ORIGINAL

Relationship between lymph node metastasis and E-cadherin expression in submucosal invasive gastric carcinomas with gastric-phenotype

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Abstract : Background : Recent advances in immunohistochemical staining have led to the proposition of a classification of gastric carcinomas based on cellular phenotypes, and the degree of biological malignancy of gastric-phenotype carcinomas has attracted particular attention.
Subjects and Methods : One hundred and seven submucosal (SM) invasive carcinomas encountered in our center were examined for their histological type, cellular phenotype, and E-cadherin expression status to clarify their relationships with lymph node metastasis.
Results : Eleven (10.3%) of 107 SM gastric carcinomas were lymph node metastasis-positive. Gastric-phenotype carcinomas accounted for 20.6%, with a lymph node metastasis rate of 27.3% (6/22), which was significantly higher ($p<0.05$) than those of intestinal-phenotype carcinomas (5.9%) and mixed-phenotype carcinomas (2.9%). In terms of E-cadherin expression, only carcinomas with reduced E-cadherin expression showed lymph node metastasis at a rate significantly higher than that of carcinomas with normal E-cadherin expression ($p<0.05$). The lymph node metastasis rate (46.2%) of gastric-phenotype carcinomas with reduced E-cadherin expression was significantly higher than those of carcinomas of other phenotypes ($p<0.05$).
Conclusion : Since gastric-phenotype differentiated carcinomas with reduced E-cadherin expression have the potential for becoming undifferentiated, the risk of lymph node metastasis should be considered. J. Med. Invest. 54 : 159-167, February, 2007

Keywords **:** *gastric carcinomas, lymph node metastasis, gastric-phenotype, E-cadherin*

INTRODUCTION

Gastric carcinomas are histological classified into differentiated and undifferentiated types (1) or intestinal and diffuse types (2). Nakamura, *et al.* (3) focused their attention on the morphological similarity between the tumor and the surrounding mucosa, considered that differentiated and undifferentiated carcinomas arose from intestinal metaplastic epithelium and the propria mucosae of the stomach, respectively, and classified the former type as intestinal and the latter type as gastric. This concept was widely accepted. However, recent advances in immunohistochemical staining have shown the exis-

Received for publication November 29, 2006 ; accepted January 9, 2007.

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tence of differentiated gastric-phenotype carcinomas and undifferentiated intestinal-phenotype carcinomas (4-6), leading to the proposition of a new classification of gastric carcinomas based on cellular phenotypes, which is not necessarily in agreement with the conventional histological typing. One reason why the new classification has been attracting attention is the degree of biological malignancy of gastric-phenotype carcinomas. It has been reported that even if differentiated, gastric-phenotype carcinomas tend to contain undifferentiated carcinomatous elements because of invasion (6), constitute an independent risk factor for lymph node metastasis (7), and have a poor prognosis (8, 9).

On the other hand, E-cadherin is one of the major cell adhesion molecules (10), and a decrease in its expression in cancer cells has recently been reported to result in reduced adhesion between tumor cells, leading to their invasion and metastasis (11) ; however, few studies have investigated the relationship between E-cadherin expression and gastric-phenotype carcinomas. Moreover, it is unclear and of interest how these factors are associated with the rates of lymph node metastasis by histological type. In this study, early SM gastric carcinomas were examined for their histological type, cellular phenotype, and E-cadherin expression status to clarify their relationship to lymph node metastasis.

MATERIALS AND METHODS

1. Materials

We examined 107 specimens of primary solitary, submucosal invasive (SM), early gastric carcinomas that had been surgically resected with D2 or greater lymph node dissection at Kagawa Prefectural Cancer Detection Center between 1986 and 2002. The resected specimens were transectioned at widths of 5-10 mm, fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. Conventional histological typing and the classification of lymphadenectomy were performed according to the Japanese Classification of Gastric Carcinoma (1). The dissected lymph nodes were cut at two levels, and were examined for metastases.

2. Methods

1) Determination of Histological classification

Histological classification was made according to the general rules established by the Japanese Research Society for Gastric Cancer (1). SM1 was defined as a submucosal invasion depth of less than 500 μm, and SM2 as that of 500 μm or deeper. The morphologic subclassifications of submucosal invasion layer were defined as follows : the solely differentiated type, comprised solely differentiated carcinomas ; the complex type, comprised differentiated and undifferentiated carcinomas ; and the undifferentiated type, comprised solely undifferentiated carcinomas.

2) Determination of cellular phenotypes

To determine cellular phenotypes, mucin staining and immunohistochemical staining were performed using the streptavidin-biotin (SAB) method after microwave treatment in citrate buffer at 100℃ for 25 min to activate antigens except in paradoxical concanavalin A-III staining.

(1) Human gastric mucin (HGM) staining

Immunohistochemical staining for HGM as a gastric-phenotype marker was performed using an anti-HGM antibody (NCL-HGM-45M1 clone 1 : 100 ; Novocastra, Newcastle, UK) to stain gastric pit epithelium (12).

(2) Paradoxical concanavalin A-III (ConA-III) staining

Type III mucin was stained using ConA (1 : 1000 ; Sigma Chemical, St. Louis) to identify pyloric gland cell-type and accessory cell-type mucins, as gastric-phenotype markers (13).

(3) MUC2 staining

Immunostaining was performed using an anti-MUC2 antibody (NCL-MUC2 clone 1 : 100 ; Novocastra, Newcastle, UK) to stain goblet cell-type mucin in intestinal metaplastic glandular ducts as an intestinal-phenotype marker (14).

(4) CD10 staining

Immunostaining was performed using an anti-CD10 antibody (NCL-CD10 clone 56C6 1 : 100 ; Novocastra, Newcastle, UK) to stain the brush border of absorptive epithelial cells in intestinal metaplasia, as an intestinal-phenotype marker (15).

Staining for HGM, ConA-III, and MUC2 was judged as positive when mucin in the cytoplasm of cancer cells in more than 5% of the cancerous mucosa was stained. CD10 staining was similarly judged as positive when the brush border of cancer cells was stained in more than 5% of the cancerous mucosa(Fig. 1a-d).

As shown in Table 1, HGM- or ConA-III-positive, CD10- and MUC2-negative carcinomas were classified as the gastric phenotype, carcinomas negative for gastric-type markers and positive for CD10 or MUC2 as the intestinal phenotype, carcinomas posi-

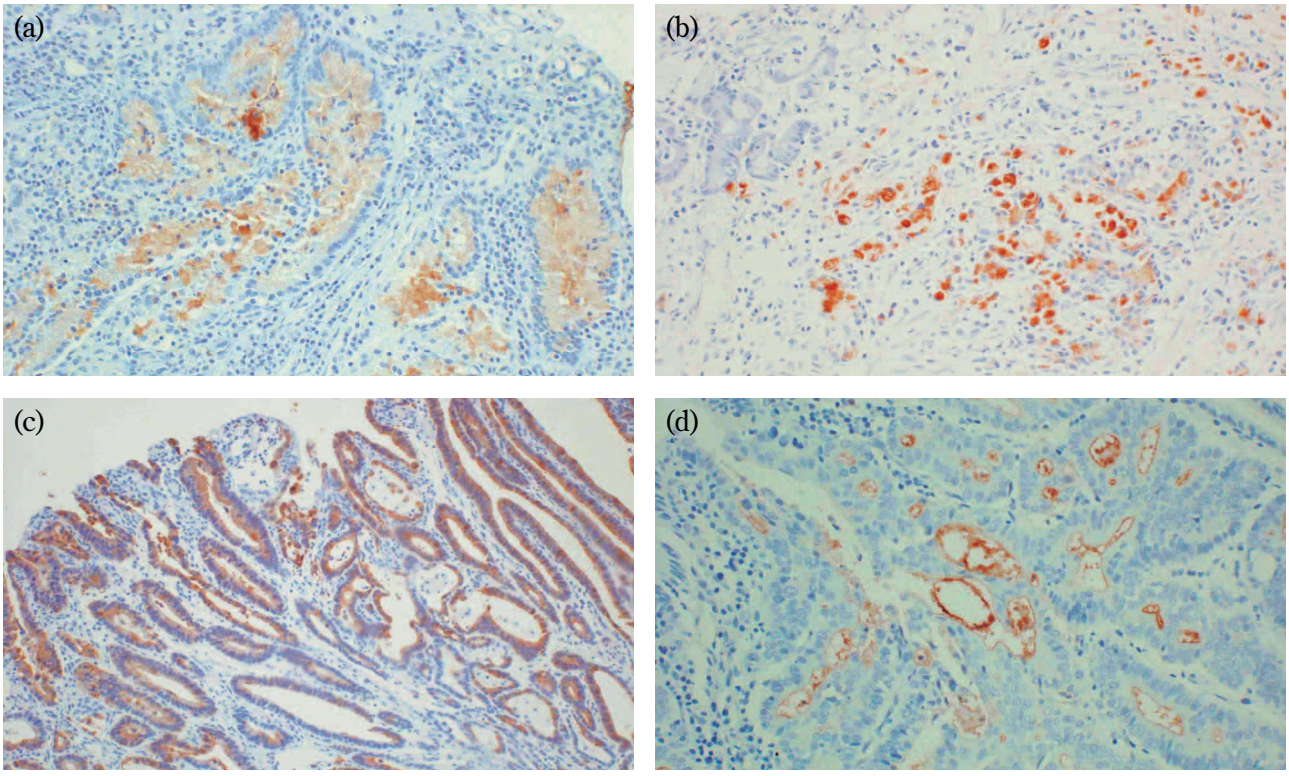

Fig. 1. mucin staining and immunohistochemical staining
(a) Human Gastric Mucin (HGM) is positive. (b) paradoxical concanavalinA-III (conA-III) stain is positive.
(c) MUC2 is positive. (d) CD10 is positive.

Table 1. Determination of mucin phenotypes of cancers.

				HGM, ConA-III	
				(-)	(+)
CD10	(+)			Intestinal type	Mixed type
	(-)	MUC-2	(+)		
			(-)	Lack of mucin (LOM)	Gastric type

tive for both gastric- and intestinal-phenotype markers as the mixed type, and carcinomas negative for all markers as lack of mucin (LOM).

3) Evaluation of E-cadherin expression

Immunostaining for the cell adhesion molecule E-cadherin was performed using an anti-E-cadherin antibody (anti-human E-cadherin, mouse monoclonal, 1 : 10,000 ; TaKaRa, Japan) (16). The stainability of the intercellular membrane in the carcinomatous area was compared with that in the normal glandular ducts in the same section : when more than 80% of the carcinomatous area was stained at a normal level, the E-cadherin expression was considered normal, and when less than 80% was so stained, the expression was considered reduced (Fig. 2a, b)

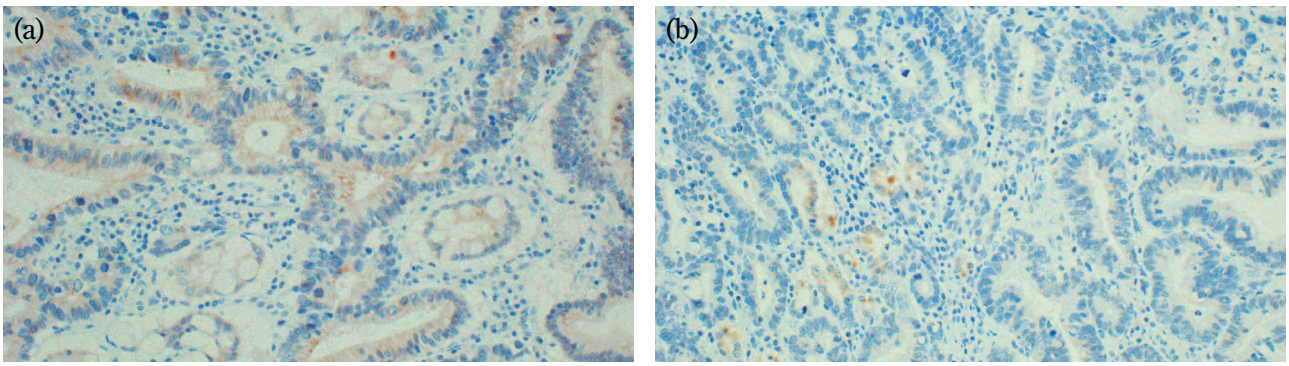

Fig. 2. Evaluation E-cadherin expression
(a) E-cadherin expression is normal. (b) E-cadherin expression is reduced.

4) Statistical analysis

The significance of differences was tested by the χ^2 test, Fisher's direct exact probability test, and Student's t-test. P values of less than 0.05 were considered significant.

RESULTS

1) Clinicopathological analysis of carcinomas according to cellular phenotypes

Table 2 shows the Clinicopathological analysis of carcinomas according to cellular phenotypes. Among the 107 SM gastric carcinomas, gastric-, intestinal-, and mixed-phenotype carcinomas and LOM carcinomas accounted for 20.6% (22/107), 31.8% (34/107), 32.7% (35/107), and 14.9% (16/107), respectively.

By phenotype, the mean age was significantly lower (56.5±9.2 years, $p<0.05$) in patients with gastric-phenotype carcinomas than in those with carcinomas of other phenotypes ; however, no differences were noted in gender, tumor size, site of involvement, or gross type among carcinomas of different phenotypes. When SM1 was defined as a submucosal invasion depth of less than 500 μm, and SM2 as that of 500 μm or deeper, no differences were ob-

Table 2. Clinicopathological parameters by mucin phenotype.

	gastric type (n=22) 20.6%		intestinal type (n=34) 31.8%		mixed type (n=35) 32.7%		LOM (n=16) 14.9%		total (n=107)	
Age, years	56.5 ±9.2		63.6 ±8.6		65.4 ±8.6		64.1±10.7		62.7 ±9.5	
Gender (M/F)	16 : 6		24 : 10		23 : 12		15 : 1		78 : 30	
Tumor size (cm)	3.3 ±1.9		3.1 ±1.8		3.9 ±1.8		3.2 ±1.9		3.4 ±1.9	
Location										
(U : M : L)	5 : 14 : 3		5 : 18 : 11		4 : 15 : 16		4 : 6 : 6		18 : 53 : 36	
Macroscopic type										
elevated	2		6		4		1		13	
depressed	15		17		22		11		65	
flat	2		0		0		0		2	
mixed	3		11		9		4		27	
Depth of invasion										
SM1	5		9		11		2		27	
SM2	17		25		24		14		80	
Histlogic type (%)										
Differentiated	11	(50.0)	31	(91.2)	25	(71.4)	12	(75.0)	79	(73.8)
Undifferentiated	11	(50.0)	3	(8.8)	10	(28.6)	4	(25.0)	28	(26.2)
Lymphatic invasion										
(-) (%)	16	(72.7)	28	(82.3)	28	(80.0)	11	(68.8)	83	(77.6)
(+) (%)	6	(27.3)	6	(17.6)	7	(20.0)	5	(31.2)	24	(22.4)
Blood vessel invasion										
(-) (%)	21	(95.5)	31	(91.2)	33	(94.3)	15	(93.8)	100	(93.5)
(+) (%)	1	(4.5)	3	(8.8)	2	(5.7)	1	(6.2)	7	(6.5)
Lymph node metastasis										
(-) (%)	16	(72.7)	32	(94.1)	34	(97.1)	14	(87.5)	96	(89.7)
(+) (%)	6	(27.3)	2	(5.9)	1	(2.9)	2	(12.5)	11	(10.3)
E-cadherin expression										
normal (%)	9	(40.9)	18	(52.9)	18	(51.3)	7	(43.7)	39	(36.4)
reduced (%)	13	(59.1)	16	(47.1)	17	(48.6)	9	(56.3)	68	(63.6)

* : $p<0.05$

U, M, L ; upper, middle, and, lower one-thirds of the stomach, (-)=negative ; (+)=positive. SM=invasion to submucosa

served in the depth of invasion among the tumor phenotypes, although SM2 was slightly more frequent in LOM carcinomas. By histological type, the percentage of the differentiated intestinal phenotype was significantly higher, at 91.2% (31/34), than those of other phenotypes ($p<0.05$). There were no significant differences in the degree of vascular invasion among the tumor phenotypes.

2)Correlation between phenotype and E-cadherin expression

No significant differences were noted among the tumor phenotypes in the expression rate of E-cadherin, a cell adhesion molecule related to tumor invasion and metastasis (Fig. 3).

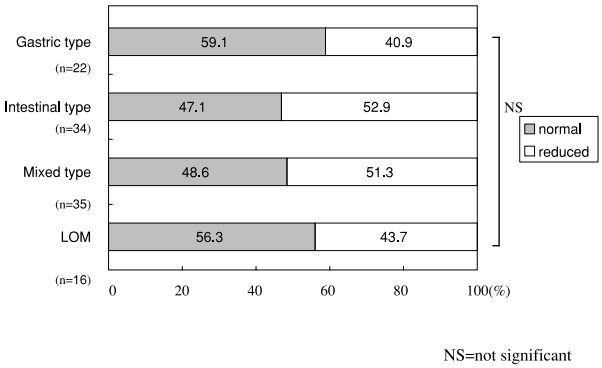

Fig. 3. Correlation between phenotype and E-cadherin expression.

3) Lymph node metastasis rates and histology, phenotype, and E-cadherin expression

(1) Lymph node metastasis and histological type (Fig. 4).

Of the 107 SM gastric carcinomas, 11 (10.3%) were lymph node metastasis-positive. No significant difference was noted in the rate of lymph node metastasis between differentiated and undifferentiated carcinomas. The lymph node metastasis rate of differentiated papillary adenocarcinomas was high, at 28.6% (2/7), but did not significantly differ from those of carcinomas of other histological types.

(2)Lymph node metastasis and phenotype or E-cadherin expression(Fig. 5).

The lymph node metastasis rate was significantly higher ($p<0.05$) in gastric-phenotype carcinomas (27.3%, or 6/22) than in intestinal-phenotype carcinomas (5.9%, or 2/34) and mixed-phenotype carcinomas (2.9%, or 1/35), but not in LOM carcinomas.

The lymph node metastasis rate of carcinomas with reduced E-cadherin expression was somewhat higher (14.5%), but not significantly, than that (5.8%) of carcinomas with normal E-cadherin expression.

(3) Lymph node metastasis rates in gastric-phenotype

Lymph node metastasis rates by histological type and E-cadherin expression status were examined in the 22 gastric-phenotype carcinomas. No difference in the metastasis rate was noted between the differentiated and undifferentiated carcinomas. However, lymph node metastasis was observed only in carcinomas with reduced expression of E-cadherin, and their metastasis rate was significantly higher than that of carcinomas with normal E-cadherin expression ($p<0.05$). As shown in Table 3, lymph node metastasis rates by the histological type in the invasive area and E-cadherin expression status were as follows : regardless of the histological type in the submucosal layer, no carcinomas with normal E-cadherin expression metastasized to lymph nodes, but even differentiated carcinomas with reduced E-cadherin expression did. Two of three complex type

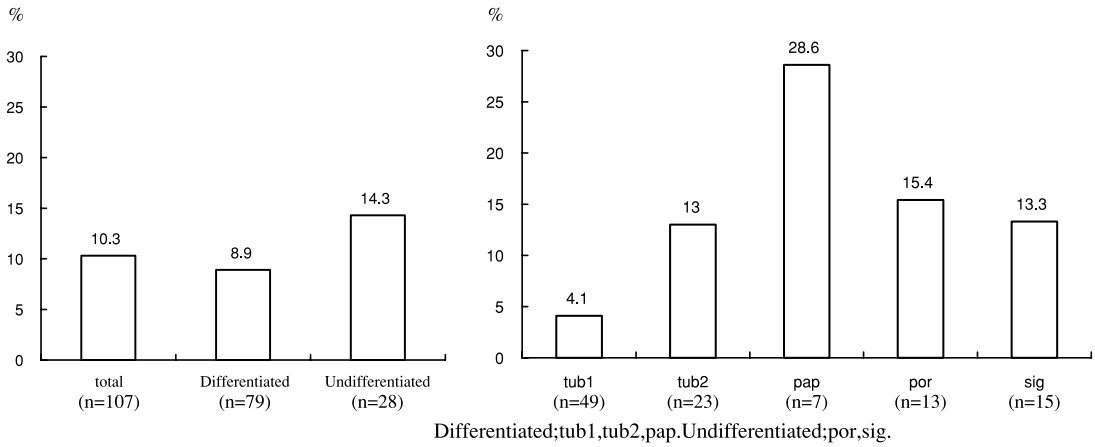

Fig. 4. Lymph node metastasis and histological type.

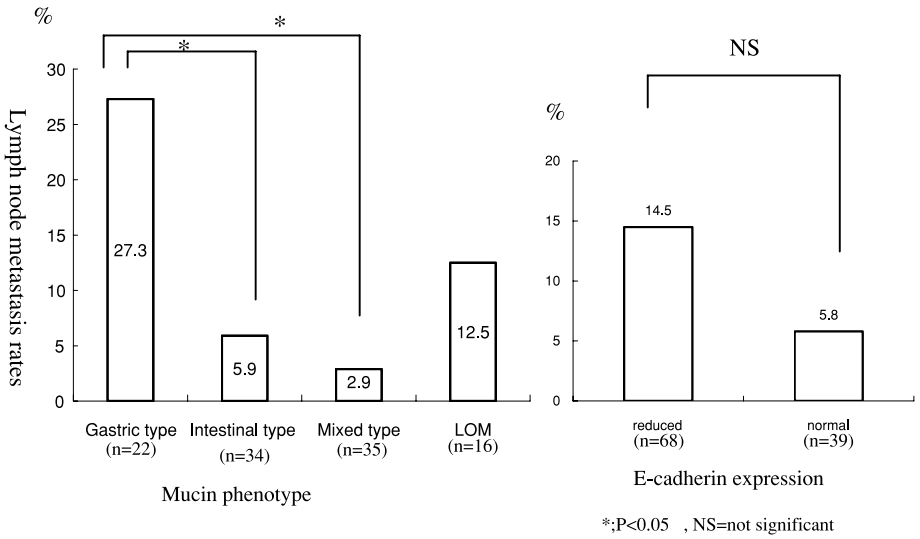

Fig. 5. Lymph node metastasis and phenotype or E-cadherin expression.

lesions, in which differentiated and undifferentiated cancer areas coexisted, metastasized to lymph nodes.

4) Lymph node metastasis rates in patients with reduced E-cadherin expression

The lymph node metastasis rate of gastric-phenotype carcinomas with reduced E-cadherin expression, which frequently metastasized to lymph nodes, was significantly higher, at 46.2%, than that of carcinomas of other phenotypes ($p<0.05$, Fig. 6). Moreover, the metastasis rate of differentiated gastric-phenotype carcinomas with reduced E-cadherin expression was significantly higher, at 60.0%, than that of carcinomas of other phenotypes ($p<0.05$, Fig. 6).

Table 3. Lymph node metastasis rates of gastric-phenotype lesions by histological type of SM invasive front and E-cadherin expression status.

(%)

		E-cadherin expression		total
		normal	reduced	
Differentiated type	solely Differentiated type	0/6(0)	1/2(50.0)	1/8(12.5)
	Complex type	0/0(0)	2/3(66.7)	2/3(66.7)
Undifferentiated type		0/3(0)	3/8(37.5)	3/11(27.3)
total		0/9(0)[a1]	6/13(46.2)[a2]	6/22(27.3)

a1 vs a2 ; $p<0.05$

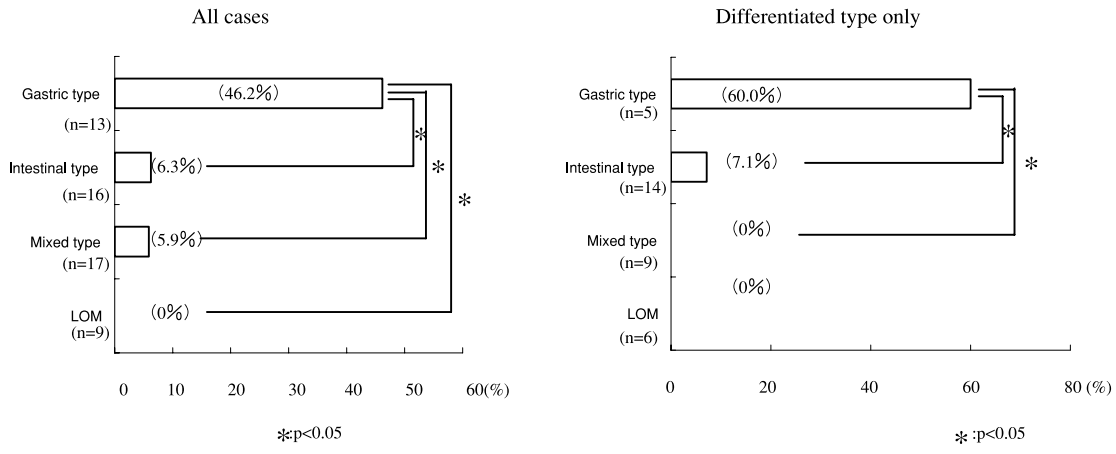

Fig. 6. Lymph node metastasis in patient with reduced E-cadherin expression.

DISCUSSION

SM gastric cancers have been reported to metastasize at rates of 14.0% to 20.3% (17-20). In this study, their metastasis rate was somewhat lower, at 10.3%. This was probably because our center is a cancer detection center where many individuals undergo endoscopic examination annually, and relatively early lesions of SM carcinomas are detected. By histological type, the lymph node metastasis rate was somewhat higher, but not significantly, in undifferentiated than in differentiated carcinomas, and in papillary adenocarcinomas among differentiated carcinomas. Among differentiated gastric carcinomas, papillary adenocarcinomas invading the subsmucosa have been reported to exhibit a high rate of lymph node metastasis (21), and to include many gastric-phenotype carcinomas (7). However, in this study, no papillary adenocarcinomas showed a gastric phenotype, presumably because of the small number of cases examined, suggesting that the histological type of papillary adenocarcinoma is a risk factor for lymph node metastasis independent of the cellular phenotype.

In this study, the gastric, intestinal, and mixed phenotypes and LOM accounted for 20.6%, 31.8%, 32.7%, and 14.9% of the SM gastric carcinomas, respectively. However, Egashira, *et al.* reported that the gastric phenotype accounted for 41.1% of differentiated intramucosal carcinomas of less than 5 mm (5), which, as Yoshikawa, *et al.* indicated (22), suggests that a phenotypic change from the gastric to intestinal phenotype occurs with enlargement and progression of the tumor. The age of patients with gastric-phenotype carcinomas was significantly younger than that of patients with carcinomas of other phenotypes, which may be related to the higher percentage of undifferentiated carcinomas among the gastric-phenotype cancers (23). In agreement with other studies (4-6), as described above, half of the gastric-phenotype carcinomas were differentiated.

With recent advances in the endoscopic treatment of early gastric cancer, indications for endoscopic therapy are expanding. The JGCA gastric cancer treatment guidelines clearly state that differentiated SM gastric carcinomas of less than 3 cm in the maximum diameter with an invasion depth of less than 500 μm have little or no potential for lymph node metastasis, and can be endoscopically treated for research purposes only (24). In this study, regardless of the phenotype, differentiated SM carcinomas with an invasion depth of less than 500 μm did not metastasize to lymph nodes (data not shown). No significant differences were observed in the percentage of lesions with an invasion depth of 500 μm or deeper among carcinomas of different phenotypes, but the lymph node metastasis rate of gastric-phenotype carcinomas was highest, at 27.3%, and was significantly higher than those of carcinomas of other phenotypes. The gastric-phenotype lesions tended to be undifferentiated, but no difference was noted in the rate of lymph node metastasis between gastric-phenotype differentiated and undifferentiated carcinomas. This suggests that the gastric phenotype is an independent risk factor for lymph node metastasis.

The mechanism of cancer metastasis involves a complex process in which cancer cells proliferate, invade blood capillaries and lymphatics, enter the blood or lymph flow, implant themselves in capillaries and lymphatics of distant organs, invade them, and proliferate. E-cadherin is a calcium-dependent cell adhesion molecule expressed in epithelial cells (10), with its intracellular domain binding to the catenin family (α-, β-, and γ-catenin) as the lining protein (25, 26). In cancer cells, mutations in cadherin-catenin genes, methylation of the cadherin promoter region, abnormalities in the signal transduction mechanism mediated by β-catenin tyrosine phosphorylation, or inhibition of cadherin-catenin complex formation involving the low-molecular-weight G-protein Rac 1 causes the abnormal expression and function of E-cadherin (27), leading to the dissociation of cancer cells from primary cancer nests (11, 28, 29). Indeed, reduced expression of E-cadherin has been reported in cancer cells at the invasive front of cancers such as digestive tract cancers other than gastric cancer, ovarian cancer, and bladder cancer (30-32). In gastric cancer, abnormal expression and reduced function of E-cadherin are associated with cancer invasion and metastasis (9, 33) ; in particular, involvement of E-cadherin abnormalities (such as gene mutations) in undifferentiated lesions has been indicated (9). Therefore, the relationship between E-cadherin expression and gastric-phenotype lesions associated with undifferentiation is of interest. Although the present examination of SM gastric carcinomas did not clarify the relationship between the E-cadherin expression status and lymph node metastasis, analysis of gastric-phenotype carcinomas alone showed that carcinomas with normal E-cadherin expression did not metastasize to lymph nodes, whereas 46.2% of carcinomas with

reduced E-cadherin expression did. This was not observed in intestinal- or mixed-phenotype lesions or LOM lesions in all or differentiated carcinomas (Figs. 5 and 6). We speculate that the biological malignancy of gastric-phenotype lesions is closely related to the E-cadherin expression status. This relationship appears to be closer than that between the histological type and gastric phenotype, because submucosally invading, differentiated carcinomas with reduced E-cadherin expression metastasized to lymph nodes, and conversely, undifferentiated carcinomas with normal E-cadherin expression did not. It has been reported that when intestinal- and diffuse-type cancer areas coexist, E-cadherin gene mutations are observed selectively in the diffuse-type area (24). However, E-cadherin function may actually be suppressed, although its expression is maintained ; therefore, the histological type is not in complete agreement with the E-cadherin expression status. Nevertheless, as this study shows, differentiated lesions of the gastric phenotype with reduced expression of E-cadherin may potentially become undifferentiated, and the risk of lymph node metastasis should be considered.

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