ORIGINAL

Clinicopathological evaluation of biological behavior of submucosal invasive gastric carcinomas : relationship among lymph node metastasis, mucin phenotype and proliferative activity

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Abstract : Background : Gastric carcinomas have been classified into the differentiated and undifferentiated type, on the basis of its tendency to gland formation. As a result of recent advances in mucin histochemistry, mucin phenotypes of gastric carcinomas have been investigated. However, no consensus on the evaluation of the grade of malignancy of early gastric carcinomas regarding mucin phenotype expression has developed. To address this issue, we evaluated the lymph node metastasis rate and proliferative activity of a submucosal invasive (sm) gastric carcinoma according to mucin phenotype expression.
Methods : In resected surgical specimens from 108 patients with a single sm gastric carcinoma, the association between clinicopathological factors and lymph node metastasis was evaluated. In all cases, immunohistochemical staining with human gastric mucin, Muc-2, and CD10 and mucin histochemical staining by paradoxical concanavalin A staining were performed. The mucin phenotypes were classified into gastric-type (G-type), intestinal-type (I-type), mixed gastric and intestinal type (M-type), or a lack of mucin (LOM), using these as markers. To evaluate the cell proliferative activity of the gastric carcinoma, proliferating cell nuclear antigen (PCNA) staining was also performed.
Results : The rate of lymph node metastasis was higher for G-type sm carcinomas. A multivariate analysis showed that the G-type and lymphatic invasion were independent factors of lymph node metastasis. However, the PCNA-labeling index (PCNA-LI) was low for G-type carcinomas irrespective of the presence or absence of lymph node metastasis. In I-type carcinomas, PCNA-LI was significantly higher in cases that were positive for lymph node metastasis than in negative cases.
Conclusion : G-type and lymphatic invasion are independent risk factors for lymph node metastasis of an sm gastric carcinoma, and proliferative activity may be a significant parameter for lymph node metastasis in cases with I-type carcinomas. J. Med. Invest. 54 : 99-108, February, 2007

Keywords **:** *submucosal invasive gastric carcinoma, mucin phenotype expression, proliferating cell nuclear antigen, lymph node metastasis*

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INTRODUCTION

Due to recent improvements in diagnostic techniques, the early detection of gastric carcinomas has been increasing(1-3), and submucosal invasive (sm) gastric carcinomas have frequently been encountered. However, lymph node metastasis is observed in 10-25% of patients with sm gastric carcinomas as early stage carcinomas, and previous studies have shown that endoscopic treatment alone is not adequate in such patients(4-9). On the other hand, it has been reported that the presence or absence of lymph node metastasis can be estimated, even in patients with sm gastric carcinomas based on histology and the depth of submucosal invasion, and endoscopic treatment is indicated in some of these patients(8, 9). In sm gastric carcinoma, the presence of lymph node metastasis is seemed to affect not only survival after surgical or endoscopic treatment, but also decision of the therapy. Therefore, establishment of supplementary diagnosis of lymph node metastasis is very important.

The histology of gastric carcinomas is conventionally classified into differentiated and undifferentiated types. The former had been regarded as intestinal-type (I-type) carcinomas closely associated with intestinal metaplasia, and the latter as gastric-type (G-type) carcinomas developing in the gastric mucosa (10, 11). However, due to advances in mucin histochemistry, the mucin phenotypes of gastric carcinoma have been investigated, and a high incidence of the G-type in differentiated-type carcinomas has been verified, and studies have extensively evaluated the histogenesis of gastric carcinomas(12-23). Many authors have reported that G-type carcinomas have various clinicopathological characteristics that differ from those of I-type carcinomas(14, 18, 20, 23, 24). However, the rate of lymph node metastasis according to mucin phenotype or the grade of malignancy such as cell proliferative activity of early stage gastric carcinomas has not been adequately evaluated. Therefore, to clarify the association between the mucin phenotypes of gastric carcinomas and the rate of lymph node metastasis or cell proliferative activity, we investigated mucin phenotypes by mucin histochemical and immunohistochemical techniques in cases of sm gastric carcinomas and evaluated their association with the rate of lymph node metastasis and cell proliferative activity.

MATERIALS AND METHODS

Subjects

This study included 108 consecutive cases of single sm gastric carcinomas (79 differentiated- and 29 undifferentiated-type carcinomas) in which surgical resection and lymph node dissection had been performed between 1986 and 1996, and in which the resected specimens showed adequate remnants of the carcinoma nest in the mucosa. Using the resected specimens from these cases, the association between clinicopathological items (location, macroscopic type, histology, depth of submucosal invasion, venous invasion, and lymphatic invasion) and lymph node metastasis was evaluated. The gastric carcinomas were histologically classified according to Nakamura's Classification(10) based on the extent of glandular formation into differentiated and undifferentiated types. A detailed histological classification was performed according to the General Rules for the Gastric Cancer Study(25). To classify the depth of carcinoma invasion into the submucosal layer, the submucosal layer was divided into 3 equal portions, and tumors with their deepest site being in the superficial layer of the submucosal layer were defined as sm1 carcinomas, those with the deepest site close to the propria muscularis as sm3 carcinomas, and those located intermediate between sm1 and sm3 were defined as sm2 carcinomas.

Mucin histochemical and immunohistochemical stainings

The gastrectomy specimens were fixed in 10% buffered formalin for 24-120 hours, cut into 5-mm sections, and embedded in paraffin. Pathological observation was performed by routine hematoxylin/eosin (HE) staining, and sections at the deepest site of the submucosal tumor invasion were selected for microscopic examination. Thin sections (3 μm) at the deepest site of the tumor were prepared using slide glasses coated with poly-L-lysine for staining. The primary antibodies used in the immunohistochemical staining were a Human Gastric Mucin mouse monoclonal antibody (NCL-HGM-45M1 clone 45M1, Novocastra ; UK, 1 : 50) as a marker for G-type, a Muc-2 Glycoprotein mouse monoclonal antibody (NCL-MUC-2 clone Ccp58, Novocastra ; UK, 1 : 100) and a CD10 mouse monoclonal antibody (NCL-CD10-270 clone 56C6, Novocastra ; UK, 1 : 80) as markers for I-type, and a proliferating cell nuclear antigen (PCNA) mouse monoclonal antibody (NCL-PCNA clone PC19, Novocastra ; UK,

1 : 100) as a marker for proliferative cells. For Muc-2 and CD10, for antigen enhancement, paraffin-embedded sections were deparaffinized, immersed in 0.01 M citrate buffer (pH, 6.0), and subjected to microwave irradiation (750 W) for 4 times for 5 minutes each, according to the method of Shi, *et al.* (26). Staining was performed by the labeled streptavidin biotin method using an LSAB kit (DAKO ; USA). As the primary antibodies, HGM, Muc-2, and CD10 were incubated at 25℃ for 60 minutes, and PCNA was incubated at 4℃ for 18 hours. After coloring with a 0.02% 3,3'-diaminobenzidine (DAB) solution (pH, 7.6), nuclear staining was performed with Mayer's hematoxylin. In addition, class III mucin was stained with Concanavalin A (Con A) (type IV, Sigma Chemical ; St. Louis, 1 : 1,000) by the method of Katsuyama and Spicer(27). Oxidation was performed by treatment with 1% periodic acid (PA) for 60 minutes, and the immersion time in the Con A solution was 60 minutes. Coloring and nuclear staining were performed by a method similar to the above. The Human gastric mucin antibody is specific for gastric foveolar cells and has a stable stainability(28). Paradoxical Con A staining is useful for the identification of pyloric gland-type mucin(27, 29, 30). The Muc-2 glycoprotein recognizes mucous core protein specific to goblet cells(31-33). CD10, also known as common acute lymphocytic leukemia antigen (CALLA), is also positive for the intestinal brush border and is useful for the identification of I-type(34, 35). Proliferating cell nuclear antigen (PCNA) is useful for the identification of cells in the proliferative phase(36). The PCNA expression rate can be a potential prognostic marker in human malignancy(37).

Determination of the mucin phenotype and PCNA-labeling index

For the identification of the G-type, only mucus of the cytoplasm of carcinoma cells was evaluated. The mucin phenotype is regarded as G-type when more than 5% of the entire carcinoma mucosal area is stained with G-type markers (HGM or Con A), and is regarded as I-type when 5% of the entire carcinoma is stained by I-type markers (Muc-2 or CD 10). For CD10, only cell membrane stainability was evaluated because the brush border of absorptive intestinal enterocytes is stained. When both the G-type and I-type were expressed, the phenotype was defined as the mixed gastric and intestinal type (M-type). The absence of expression of either G-type or I-type was regarded as a lack of mucin (LOM) (Table 1). In PCNA immunostaining, more than

Table 1. Immunohistochemistry and mucin histochemistry for the classification of gastric and intestinal mucin phenotypes

Immunohistochemistry			
Antibody	Clone	Mucin histochemistry	Specificity
Gastric type markers			
HGM	45M1	—	Peptide core of human gastric mucin
—	—	PCS (Class III)	Pyloric gland type mucin
Intestinal type markers			
Muc-2	Ccp58	—	Muc-2 glycoprotein
CD 10	56C6	—	Brush border on luminal surface

HGM : human gastric mucin ; PCS : paradoxical concanavalin A staining

Table 2. Relationship between clinicopathological factors and lymph node metastasis associated with 108 gastric carcinomas with submucosal invasion

Characteristic	Value	Lymph node metastasis(%)	*P* value
Age (yrs) : Mean ± SD (range)	62.7 ± 9.5 (35-82)	—	—
Gender (Male : Female)	78 : 30	7.7 : 16.7	NS
Location (U : M : L)	18 : 53 : 37	0 : 13.2 : 10.8	NS
Macroscopic type (elevated : depressed : mixed[a])	13 : 73 : 22	7.7 : 11.0 : 9.1	NS
Size (cm) : Mean ± SD (range)	3.4 ± 1.9 (0.6-9.8)	—	—
Histology (differentiated : undifferentiated type)	79 : 29	8.9 : 13.8	NS
Depth of submucosal invasion (sm 1 : sm 2 : sm 3)	22 : 46 : 40	13.6 : 4.3 : 15.0	NS
Venous invasion (negative : positive)	101 : 7	7.9 : 42.9	<0.01
Lymphatic invasion (negative : positive)	84 : 24	3.6 : 33.3	<0.001

U, M, L : upper, middle, and lower one-thirds of the stomach, respectively. SD : standard deviation
[a] mixed type consists of both of elevated and depressed components.

500 carcinoma cells in areas showing relatively uniform stainability at the invasive front were observed by microscopy, and cells in which the nucleus was stained were considered to be positive. Those, which did not show adequate stainability in the proliferative zone of the non-carcinoma portion of the tissue section, were excluded from evaluation. Positive cells were expressed in terms of percentages as the PCNA-labeling index (PCNA-LI).

Statistical analysis

Lymph node metastasis rate was analyzed by the χ^2 test and Fisher's exact probability method, and PCNA-LI was analyzed by the unpaired t-test and ANOVA with Stat View software. A multivariate analysis by logistic regression was also performed with SAS software. The odds ratios were presented with a 95% confidence interval by multivariate analysis. $P < 0.05$ was considered to be significant.

RESULTS

Clinicopathological factors and the lymph node metastasis rate

Of the 108 cases of sm gastric carcinomas, 11 (10.2%) were positive for lymph node metastasis. Table 2 summarizes the association between clinicopathological factors for sm gastric carcinomas and lymph node metastasis. Venous invasion and lymphatic invasion were associated with lymph node metastasis. The lymph node metastasis rate was 8.9% (7/79) for differentiated-type gastric carcinomas and 13.8% (4/29) for undifferentiated-type gastric carcinomas, with no significant difference. The rate of lymph node metastasis as a function of the depth of sm invasion was 13.6% for sm 1, 4.3% for sm2, and 15.0% for sm3, being high even for sm1 and suggesting no association between the depth of sm invasion and the rate of lymph node metastasis. The rate of lymph node metastasis was also not associated with sex, location, or macroscopic type.

The incidence of expression of each mucin phenotype and its association with the lymph node metastasis rate

Figs. 1 A～D and 2 A～C show typical cases that were found to be positive for G-type and I-type mucin histochemical staining and immunohistochemical staining. Table 3 summarizes the incidences of mucin phenotypes and rates of lymph node metastasis, as evidenced by histology. In the differentiated-type

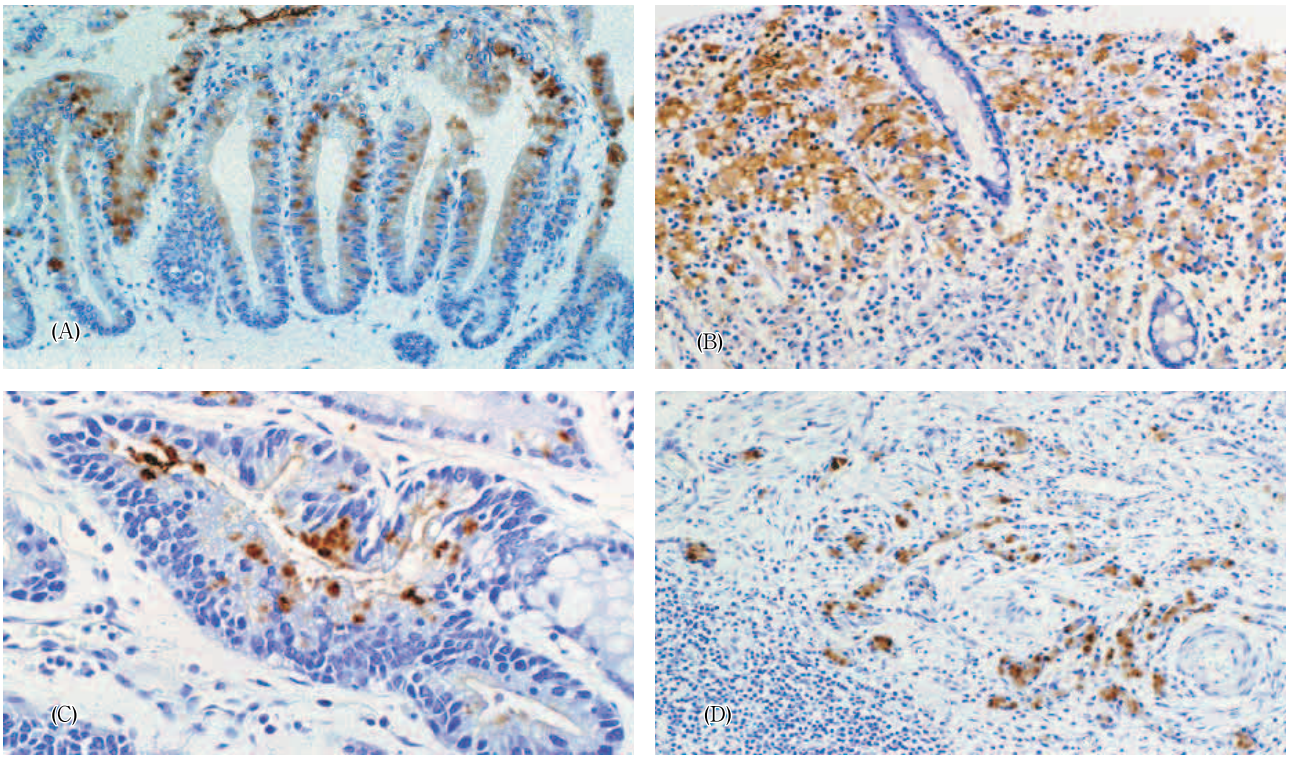

Fig. 1. Expression of gastric type mucin
Carcinoma cells are positive for human gastric mucin (45M-1). (A) differentiated type carcinoma (× 50) (B) undifferentiated type carcinoma (× 50)
ConA III staining is partially positive in the carcinoma. (C) differentiated type carcinoma (× 100) (D) undifferentiated type carcinoma (× 50)

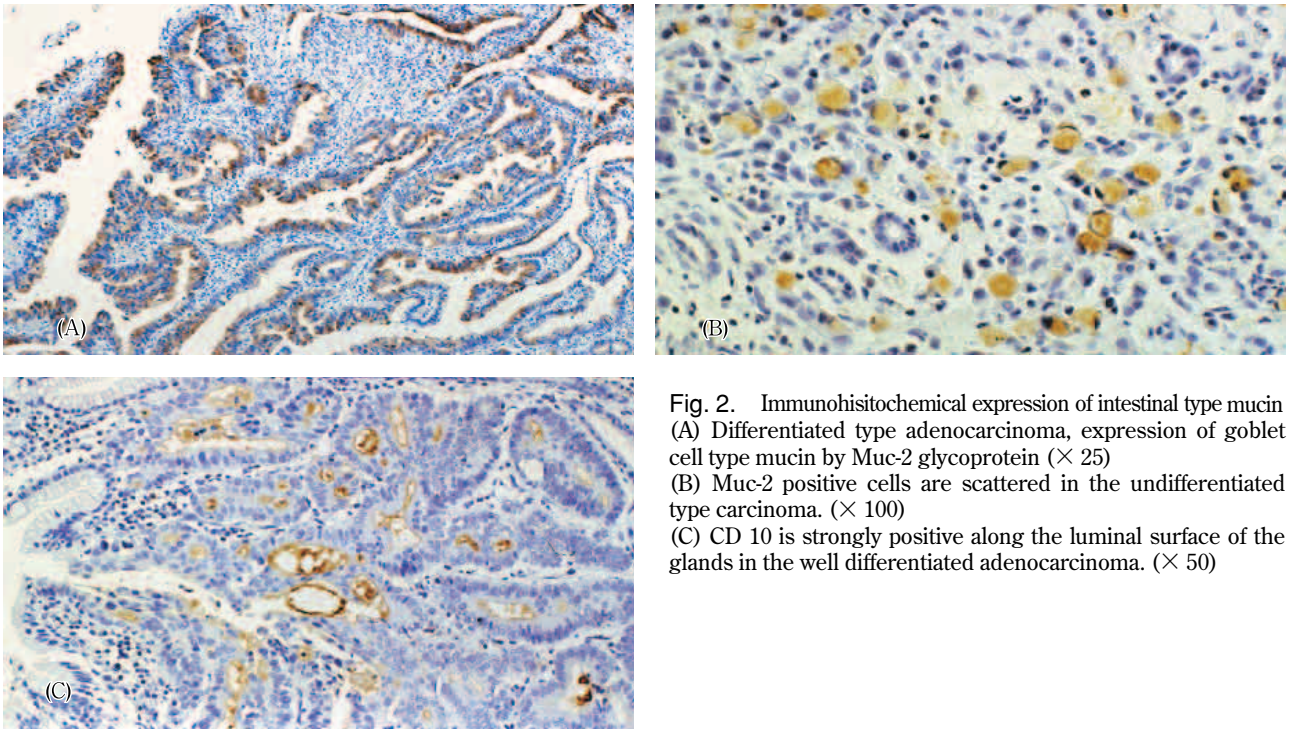

Fig. 2. Immunohisitochemical expression of intestinal type mucin
(A) Differentiated type adenocarcinoma, expression of goblet cell type mucin by Muc-2 glycoprotein (× 25)
(B) Muc-2 positive cells are scattered in the undifferentiated type carcinoma. (× 100)
(C) CD 10 is strongly positive along the luminal surface of the glands in the well differentiated adenocarcinoma. (× 50)

Table 3. Relationship between mucin phenotype and lymph node metastasis

	Histological type			
Mucin phenotype	Differentiated	Undifferentiated	Total	
G-type	3 / 11 (27.3)[a]	3 / 12 (25.0)	6 / 23	(26.1)[c]
I-type	2 / 31 (6.5)	0 / 3 (0)	2 / 34	(5.9)
M-type	0 / 25 (0)[b]	1 / 10 (10.0)	1 / 35	(2.9)[d]
LOM	2 / 12 (6.7)	0 / 4 (0)	2 / 16	(12.5)
Total	7 / 79 (8.9)	4 / 29 (13.8)	11 / 108	(10.2)

( ) : %
a vs b, c vs d : $p<0.05$

Table 4. Multivariate logistic regression analysis of lymph node metastasis

Characteristic	Odds ratio	95% CI	*P* value
G-type	5.128	0.552 - 47.656	0.151
I-type	0.565	0.048 - 6.648	0.650
M-type	0.221	0.013 - 3.869	0.302
Venous invasion	9.266	0.894 - 96.081	0.062
Lymphatic invasion	12.942	2.481 - 67.502	0.002

95% CI : 95% confidence interval

gastric carcinomas, the I-type was the most frequently observed (39.2%, 31/79), followed by the M-type (31.6%, 25/79), LOM (15.2%, 12/79), and G-type (13.9%, 11/79). In the undifferentiated gastric carcinomas, the G-type was most frequently observed (41.4%, 12/29), followed by the M-type (34.5%, 10/29), LOM (13.8%, 4/29), and I-type (10.3%, 3/29). The rate of lymph node metastasis according to mucin phenotype was higher for the G-type (26.1%, 6/23) than for the I-type (5.9%, 2/34) or the M-type (2.9%, 1/35), and a significant difference was recognized between G-type and M-type ($p < 0.05$). This tendency was also observed in a separate evaluation of differentiated and undifferentiated carcinomas.

Multivariate analysis of lymph node metastasis

Based on the above results on the lymph node metastasis rate, we established a hypothesis that lymphatic invasion, venous invasion, and the mucin phenotype are important variables as factors as-

sociated with lymph node metastasis. To confirm this hypothesis, multivariate analysis using a logistic model was performed. The results of analysis using 5 variables first selected (lymphatic invasion, venous invasion, G-type, I-type, and M-type) are shown in Table 4. Subsequently, variable selection by the backward elimination procedure was performed, and two variables (lymphatic invasion and G-type) remained as significant variables in the final model (Lymphatic invasion : odds ratio, 16.846 ; 95% confidence interval, 3.454-82.171 ; $p < 0.001$ G-type : odds ratio, 7.690 ; 95% confidence interval, 1.609-36.748 ; $p = 0.011$).

Clinicopathological factors and PCNA-LI

To evaluate proliferative activity, PCNA staining was performed in all 108 cases. As a result, only 53 of 108 cases could be immunohistochemically evaluated in the point of their stainability. It was considered that PCNA immunoreactivity possibly had been greatly reduced or abolished because the time of fixation in formalin solution was too long in many cases(36). The association between the clinicopathological factors and PCNA-LI in 53 cases in which it was possible to immunohistochemically evaluate is shown in Table 5. PCNA-LI was significantly higher ($p < 0.05$) in cases that were positive for lymph node metastasis (43.4 ± 27.4%) than in negative cases (27.7 ± 19.0%). PCNA-LI according to the depth of sm invasion was significantly higher for sm3 (45.1 ± 24.3%) than for sm1 (21.3 ± 15.0%) or sm2 (25.5 ± 17.5%), and that according to histology was significantly higher ($p < 0.01$) for papillary adenocarcinomas (58.8 ± 26.2%) than for the other histological types. PCNA-LI according to mucin phenotype did not significantly differ among I-type (36.2 ± 24.0%), M-type (27.1 ± 21.4%), LOM (41.9 ± 23.2%), and G-type (22.7 ± 15.1%) but was the low-

Table 5. Relationship between clinicopathological factors and PCNA-LI

Clinicopathological factors	Number of cases	PCNA-LI, % Mean ±SD	*P* value
Lymph node metastasis			
+	11	43.4 ±27.4	<0.05
−	42	27.7 ±19.0	
Submucosal invasion			
sm1	15	21.3 ±15.0 [a]	
sm2	20	25.5 ±17.5 [b]	<0.01 (c vs a,b)
sm3	18	45.1 ±24.3 [c]	
Histological type			
pap	7	58.8 ±26.2 [d]	
well	14	25.8 ±14.4 [e]	
moderate	12	28.0 ±24.2 [f]	<0.01 (d vs e,f,g,h)
por	8	25.9 ±17.1 [g]	
sig	12	27.2 ±16.3 [h]	
Mucin phenotype			
G-type	12	22.7 ±15.1	
I-type	12	36.2 ±24.0	NS
M-type	19	27.1 ±21.4	
LOM	10	41.9 ±23.2	
Lymphatic invasion			
+	24	36.4 ±23.1	NS
−	29	26.4 ±19.8	
Venous invasion			
+	5	40.6 ±33.9	NS
−	48	30.0 ±20.3	

pap : papillary adenocarcinoma ; well : well differentiated adenocarcinoma ; moderate : moderately differentiated adenocarcinoma
por : poorly differentiated adenocarcinoma ; sig : signet ring cell carcinoma

est for G-type. PCNA-LI according to mucin phenotype in the presence or absence of lymph node metastasis was evaluated. In cases positive for lymph node metastasis, PCNA-LI for the G-type (23.5 ± 15.0%) was significantly lower ($p < 0.05$) than that for the I-type (75.2 ± 18.8%) or LOM (64.7 ± 23.3%). PCNA-LI for the G-type was similar between cases that were positive (23.5 ± 15.0%) and those that were negative (21.9 ± 16.7%) for lymph node metastasis, while that for the I-type was significantly higher in cases positive for lymph node metastasis (75.2 ± 18.8%) and negative cases (28.4 ± 16.0%) (Table 6). Fig. 3 A, B shows Muc-2 and PCNA staining micrographs in the case of an I-type differentiated adenocarcinoma that was positive for lymph node metastasis.

Table 6. Relationship between mucin phenotype and PCNA-LI associated with lymph node metastasis

	Lymph node metastasis		
Mucin phenotype	+	-	Total
G-type	23.5 ±15.0[a](n=6)	21.9 ±16.7(n= 6)	22.7 ±15.1 (n=12)
I-type	75.2 ±18.8[b](n=2)	28.4 ±16.0[d](n=10)	36.2 ±24.0 (n=12)
M-type	56.7(n=1)	25.5 ±20.7(n=18)	27.1 ±21.4 (n=19)
LOM	64.7 ±23.3[c](n=2)	36.3 ±20.7(n= 8)	41.9 ±23.2 (n=10)

a vs b : $p<0.01$; a vs c : $p<0.05$; b vs d : $p<0.01$

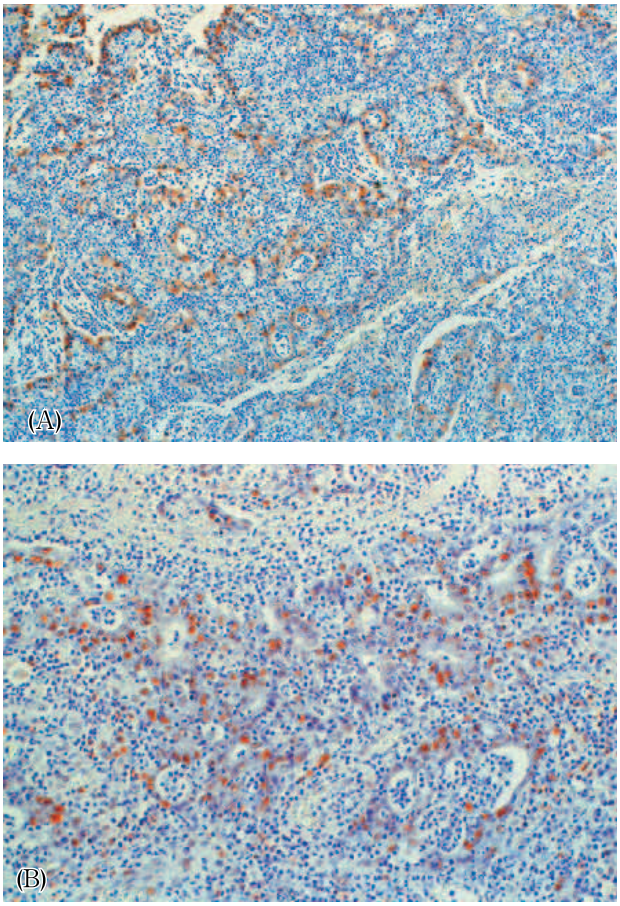

Fig. 3. Moderately differentiated adenocarcinoma with lymph node metastasis, intestinal type
(A) Carcinoma cells are positive for Muc-2. (× 25)
(B) PCNA-positive cells were clearly identified in the invasive front of the cancer, as evidenced by brown nuclear staining. PCNA-LI is 61.9%. (× 50)

DISCUSSION

Due to recent advances in mucin histochemical techniques, a considerable number of differentiated-type carcinomas showing G-type and undifferentiated carcinomas showing I-type have been reported (12, 14, 17-23). In this study, according to the mucin phenotypes, the incidence of G-type was 13.9% (11/79) in differentiated-type adenocarcinomas, and that of the I-type was 10.3% (3/29) in undifferentiated-type adenocarcinomas. As previously reported (14, 20, 23, 38), there is no consistency between mucin phenotype expression and histological type.

The rate of lymph node metastasis for sm G-type carcinomas was 26.1%, clearly higher than that for the other mucin phenotypes, suggesting its high grade of malignancy. A multivariate analysis showed that the G-type is an independent factor associated with lymph node metastasis. Noda (38) analyzed 18 cases of early gastric carcinomas showing lymph node metastasis and observed a high incidence of G-type (15/18, 84%). Oya, *et al.* (39) identified the mucin phenotype in 47 cases of early gastric carcinomas that were positive for lymph node metastasis and 91 not showing lymph node metastasis, and suggested that early gastric carcinomas showing the G-type in an early stage are biologically aggressive. Koseki, *et al.* (24) evaluated cases of differentiated sm gastric carcinomas and observed a high rate of lymph node metastasis for G-type sm carcinomas (11/29, 37.9%) and the highest correlation between lymph node metastasis and the G-type. The results of this study support their findings.

Kushima, *et al.* (18) examined 1-2 cm diameter intramucosal carcinomas and reported that only 30% of the gastric differentiated adenocarcinomas were I-type from the initial stage, and most were the G-type or M-type. Tatematsu, *et al.* (17) compared mucin phenotype expression between m carcinomas and carcinomas showing sm or deeper invasion and observed a significantly higher incidence of the I-type in the latter. Egashira, *et al.*, who studied differentiated gastric microcarcinomas (< 5 mm), also observed the G-type in 41.1% and the addition of the I-type with an increase in tumor diameter (21).

On the other hand, in undifferentiated-type carcinomas, changes in the mucin phenotype have also been reported. Various studies have shown complete preservation of the G-type in the early stage of signet ring cell carcinomas but acquisition of the I-type in the process of progression (13, 16, 19). Thus, it has been gradually clarified that the mucin phenotype can change during the process of progression. Considering these changes, lymph node metastasis may tend to develop in cases showing the G-type from the initial stage and preservation of the G-type without changes in the mucin phenotype, even after sm invasion.

To clarify the reason for the high incidence of lymph node metastasis in G-type carcinomas, we used PCNA-LI as a parameter for proliferative activity. PCNA-LI was significantly higher in cases that were positive for lymph node metastasis, showing its usefulness for evaluating proliferative activity. However, despite the high rate of lymph node metastasis for G-type sm gastric carcinomas, their proliferative activity was generally and unexpectedly low. These results suggest that proliferative activity may not be a parameter of lymph node metastasis in G-type sm gastric carcinomas. In G-type carcinomas, even when the proliferative activity is low, lymph node metastasis can occur. In the G-type, no difference in carcinoma cell proliferative activity was found between metastasis-positive and -negative cases. In contrast, in the case of the I-type, proliferative activity significantly differed between metastasis-positive and-negative cases. Kushima, *et al.* (18) examined gastric intramucosal carcinomas and reported that the number of PCNA-positive cells tended to be lower in G-type adenocarcinomas than in other-type adenocarcinomas. They speculated that this is because many cancer cells in G-type adenocarcinomas contain foveolar-epithelium-type or pyloric-gland -type mucin and have lost the ability to proliferate, or that they have mimicked the differentiation mode of normal cells in the gastric proper mucosa, and most cancer cells drop out of the cell cycle and differentiate and mature into cells that resemble surface mucous cells and pyloric gland cells. Saito, *et al.* (22) reported in their study of early gastric carcinomas that differentiated adenocarcinomas with the G-type histologically transform into undifferentiated-type adenocarcinomas with an increase in tumor size. Certain factors other than proliferative activity may be involved in the lymph node metastasis of G-type sm gastric carcinomas. Such factors were not clarified in this study. However, we speculate that G-type sm gastric carcinomas, despite their low proliferative activity, transform into the undifferentiated type with the progression of invasion, which facilitates carcinoma cell separation from the glands, leading to vascular invasion and lymph node metastasis. In this study, in I-type sm gastric carcinomas, proliferative activity was significantly higher in cases that were positive for lymph node metastasis than in negative cases, and proliferative activity can be a parameter for lymph node metastasis. Thus, it would be of interest to determine if the incidence of lymph node metastasis and proliferative behavior differed among mucin phenotypes.

In conclusion, G-type expression in sm carcinomas may be an important risk factor for lymph node metastasis, and attention should be paid to G-type carcinomas even if they are clinically of the differentiated type. Furthermore, it is seemed that proliferative activity is a significant parameter for lymph node metastasis in cases with I-type carcinomas. Therefore, in addition to the conventional examination of histological type, a preoperative examination of the mucin phenotype may be an important supplementary diagnosis.

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