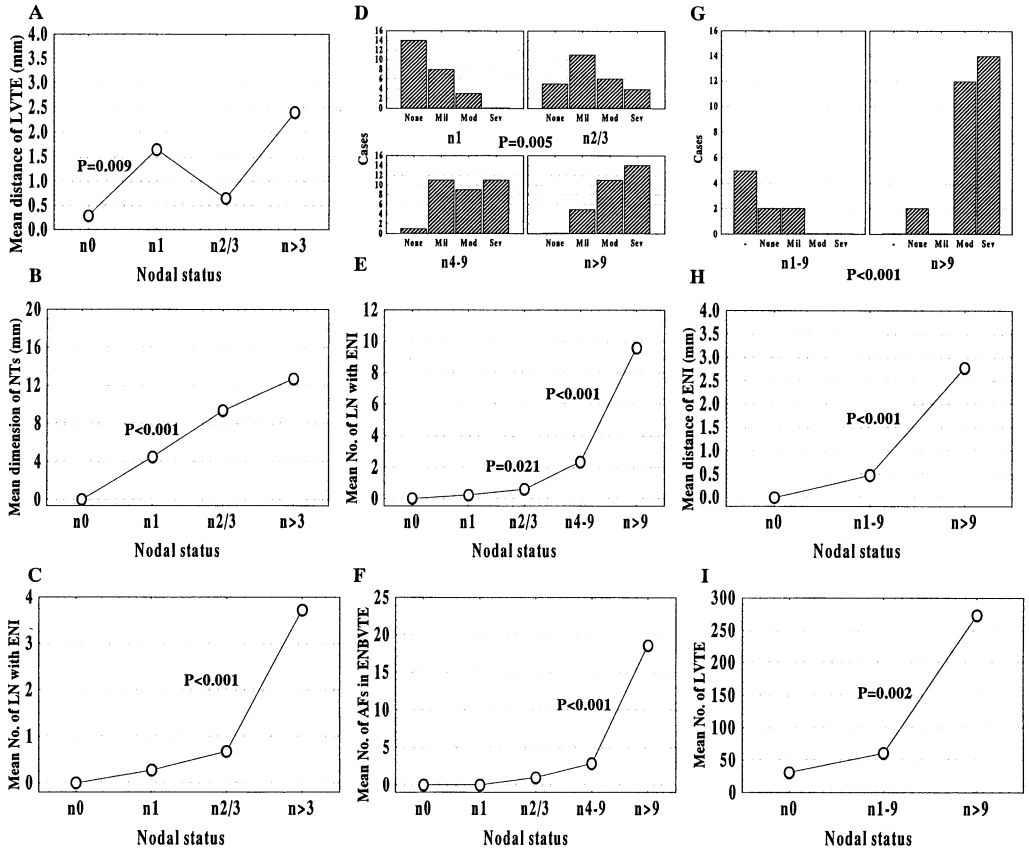

Fig. 4. (A) Mean distances of lymph vessel tumor emboli from lymph nodes according to nodal status of IDCs ≤20 mm in size. IDCs with 1 nodal metastasis are associated with a significantly longer mean distance of lymph vessel tumor emboli from the primary-invasive tumor margin than IDCs without nodal metastasis (P=0.009). (B) Mean sizes of nodal tumors according to nodal status of IDCs ≤20 mm in size. IDCs with 2 or 3 nodal metastases are associated with a significantly larger mean nodal tumor size than IDCs with 1 nodal metastasis (P<0.001). (C) Mean number of lymph nodes with extra-nodal invasion according to nodal status of IDCs ≤20 mm in size. IDCs with 4 or more nodal metastases are associated with a significantly larger mean number of lymph nodes with extra-nodal invasion than IDCs with 2 or 3 nodal metastases (P<0.001). (D) Nodal tumor stroma. Number of cases with nodal tumors having no, mild, moderate, and severe nodal stromal fibrosis in each nodal class of IDCs >20 to ≤50 mm in size. The number of cases of nodal tumors with mild, moderate or severe stromal fibrosis is significantly larger in IDCs with 2 or 3 nodal metastases than in IDCs with 1 nodal metastasis (P=0.005). (E) Mean number of lymph nodes with extra-nodal invasion among IDCs >20 to ≤50 mm in size. IDCs with 4 to 9 nodal metastases are associated with a significantly larger mean number of cases with lymph nodes exhibiting extra-nodal invasion than IDCs with 2 or 3 nodal metastases (P=0.021), and IDCs with 10 or more nodal metastases are associated with a significantly larger mean number of cases with extra-nodal invasion than IDCs with 4 to 9 nodal metastases (P<0.001). (F) Mean numbers of apoptotic figures in extra-nodal blood vessel tumor emboli in IDCs >20 to ≤50 mm in size. Mean numbers of extra-nodal blood vessel tumor emboli are significantly larger among IDCs with 10 or more nodal metastases than IDCs with 4 to 9 nodal metastases (P<0.001). (G) Extra-nodal invasive tumor stroma. Among IDCs >50 mm in size, those with 10 or more nodal metastases are associated with significantly larger numbers of lymph nodes with moderate to severe stromal fibrosis in extra-nodal invasions than those with 1 to 9 nodal metastases (P<0.001). (H) Mean distances of extra-nodal invasive tumors from lymph nodes among IDCs >50 mm in size according to their nodal class. Mean distances of extra-nodal invasive tumors from lymph nodes are significantly longer among IDCs with 10 or more nodal metastases than in those with 1 to 9 nodal metastases (P<0.001). (I) Mean numbers of lymph vessel tumor emboli among IDCs >50 mm in size according to nodal status. Mean numbers of lymph vessel tumor emboli are significantly larger among IDCs with 10 or more nodal metastases than those with 1 to 9 nodal metastases (P=0.002). n0, no nodal metastasis; n1, 1 nodal metastasis; n2/3, 2 or 3 nodal metastases; n>3, 4 or more nodal metastases; n4–9, 4 to 9 nodal metastases; n1–9, 1 to 9 nodal metastases; n>9, 10 or more nodal metastases; LVTE, lymph vessel tumor emboli; NT, nodal tumor; LN, lymph node; ENI, extra-nodal invasion; Mil, mild; Mod, moderate; Sev, severe; –, no nodal metastasis.

primary-invasive tumor cells or of tumor cell emboli in lymph vessels. In the univariate analysis some of the histological characteristics of the lymph vessel tumor emboli were significantly associated with 3 or more nodal metastases in IDCs ≤20 mm in size and with 10 or more nodal metastases in IDCs >20 to ≤50 mm in size. None of them, however, could independently predict increased nodal metastases, and the occurrence of 2 or more nodal metastases significantly depended only on the histological characteristics of nodal metastatic tumors in IDCs ≤20 and >20 to ≤50 mm in size. We therefore concluded that histological characteristics of nodal metastatic tumors play the most important role in the establishment of 2 or more nodal metastases in IDCs ≤50 mm in size. Based on the findings of this study, we hypothesize that in IDCs ≤20 mm in size, tumor cells of the primary-invasive tumor invade the lymph vessels, and the tumor cells that have the ability to widely spread via the lymph vessel system arrive at lymph nodes where they establish metastases. Nodal metastatic tumors that consist of highly proliferative tumor cells with severe stromal fibrosis probably increase the tumor's size and invade beyond the nodal capsule, resulting in the establishment of 3 or more nodal metastases (Fig. 5A). Similarly, in IDCs >20 to ≤50 mm in size, nodal metastatic tumors with severe stromal fibrosis invade extra-nodal areas resulting in 3 or more nodal metastases. Some extra-nodal invasive tumors probably invade extra-nodal blood vessels (Fig. 2F), and their tumor cells with large numbers of apoptotic figures can spread widely via extra-nodal blood vessels, resulting in 10 or more nodal metastases (Fig. 5B).

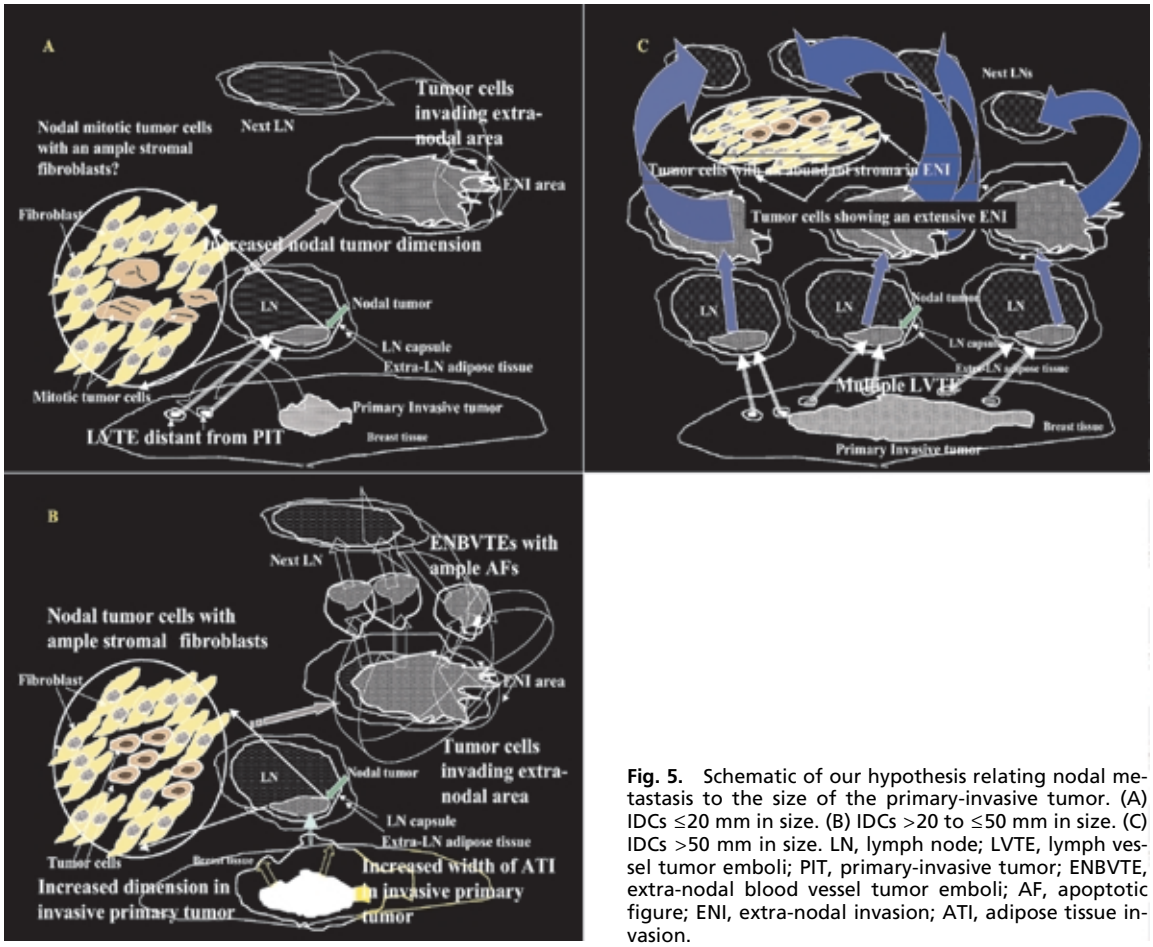

Fig. 5. Schematic of our hypothesis relating nodal metastasis to the size of the primary-invasive tumor. (A) IDCs ≤20 mm in size. (B) IDCs >20 to ≤50 mm in size. (C) IDCs >50 mm in size. LN, lymph node; LVTE, lymph vessel tumor emboli; PIT, primary-invasive tumor; ENBVTE, extra-nodal blood vessel tumor emboli; AF, apoptotic figure; ENI, extra-nodal invasion; ATI, adipose tissue invasion.

In contrast to IDCs ≤50 mm in size, more than half the number of cases with IDCs >50 mm in size were associated with 10 or more nodal metastases, which strongly suggests that the mechanism responsible for the higher number of nodal metastasis by IDCs ≤50 mm than IDCs >50 mm in size is different. In IDCs >50 mm in size, in addition to the histological characteristics of the extra-nodal invasive tumors, the number of lymph vessels invaded was significantly associated with 10 or more nodal metastases, strongly suggesting that several nodal metastatic tumor foci are completely formed by multiple lymph vessel tumor emboli at the first step of nodal metastasis in more than half of IDCs >50 mm in size, and nodal metastatic tumors with severe stromal fibrosis most likely produce extensive extra-nodal invasions or extra-nodal invasions with severe stromal fibrosis resulting in 10 or more nodal metastases (Fig. 5C).

The results of this study clearly demonstrate that extra-nodal invasion is the most important parameter responsible for the formation of many nodal metastases in IDCs of the breast. The extra-nodal area consists mainly of adipose tissue, and several microvessels are present within the extra-nodal adipose tissue. Thus, tumor cells extending through the nodal capsule most likely invade microvessels within extra-nodal adipose tissues, resulting in the establishment of many nodal metastases via extra-nodal microvessels. Next, it will be very important to investigate whether the microvessel density in the extra-nodal adipose tissue is closely associated with many nodal metastases in IDCs.

In the current study, we evaluated the histological characteristics of nodal metastatic tumors by examining a single routinely HE-stained section of the lymph nodes, not serial HE-stained sections. Information about nodal metastatic tumor characteristics obtained by examining serial sections of lymph nodes may be more accurate than that obtained by examining only a single section, and the information obtained by examining serial sections of lymph nodes may provide better support for the view that nodal metastatic tumors play the most important role in nodal metastases of IDCs. However, the information on the characteristics of nodal metastatic tumors obtained by examining a single section of lymph nodes in this study clearly showed that the characteristics of the nodal metastatic tumors play a more important role in nodal metastasis of IDCs than those of the lymph vessel tumor emboli or of the primary-invasive tumors. Thus, one can conclude that nodal metastatic tumors play the most important role in the establishment of many nodal metastases in IDCs of the breast.

In conclusion, this is the first study to clarify the mechanism of nodal metastasis based on an investigation that evaluated the histological characteristics of tumors in lymph vessels and lymph nodes as well as those of primary-invasive tumors. Among the histological characteristics of nodal metastatic tumors, the characteristics of extra-nodal invasive tumors and severe stromal fibrosis of nodal tumors played a very important role in the establishment of many nodal metastases in IDCs, and they were closely associated with each other. These findings strongly suggest that stromal cells help intra-nodal tumor cells to invade extra-nodal areas and that tumor cell-stromal cell interaction[15–17] in intra- and extra-nodal metastatic tumors plays a very important role in the establishment of many nodal metastases in IDCs of the breast. The current study provides an important clue to elucidation of the mechanism of nodal metastases in IDCs of the breast.

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