J Med Dent Sci 2009; 56: 25–35

Original Article

Relationship between mucin expression of gastric intramucosal signet ring cell carcinoma and its background mucosa

Takayuki Seki[1)], Tateki Ito[2)], Hiroshi Kawachi[3)], Masaki Sekine[3)], Nobuaki Funata[4)], Touichiro Takizawa[1)]

1) Department of Molecular Pathophysiology, Graduated School of Allied Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan
2) Divisions of Pathology, National Medical Center of Japan, Tokyo, Japan
3) Department of Surgical Pathology, Tokyo Medical and Dental University, Tokyo, Japan
4)Department of Pathology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

The intramucosal lesion of gastric signet ring cell carcinoma (SIG) is known to form a layered structure (LS) that simulates mucin expression in ordinary gastric mucosa. In this study, we suspected the influence of background mucosa on the formation of LS and performed histopathological analysis. We examined 35 cases of intramucosal SIG with a maximum diameter of 30 mm or less. The LS patterns were classified into those with a layer of MUC6-positive cells (complete pattern, CP) and those lacking this layer (incomplete pattern, ICP). The relationship between LS patterns and the characteristics of the background mucosa, the expression of MUC2 (intestinal-type mucin antigen), MUC5AC (foveolar-type mucin antigen), and Ki-67 (the marker of cell proliferation activity) was examined by histochemistry and immunohistochemistry. Intestinal metaplasia in the background mucosa and MUC2 expression were frequently observed in cases with ICP. Ki-67-positive cells were much more and they were distributed more widely in the lesion of cases with ICP alone than in the other cases. Mucin expression and LS formation of gastric SIG are strongly influenced by its background mucosa. The cases completely lacking MUC6 expression may have higher malignant potential.

Key words: signet ring cell carcinoma, stomach, mucin expression, intestinal metaplasia, immunohistochemistry

Corresponding Author: Takayuki Seki
Affiliation; Department of Molecular Pathophysiology, Graduated School of Allied Health Sciences, Tokyo Medical and Dental University,1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8510, Japan
Tel; +81-3-5803-5371 Fax; +81-3-5803-5371
E-mail; sekimp@tmd.ac.jp
Received October 9: Accepted November 28, 2008

Introduction

Signet ring cell carcinoma (SIG) of the stomach is classified into the diffuse or undifferentiated types as described by Laurén and Nakamura et al. [1, 2], respectively. SIG is characterized by the presence of signet ring cells with nuclear dislocation due to intracytoplasmic mucin.

Since the concanavalin A-horseradish peroxidase method had been developed by Katsuyama et al. [3], pyloric-gland-type mucin can be distinguished from the other neutral types of mucin, including foveolar-type mucin. By this method, several authors have observed that mucin expression in the intramucosal lesions of SIG results in layered structure (LS) similar to those in ordinary gastric mucosa [4, 5, 6]. They reported that the LS comprised three layers: an upper layer composed of signet ring cells with a mucin type other than the pyloric-gland-type mucin, a middle layer composed of immature small round cells, and a lower layer composed of signet ring cells with pyloric-gland-type mucin. In particular, Akamatsu et al. [4] concretely

Table 1 Summary of cases

Age (years, mean ± S.D.)	55.8 ± 13.9
Sex (male: female)	14:21
Location (U:M:L)	0:20:15
Macroscopic type (elavated:flat:depressed)	0:3:32
Size (mm, mean ± S.D.)	18.7 ± 7.3

Table 2 Monoclonal antibodies for immunohistochemistry

Antibody (clone/manufacture)	Working dilution	Specificity [Reference]
Anti-MUC2 (Ccp58/Novocastra, Newcastle, UK)	1:100	Intestinal-type mucin [7]
Anti-MUC5AC (CLH2/Novocastra)	1:200	Foveolar-type mucin [8]
Anti-MUC6 (CLH5/Novocastra)	1:50	Pyloric-gland-type mucin [8]
Anti-Ki-67 (MIB-1/Dako, Copenhagen, Denmark)	1:100	Proliferating cell [9]

classified LS, termed "intramucosal laminated structure" in their paper, into complete, incomplete, and inverted patterns. Complete patterns comprised the three layers as mentioned above, incomplete patterns comprised two layers lacking the lower layer, and the inverted patterns also comprised two layers but lacked the upper layer. On the other hand, Sugihara et al. [6] reported that proliferating cancer cells were localized in the middle layer of the LS and that cases without LS tended to invade the submucosa or deeper layers.

No studies have elucidated the factors participating in LS formation. Although Akamatsu et al. [4] have reported that the degree of peripheral intestinal metaplasia was not correlated with the LS patterns, they did not present detailed data based on statistical analyses. Furthermore, other authors have not investigated the relationship between the LS and its background mucosa.

We hypothesize that mucin expression and LS formation of SIG reflects the characteristics of its background mucosa. In this study, we performed the histopathological analyses by using cases of intramucosal SIG to elucidate the relationship between LS and its background mucosa.

Materials and methods

Cases

We examined 35 cases of intramucosal SIG with a maximum diameter of 30 mm or less, resected at Tokyo Medical and Dental University Hospital and Tokyo Metropolitan Komagome Hospital. Cases with apparent tubular components were excluded. A summary of cases is presented in Table 1.

Monoclonal antibodies

We examined the expression of mucin-related glycoprotein (MUC2, MUC5AC, MUC6) and Ki-67 by immunohistochemistry. Mouse monoclonal antibodies used in this study and their working dilutions, specificity, and corresponding references [7, 8, 9] are listed in Table 2.

Histochemistry and Immunohistochemistry

To examine the relationship between the LS and its background mucosa in detail, all paraffin blocks containing the lesion were prepared (1–8 blocks per case; mean, 4.37 blocks per case; total, 153 blocks per 35 cases) and tissue sections of each block were subjected to all staining and evaluation procedures (153 sections). Formalin-fixed and paraffin-embedded tissue sections 4 μ m thick were prepared, dewaxed

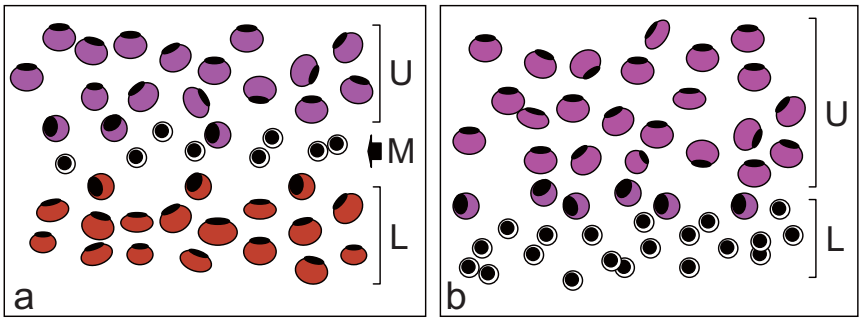

Fig. 1 Schema of the LS pattern. U, M, and L, upper, middle, and lower layers. (a) Complete pattern (CP). CP is a triple-layer pattern consisting of an upper layer of foveolar-type cells (indicated in purple), a middle layer of immature-type cells, and a lower layer of gland-type cells (indicated in brown). (b) Incomplete pattern (ICP). ICP is a double-layer pattern comprising an upper layer of foveolar-type cells (indicated in purple), and a lower layer of immature-type cells.

in xylene, rehydrated by a series of graded ethanol, and stained with hematoxylin and eosin. Similarly, the adjacent serial sections were subjected to alcian blue (pH 2.5)-periodic acid Schiff (AB-PAS) staining [10] and immunohistochemistry. Immunohistochemistry was performed as follows: dewaxed and rehydrated tissue sections were autoclaved (121 ° C for 20 min) in 0.01 M citrate buffer (pH 6.0) for antigen retrieval. After the sections were rinsed with 0.05 M phosphate-buffered saline (PBS), endogenous peroxidase was blocked with 3% H_2O_2 in ethanol for 20 min. After being rinsed with PBS, the sections were treated with Block Ace (Dainippon Sumitomo Pharma, Osaka, Japan) for 15 min to block nonspecific reactions and then incubated with one of the primary antibodies in a humid chamber overnight at 4°C. After being rinsed with PBS, the sections were incubated with peroxidase-labeled secondary antibody (Histofine MAX-PO (MULTI) kit, Nichirei, Tokyo, Japan) for 30 min. The bound antibody complex was visualized as brown by reaction in 3,3'-diaminobenzidine (Histofine DAB, Nichirei) for 20 min. After being washed with running water, the sections were then continuously stained by AB-PAS. Subsequently, the sections were lightly counterstained with Mayer's hematoxylin.

Evaluation of LS and classification of cases

To evaluate LS, cancer cells were classified into three types as follows: cells in the superficial area of the mucosa and with abundant mucin were classified as foveolar-type cells. Cells in the deeper area and whose mucin was immunohistochemically stained for MUC6 were classified as gland-type cells. Cells with low amounts of mucin representing a high nuclear-cytoplasmic ratio were classified as immature-type cells. Further, based on the distribution of each cell type, the LS was classified into complete pattern (CP) and incomplete pattern (ICP), according to the classification by Akamatsu et al. [4]. The CP is a triple-layer pattern (comprising an upper layer of foveolar-type cells, a middle layer of immature-type cells, and a lower layer of gland-type cells). The ICP is a double-layer pattern lacking the gland-type cell layer (comprising an upper layer of foveolar-type cells and a lower layer of immature-type cells). The schemas of each LS pattern are shown in Fig. 1. Subsequently, we classified all the cases into one of three groups based on the pattern and degree of LS distribution: those with CP alone (group A), both CP and ICP (group B), and with ICP alone (group C).

Evaluation of MUC2 and MUC5AC expression

Positive staining for MUC2 was graded as diffuse (2+: greater than 90% of cells), sporadic or focal (1+: greater than 10% of cells), or negative (–: 10% or less of cells). The staining results for MUC5AC were regarded as positive when positive cells were diffusely observed in the lesion.

Evaluation of Ki-67-positive cancer cell localization in the LS and the Ki-67 index

It has been reported that proliferating cancer cells

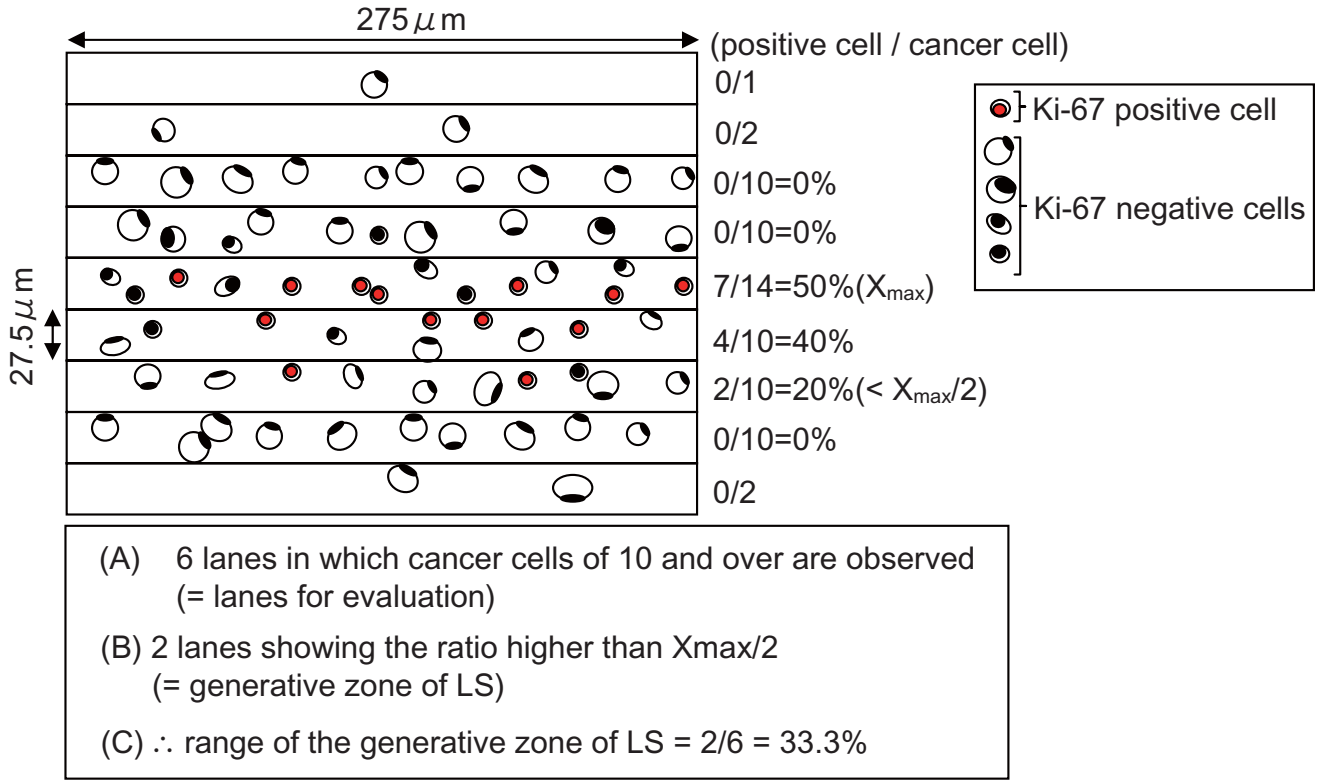

Fig. 2 Identification of the generative zone of LS and calculation of the range. The schema indicates the LS of the intramucosal lesion (complete pattern in this schema). Ki-67-positive cancer cells are indicated in red and Ki-67-negative ones are indicated in black. The frame enclosing LS represents the outer frame of the square micrometer. The horizontal lines in the frame represent those of the square micrometer, which are drawn at intervals of 27.5 μ m. (A) The horizontal lanes, in which cancer cells of 10 cells and over were observed, were regarded as the lanes for evaluation. In this schema, there are 6 lanes. (B) The maximum ratio of the positive cells/total cancer cells in a lane was represented as Xmax (50% in this schema). Lanes representing the ratio higher than Xmax/2 were regarded as the generative zone of LS (2 lanes in this schema). (C) The ratio of the generative zone of LS/lanes for evaluation was regarded as the range of the generative zone of LS (2/6 = 33.3% in this schema).

are localized in the middle layer of the LS [6]. However, this localization was observed only in case of the triple-layer pattern or CP in this study. To investigate whether proliferating cells are localized not only in CP but also in ICP and whether there are any differences of localization between the abovementioned groups, we used sections immunohistochemically stained for Ki-67 for an original evaluation. An example of this evaluation method is presented in Fig. 2. Initially, we selected foci in which the LS layers were the most clearly defined in the section observed for evaluation. Both CP and ICP in the lesions of groups B were selected for evaluation. Secondly, the focus was restricted to a width of 275 μ m by using a square micrometer (20.4-#10/10 × 10, Olympus, Tokyo, Japan). Subsequently, we enumerated both Ki-67-positive and Ki-67-negative cancer cells in the area and calculated the range of the "generative zone of LS" as illustrated in Fig. 2. In addition, the Ki-67 index of total cancer cells in the area was also calculated.

Statistical analysis

Statistical analysis was performed using Kyplot 4.0 (KyensLab Inc., Tokyo, Japan). The data of sex, location, macroscopic type, histological location, and intestinal metaplasia in background mucosa was analyzed by the chi-square test. One of age and size was analyzed by Mann-Whitney U test. And the data of MUC2 expression was analyzed by Kruskal-Wallis test. Probability values of less than 0.05 were regarded as significant.

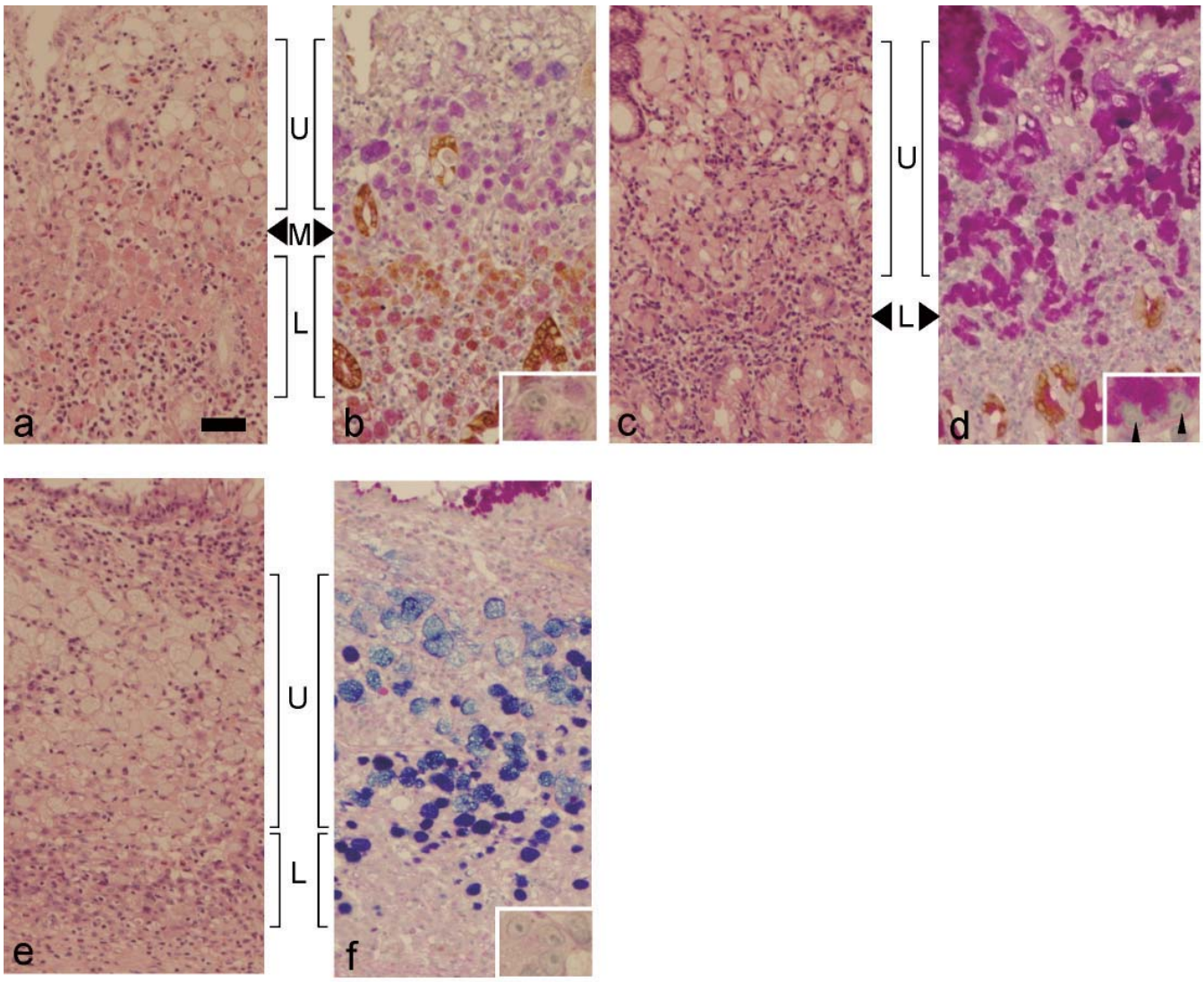

Fig. 3 Histological appearance of LS. U, M, and L, upper, middle, and lower layers. (a, b) Complete pattern. The inset illustrates immature-type cells in the middle layer. (a: H.E.; b: anti-MUC6 + AB-PAS) (c, d) Incomplete pattern in group B. The inset illustrates immature-type cells (arrowheads) in the lower layer. (c: H.E.; d: anti-MUC6 + AB-PAS,) (e, f) Incomplete pattern in group D. The inset illustrates immature-type cells in the lower layer. (e: H.E.; f: anti-MUC6+AB-PAS) Scale bar: 50 μ m

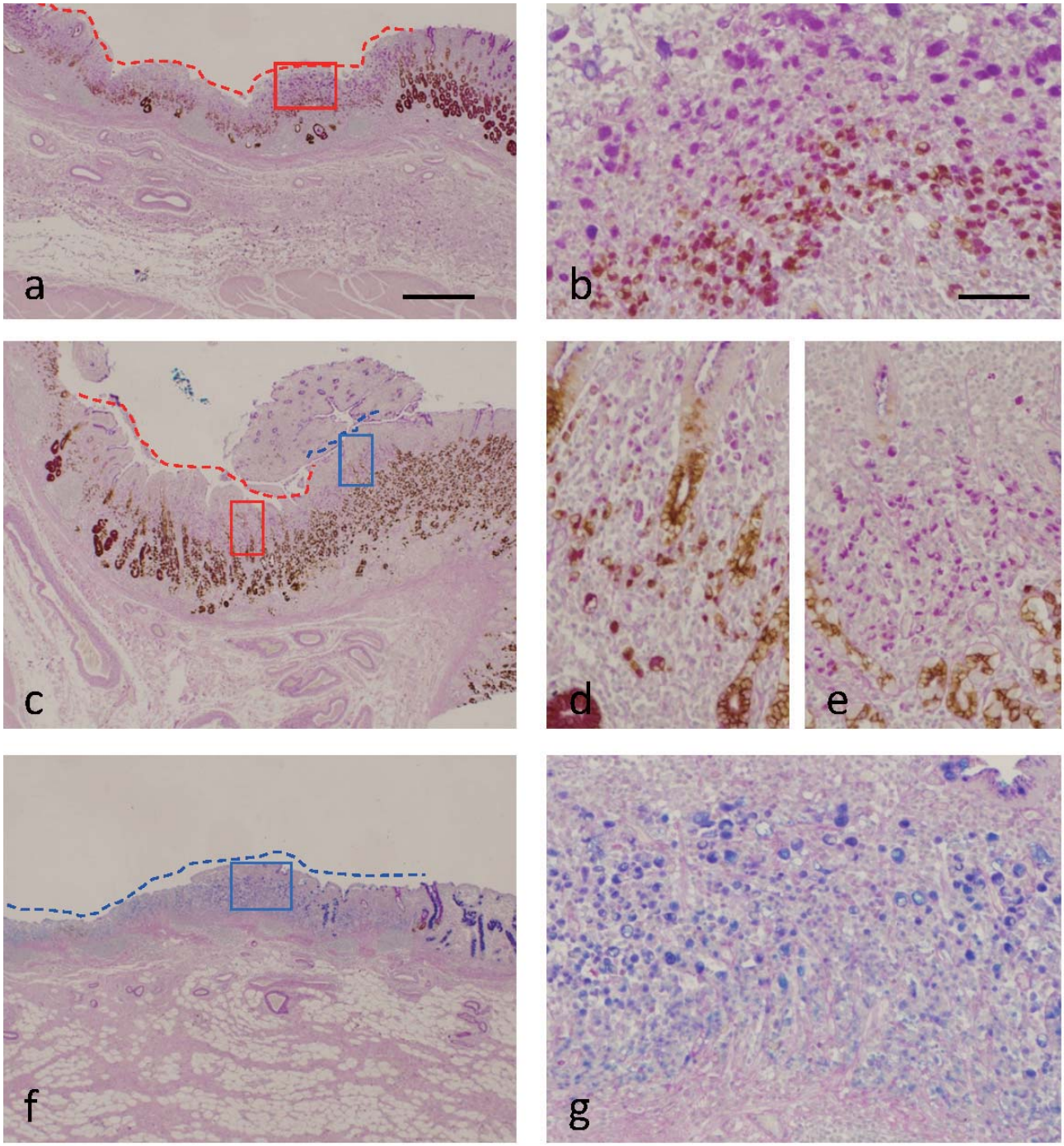

Fig. 4 Representative cases from each group. The red dotted lines indicate the extent of complete pattern. The blue dotted lines indicate the extent of incomplete pattern. (a) A case in group A (anti-MUC6 + AB-PAS). (b) High-power magnification of the region enclosed by the red square in a. (c) A case in group B (anti-MUC6 + AB-PAS). (d) High-power magnification of the CP enclosed by the red square in c. (e) High-power magnification of the ICP enclosed by the blue square in c. (f) A case in group C. (anti-MUC6 + AB-PAS) (g) High-power magnification of the region enclosed by the blue square in f. (a,c,f), Scale bar: 300 μ m. (b,d,e,g), Scale bar: 50 μ m.

Table 3 Histological and cytological features of ICP

	ICP in	
	group B	group C
Position in the mucosa	Middle to superficial	Entire mucosal layer
Thickness of the lower layer	Thin	Thick
Mucin properties of most cancer cells	PAS positive	Alcian blue positive

ICP, incomplete pattern; PAS, periodic acid-Schiff

Table 4 Clinicopathological features

	group A (n=14)	group B (n=17)	group C (n=4)
Age (years, mean ± S.D.)	58.4 ± 14.6	54.1 ± 13.8	54.0 ± 13.6
Sex (male: female)	6:8	6:11	2:2
Location (U:M:L)	0:9:5	0:9:8	0:2:2
Macroscopic type (flat:depressed)	2:12	1:16	0:4
Size (mm, mean ± S.D.)	15.6 ± 7.2[a]	20.9 ± 5.8[a]	20.3 ± 11.2

U, M, and L, upper, middle, and lower one-third of the stomach. [a]P = 0.0438

Table 5 Histological location of the lesion and intestinal metaplasia in background mucosa

	group A (n=14)	group B (n=17)	group C (n=4)
Histological location of the lesion			
Fundic gland area	11	10	3
Intermediate zone	0	2	0
Pyloric gland area	3	5	1
IM in background mucosa (%)[a]	4 (29)	11 (65)	4 (100)

IM, intestinal metaplasia. [a]P = 0.0198

Results

Histological appearance of LS and classification of cases

There were a few immature-type cells in the CP due to which the middle layer was thin (Fig. 3a, b). The middle layer was frequently localized at the neck region of the mucosa. The features of the CP were almost identical in groups A and B. In contrast, certain differences in the features of ICP were observed between the groups. A majority of the ICP in group B were positioned from the middle to the superficial areas of the mucosa. The ICP had thin immature-type cell layers, which were frequently localized at the neck region similar to those in CP (Fig. 3c, d). On the other hand, a majority of the ICP in group C comprised the entire mucosal layer. Moreover, they had signet ring cells with mucin that could be stained by alcian blue, and thick lower layers, which was distributed from the bottom to the middle areas of the mucosa (Fig. 3e, f). The differences in the features of ICP between the groups are summarized in

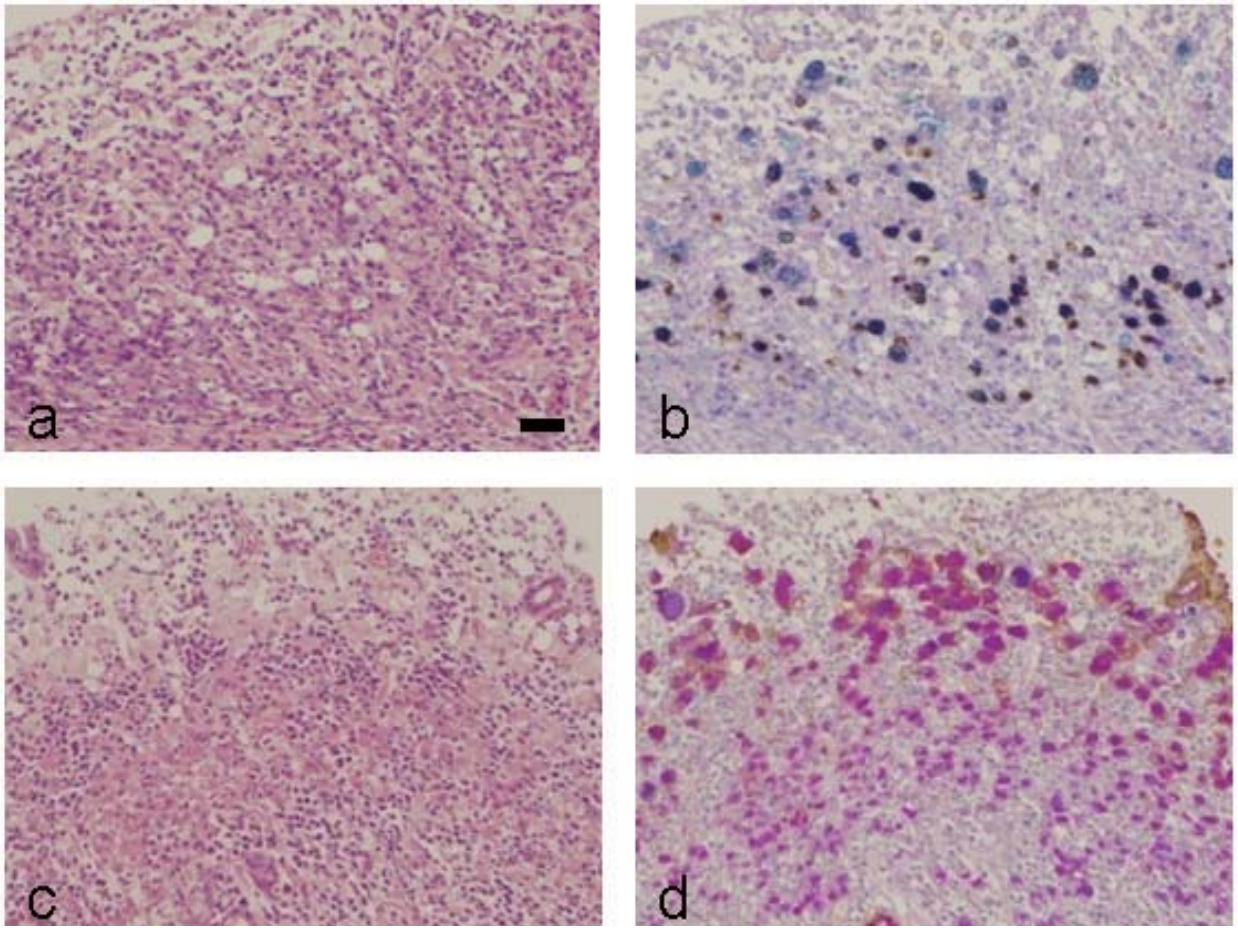

Fig. 5 MUC2 and MUC5AC expression. (a, b) Lesion with MUC2 expression. (a: H.E.; b: anti-MUC2 + AB-PAS) (c, d) Lesion with MUC5AC expression. (c: H.E.; b: anti-MUC5AC + AB-PAS) Scale bar: 50 μ m.

Table 3.
Of all the 35 cases, fourteen (40%), seventeen (49%), and four (11%) cases were classified into groups A, B, and C respectively. The representative cases from each group are shown in Fig. 4.

Relationship between the clinicopathological features and groups

The clinicopathological features of each group are summarized in Table 4. The size of group A cases was smaller than that of group B ones (P = 0.0438). There was no significant correlation between the other clinicopathological features and groups.

Histological location and intestinal metaplasia in background mucosa (Table 5)

We focused on and evaluated the factors related to the background mucosa of the lesion, namely, the histological location (the fundic gland area, intermediate zone, and pyloric gland area) and intestinal metaplasia in the background mucosa. There was no significant difference in the histological location between groups. The number of cases with intestinal metaplasia was four (29%) cases of group A, eleven (65%) of group B, and four (100%) of group C (P = 0.0198).

MUC2 and MUC5AC expression

Immunohistochemistry for MUC2 revealed that most MUC2-positive cancer cells were stained by alcian blue (Fig. 5a, b). MUC2 expression was observed in four (29%) cases of group A, thirteen (76%) of group B, and four (100%) of group C. Furthermore, in three of four cases in group C, MUC2 expression was diffuse in the lesion. There was a significant difference in the degree of MUC2 expression between groups (P = 0.0006; Table 6). Immunohistochemistry for MUC5AC

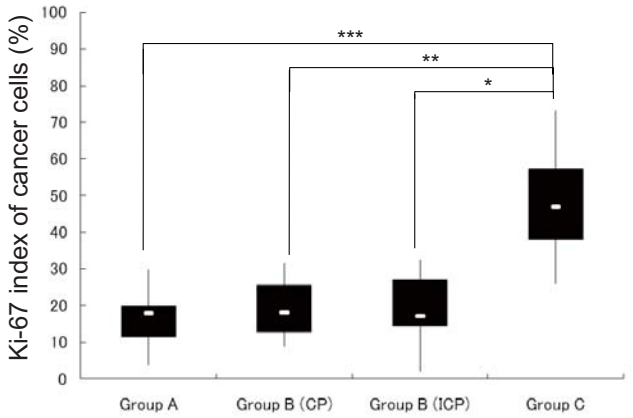

Fig. 6 Ki-67 index of cancer cells. The box indicates interquartile range. The vertical bar indicates the difference between the minimum and maximum ratios. The horizontal bar indicates the median. *P = 0.0107, **P = 0.0063, ***P = 0.0049; Mann-Whitney U test.

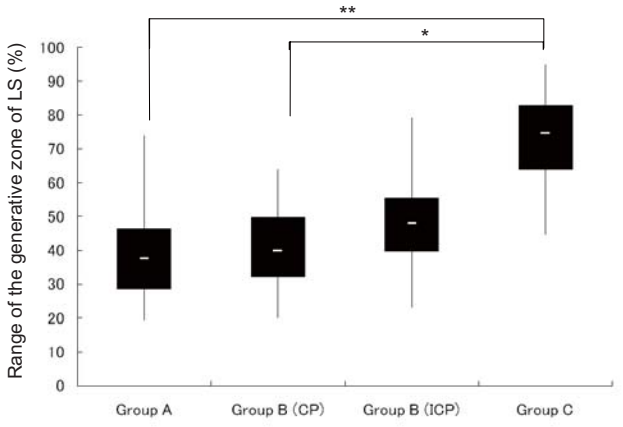

Fig. 7 Range of the generative zone of LS. The box indicates interquartile range. The vertical bar indicates the difference between the minimum and maximum ratios. The horizontal bar indicates the median. *P = 0.0224, **P = 0.0224; Mann-Whitney U test.

Table 6 Degree of MUC2 expression in lesions of each group

	Number (%) of cases in		
	group A (n=14)	group B (n=17)	group C (n=4)
-	10 (71)	4 (24)	0 (0)
1+	4 (29)	13(76)	1 (25)
2+	0 (0)	0 (0)	3 (75)

revealed that MUC5AC-positive cancer cells were distributed in the superficial area in the lesions, which corresponded to the upper layer of the LS (Fig. 5c, d). MUC5AC expression was observed in all cases with one exceptional case of group B.

Ki-67 index and range of the generative zone of LS

In the CP of groups A and B, only a few Ki-67-positive cells were present, which were localized in the middle layer of immature-type cells. In a majority of the ICP in group B, similar to CP, a few Ki-67-positive cells were present, and these cells were localized in the lower layer of immature-type cells. On the other hand, in most ICP lesions of group C, although positive cells mainly aggregated in the lower layer, they were also distributed widely from the bottom to the middle or to the superficial areas of the LS. The Ki-67 index of cancer cells was higher in group C than those in the other groups (Fig. 6). Furthermore, the range of the generative zone of LS was also higher in group C than those in the other groups (Fig. 7).

Discussion

In this study, we performed histopathological analyses to elucidate the influence of background mucosa on the mucin expression and LS formation of gastric intramucosal SIG. We obtained the results which suggest that SIG may express gastric-type or intestinal-type mucin corresponding to its background mucosa. We also obtained the results which suggest that the cases completely lacking the differentiation into gland-

type cells may have higher malignant potential.

Although Akamatsu et al. [4] attempted to confirm the relationship between the LS patterns and their background mucosa as we did in this study, they did not detect any significant correlation. In this study, we evaluated not only the section showing maximum diameter but also all lesion-containing sections. Therefore, these results may considerably differ from those of Akamatsu et al., and this method enabled us to more precisely evaluate the relationship between the LS patterns and their background mucosa.

We classified the LS into the CP and ICP. CP was composed of three layers including a layer of MUC6-positive cells, whereas ICP was composed of two layers without the MUC6-positive cell layer. The upper, middle, and lower layers of CP correspond to the superficial layer of foveolar epithelium, neck region of immature cells, and the glandular cell layer in ordinary gastric mucosa, respectively. Therefore, CP appears to precisely simulate the ordinary gastric mucosa. On the other hand, in ICP, cancer cells of the upper and lower layers may correspond to the foveolar epithelium of the superficial layer and immature cells of the neck region, respectively. However, the distribution of cancer cells in the mucosa differed among the ICP. In other words, certain ICP structures were positioned from the middle to the superficial areas of the mucosa with non-neoplastic gastric glands, while others were entire mucosal layers without non-neoplastic glands. The former ICP structures were mainly observed in group B whereas the latter, mainly observed in group C. The difference between the two is the existence of non-neoplastic glands. ICP structures without non-neoplastic glands may reflect atrophic changes in the background mucosa with loss of proper gastric glands.

Among the groups, the frequency of cases with intestinal metaplasia in the background mucosa was the lowest in group A and the highest in group C. These results are consistent with the relationship between LS and the background mucosa. The lesions of group A may simulate their background mucosa, which primarily comprises gastric mucosa without intestinal metaplasia. The lesions of groups B and C may also simulate their background mucosa; however, this mainly comprises intestinal metaplasia. Therefore, cancer cells in the lesions of groups B and C may result in the paucity or absence of the differentiation into gland-type cells. Although the lesions of group A may be influenced by intestinal metaplasia in the background mucosa, the degree of influence appears weaker than that in groups B and C.

MUC2 glycoprotein, also known as the intestinal-mucin related protein antigen, is a major colonic apomucin and is known to be expressed in goblet cells, including metaplastic cells in the stomach and other parts of the alimentary tract [7, 11, 12]. MUC2 expression was observed more frequently in groups B and C than in group A. In addition, in three of four cases in group C, MUC2 was diffusely expressed in the lesion. Cases of group C appear to have almost identical properties as those of intestinal metaplasia. Yamachika et al. [13] reported that the phenotype of gastric SIG changes from the gastric to the intestinal type with progression. On the other hand, Bamba et al. [14] reported that the intestinal phenotype is time dependent (size dependent) and plays a neutral role in the progression of SIG. Although there were differences between the progression-dependent or time-dependent phenotypes, these reports indicate that the intestinal phenotype of SIG is expressed as the tumor develops. The results in this study, however, suggest that certain cases such as those in groups B and C may express the intestinal phenotype regardless of tumor size or progression. Of the three cases with diffuse MUC2 expression, the minimum tumor was 5 mm in maximum diameter. Although further minute cases have to be prepared to confirm these details, SIG may express gastric-type or intestinal-type mucin corresponding to its background mucosa in the incipient phase.

MUC5AC glycoprotein was generally recognized as the foveolar-type mucin antigen [8]. Almost all the MUC5AC-positive cells were foveolar-type cells and were present in the upper layer. This indicates that they definitely possess the same cytological properties as the foveolar epithelium. MUC5AC expression was observed in almost all cases, regardless of groups. The gastric phenotype of SIG may be maintained well regardless of either the LS patterns or the expression of intestinal phenotype.

Ki-67-positive cells in most lesions of groups A and B were localized in the immature-type cell layers and there were a few positive cells. On the other hand, in most lesions of group C, positive cells were widely distributed from the bottom to the middle or to the superficial areas of the LS. These results suggest that the proliferation of cancer cells may be related to the differentiation into gland-type cells. Cases of group C may grow more rapidly and consequently have higher malignant potential than those of the other groups. Complete absence of the differentiation into gland-type cells may be a predictable factor of the high malignant potential of SIG.

Acknowledgements

We thank Yoshiyuki Kasuga, and the staffs of both Tokyo Medical and Dental University Hospital and Tokyo Metropolitan Komagome Hospital for technical assistance.

References

1. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma. APMIS 1965;64:31-49
2. Nakamura K, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann 1968;59:251-258
3. Katsuyama T, Spicer SS. Histochemical differentiation of complex carbohydrates with variants the Concanavalin A-horseradish peroxidase methods. J Histochem Cytochem 1978;26:233-250
4. Akamatsu T, Katsuyama T. Histochemical demonstration of mucins in the intramucosal laminated structure of human gastric signet ring cell carcinoma and its relation to submucosal invasion. Histochem J 1990;2:416-425
5. Katsuyama T, Tsukahara M, Nasu T. Application of paradoxical concanavalin A staining to gastrointestinal pathology; II. Mucosubstance histochemistry of human gastric carcinoma. Igakunoayumi 1979;111:156-158 (in Japanese)
6. Sugihara H, Hattori T, Fukuda M, et al. Cell proliferation and differentiation in intramucosal and advanced signet ring cell carcinomas of the human stomach. Virchows Arch 1987;411:117-127
7. Xing PX, Prenxoska J, Layton GT, et al. Second-generation monoclonal antibodies to intestinal MUC2 peptide reactive with colon cancer. J Natl Cancer Inst 1992;84:699-703
8. Reis CA, David L, Correa P, et al. Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. Cancer Res 1999;59(5):1003-1007
9. Cattoretti G, Becker MHG, Key G, Duchrow M, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB-1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992;168:357-63
10. Spicer SS, Horn RG, Leppi TJ. Histochemistry of connective tissue mucopolysaccharides. In: Wagner BM, Smith DE (eds) The Connective Tissue. Williams and Wilkins, Baltimore, 1967;pp 251-303
11. Blank M, Klussmann E, Krüger-Krasagakes S, et al. Expression of MUC2-mucin in colorectal adenomas and carcinomas of different histological types. Int J Cancer 1994;59:301-306
12. Gum JR Jr, Byrd JC, Hicks JW, et al. Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphism. J Biol Chem 1989;264:6480-6487
13. Yamachika T, Inada K, Fujimitsu Y, et al. Intestinalization of gastric signet ring cell carcinomas with progression. Virchows Arch 1997;431:103-110
14. Bamba M, Sugihara H, Kushima R, et al. Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the stomach. Virchows Arch 2001;438:49-56