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Morphological changes in human gastric tumours after eradication therapy of Helicobacter pylori *in a short-term follow-up*

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SUMMARY

Background: It is controversial as to whether the development of gastric cancer is influenced by *Helicobacter pylori* eradication. If eradication itself influences the tumour morphology, this may affect the tumour discovery rate.

Aim: To investigate the morphological changes in the gastric neoplasm after *H. pylori* eradication.

Methods: We studied 37 patients with eradication therapy. After a 1-month follow-up, endoscopic re-evaluation was performed and the appearance was compared with first image. All lesions were resected endoscopically, and were subjected to histological assessment and to immunohistochemistry. Serum gastrin levels were determined before and after eradication.

Results: Twenty-nine of 37 patients underwent successful eradication. The appearance of 11 lesions (33% of 33 lesions) became indistinct after successful eradication. All lesions were of the superficial-elevated type and the height of the lesions decreased. We detected normal columnar epithelium over the neoplasm in eight of the lesions. Higher expression of single-stranded deoxyribonucleic acid in the deep area was characteristic in tumours with an indistinct appearance. These changes did not correlate with the serum gastrin levels.

Conclusions: The morphology of the gastric neoplasm change after eradication in the short-term. This may contribute to the decreased tumour discovery rate.

INTRODUCTION

Helicobacter pylori plays an important role in the promotion of atrophic gastritis.[1] Long-term infection of *H. pylori* results in glandular atrophy and intestinal metaplasia. It has been accepted that there is a strong association between *H. pylori*-associated gastritis and gastric cancer.[2–4] Uemura *et al.* clearly demonstrated that gastric cancer developed only in patients with *H. pylori* infection by prospective study.[5] *Helicobacter pylori* eradication therapy is widely accepted as a prevention of peptic ulcer. We have previously demonstrated that the extent of gastric atrophy and intestinal metaplasia improved in some cases after successful eradication therapy.[6] Severe gastric atrophy induced by *H. pylori* is thought to be an important risk factor in the development of gastric carcinoma; therefore, it is speculated that control of histological gastritis is linked to the control of gastric cancer developments. Indeed, Uemura *et al.* had reported that eradication therapy of *H. pylori* decreased the occurrence of second gastric cancer in patients with pre-treated gastric cancer by

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endoscopic mucosal resection.[7] We also found a low Ki-67 labelling index in gastric cancer cells in *H. pylori*-negative gastric cancer tissue compared with *H. pylori*-positive tissue, suggesting that *H. pylori* has a growth promoting role on gastric cancer cells.[8] However, it is still controversial as to whether eradication therapy of *H. pylori* diminishes the incidence of gastric cancer.

One of the difficulties of this field seems to be based on the methodology used to evaluate the gastric carcinogenesis. Researchers can evaluate the degree of carcinogenesis only by the discovery rate of gastric cancer by endoscopic examination. Due emphasis must be placed on the differences in diagnostic ability of each examination. Moreover, endoscopic morphology might be influenced directly by eradication therapy, this affecting the discovery rate of gastric cancer.

In the present study, we focused on the morphological changes in gastric neoplasms after the eradication therapy with a short-term follow-up study. We then examined the histological and molecular biological changes induced by eradication therapy, and discussed the clinical implication.

METHODS

Patients

Thirty-eight patients with gastric neoplasm (27 men, mean age: 69.1 year old) were included in this study, and 45 lesions (28 gastric carcinomas and 17 gastric adenoma) were studied. All patients received an endoscopic examination and the endoscopic features were recorded in a database. No patients who had undergone gastrectomy were included in the study. All patients had histological gastritis in both corpus and antrum and were confirmed as being *H. pylori*-positive by rapid urease test (PyloriTek, Serim Research, Elkhart, IN, USA), Giemsa staining, ^{13}C-urea breath test (UBT; Otsuka UBiT-IR200, Tokushima, Japan) or the presence of serum IgG antibodies against *H. pylori* (E-plate, Eiken, Tokyo, Japan). Patients were considered as *H. pylori*-positive if at least two of them were positive. After diagnosis of the *H. pylori* infection, all patients received eradication therapy by the use of a proton-pump inhibitor (lansoprazole 60 mg, twice daily), amoxicillin (1500 mg, twice daily) and clarithromycin (400 mg, twice daily) for 1 week. The successful clearance of *H. pylori* was judged more than 4 weeks later by UBT or the *H. pylori* stool antigen test (Meridian Diagnostics, Cincinnati, OH, USA). A second endoscopic observation was performed prior to endoscopic mucosal resection (average 33.9 days) of the gastric tumour. From the patients we received written informed consent and the Ethical Committee of Hiroshima University approved our protocol.

Evaluation of endoscopic findings

First, endoscopic pictures were saved in the database. Secondly, endoscopic observations were performed using the same endoscopic system and saved in the same manner. Later, the pictures were printed out and three specialists judged the alterations of endoscopic appearance independently, unaware of the clinical information including the evaluation of the eradication therapy. They evaluated the endoscopic changes concerning: (i) difficulties to point out the tumour itself or its margin (whether tumour became indistinct or not), (ii) tumour height or depth, (iii) tumour surface and (iv) the degree of redness in background mucosa. If more than two specialists recognized the finding, we regarded it as being significant.

Determination of serum pepsinogen and gastrin levels

Fasting serum was collected from all patients. The samples were centrifuged immediately at 4 °C and stored at −20 °C until use. Serum concentrations of pepsinogens (PGs) and gastrin were determined by enzyme-linked immunosorbent assay and modified radioimmunoassay.[9]

Immunohistochemistry

About 4-μm sections of formalin-fixed paraffin-embedded tissues were used for immunohistochemical staining. After deparaffinization and hydration, internal peroxidase was blocked by incubating with 0.3% H_2O_2 in methanol for 15 min. After incubation with 5% skim milk/phosphate-buffered saline (PBS) for 20 min, the sections were reacted with the primary antibody (diluted with PBS) for 2 h at room temperature. The primary antibodies used were anti-single-stranded DNA (ssDNA) polyclonal antibody (dilution of 1:300; Dako, Kyoto, Japan),[10] and antihuman Ki-67 antigen (MIB-1, dilution of 1:100; Dako).[8] We performed the immunostaining using an LSAB2 kit (Dako). Antigen retrieval was carried out with microwave

treatment before reacting with anti-Ki-67. Strong signals in the nuclei of the epithelial cells were taken to be positive result.

Statistics

Results are reported as mean ± s.d. Statistical analysis was performed by chi-square test with STATVIEW software (SAS Institute Inc., Cary, NC, USA). A *P*-value of <0.05 was considered statistically significant.

RESULTS

Clinical features of patients and changes in endoscopic findings

Following initial enrolment of 38 patients, one patient dropped out of this protocol because of a suspicious tumour invasion into the submucosal layer, which was followed by an operation. Therefore, 37 patients with 44 lesions (27 carcinomas and 17 adenomas) were finally enrolled. *Helicobacter pylori* eradication therapy succeeded in 29 patients (78%) with 33 lesions. The clinical features are summarized in Table 1. In 11 of the lesions, we found that the presence of the lesion came to be indistinct compared with the primary image (Table 1). All of these lesions were found in patients who underwent successful eradication therapy and no lesions in the cases of failed eradication showed this alteration.

Table 1. Clinical features of patients and alterations of tumour findings

	Eradicated (n = 29)	Non-eradicated (n = 8)	*P*-value
Clinical features			
Mean age (range)	69.8 (48–84)	69.3 (54–78)	N.S.
Gender (male/female)	19/10	8/0	N.S.
Period (days)	33.3	36.0	N.S.
Lesions			
Number	33	11	
Elevated/depressed	20/13	3/8	N.S.
Tumour diameter (mm, mean ± s.d.)	15.9 ± 5.6	13.1 ± 10.7	N.S.
Carcinoma/adenoma	21/12	6/5	N.S.
Endoscopic change			
Indistinct	11 (33%)	0 (0%)	**0.03**[1]

[1] Chi-square test.

Table 2. Clinicopathological features of 33 gastric tumours with successful eradication; comparison between adenoma and carcinoma

	Adenoma (n = 12)	Carcinoma (n = 21)	*P*-value
Tumour features			
Elevated/depressed	11/0	9/13	
Mucosal/submucosal	12/0	21/0	
Diameter (average; mm)	11.5	18.5	
Endoscopic alterations			
Indistinct	6 (50%)	5 (24%)	0.12[1]

[1] Chi-square test.

Comparison between adenoma and carcinoma

We compared the endoscopic alteration in patients with gastric adenoma and in those with adenocarcinoma. The clinicopathological features of patients with the adenomas and carcinomas were summarized in Table 2. All adenocarcinoma tissues were confirmed histologically to be limited in the mucosal layer. We could find the endoscopic alteration not only in six adenomas but also in five carcinomas (Table 2). The representative endoscopic features were demonstrated in Figures 1 and 2. After eradication, the tumours became flattened and indistinct, and it was difficult to point out the tumour itself or to set the clear horizontal margin of the tumours. Although this alteration was frequently detected in adenoma tissue, we could not find the statistical difference in the endoscopic change of tumours between two groups.

Characteristics of the lesions that became unclear after eradication

We tried to clarify the characteristics of the lesions that became indistinct after successful eradication therapy. As shown in Table 3, this phenomenon was characteristically found in elevated lesions. Moreover, a flattened appearance had a close association with the incidence of unclear change. Although it is well-known that the redness of the background mucosa often diminishes after eradication therapy, it was not associated with the indistinct appearance.

Changes in histological findings by eradication

We then examined the histological features using sections taken from the endoscopic resection stained

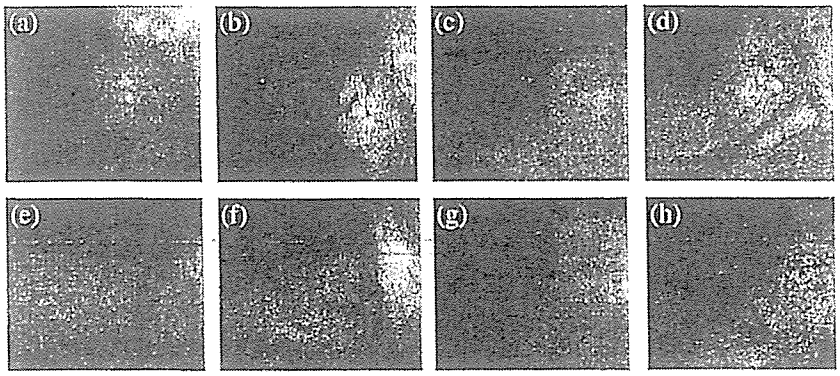

Figure 1. Endoscopic features of the gastric adenoma at pre- (a–d) and post-eradication therapy (e–h). Patients were 71 years female (a, b, e, f) and 67 years male (c, d, g, h). Ordinary (a, c, e, g) and dye-endoscopic (b, d, f, h) observation. Tumours became flattened and indistinct after eradication therapy.

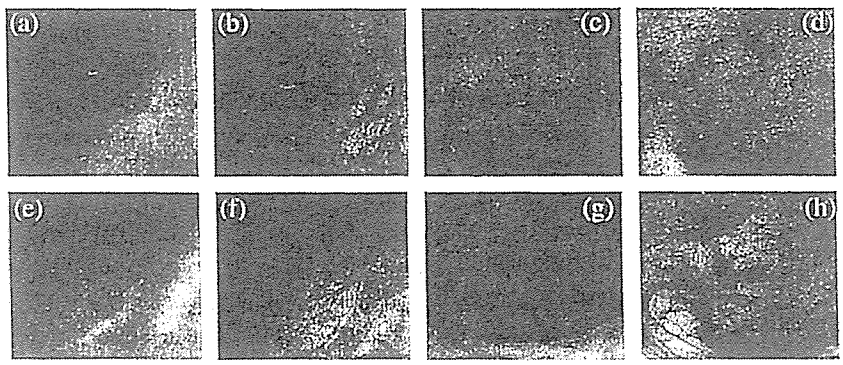

Figure 2. Endoscopic features of the gastric adenocarcinoma at pre- (a–d) and post-eradication therapy (e–h). Patients were 64 years male (a, b, e, f) and 75 years male (c, d, g, h). Ordinary (a, c, e, g) and dye-endoscopic (b, d, f, h) observation. Tumours became flattened and indistinct after eradication therapy as seen in cases with adenoma.

Table 3. Characteristics of the lesions, which became indistinct after successful eradication therapy

	Indistinct (n = 11)	No change (n = 22)	P-value
Tumour features			
Elevated/depressed	11/0	9/13	<0.01[1]
Carcinoma/adenoma	5/6	16/6	0.12[1]
Endoscopic alterations			
Flattened	11 (100%)	2 (9%)	<0.01[1]
Diminished redness	6 (55%)	6 (27%)	0.12[1]
Histological alterations			
Normal columnar			
Epithelium over the tumour	8 (73%)	3 (14%)	<0.01[1]
Serum pepsinogens (pre-eradication)			
PG I (ng/mL, mean ± s.d.)	33.0 ± 23.9	30.0 ± 25.5	N.S.
PG II (ng/mL, mean ± s.d.)	18.2 ± 7.9	19.2 ± 11.9	N.S.
PG I/II (mean ± s.d.)	1.73 ± 0.97	1.49 ± 0.87	N.S.

[1] Chi-square test.

with haematoxylin and eosin. We could detect the appearance of normal columnar epithelium to various degrees over the tumour tissue (Figure 3) in 12 lesions. Of 12, 11 were found in patients who underwent successful eradication therapy. Especially, in three cases, the atypical epithelium covers the bulk of the tumour tissue. This change was found not only in adenoma (nine lesions) but also in carcinoma tissue (three lesions) and showed a close association with the endoscopic finding of unclear margin (Table 3).

Serum pepsinogen levels and endoscopic alteration

Sera from patients were collected before eradication therapy and the serum level of PGs was estimated. We examined the relationship between serum levels of PGs

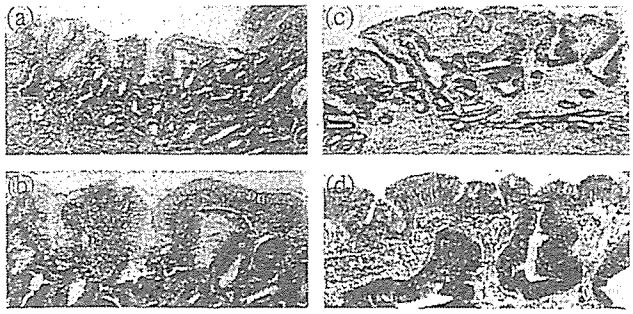

Figure 3. Histological features of gastric neoplasm at posteradication. Cases of gastric adenoma (a, b) and adenocarcinoma (c, d) with successful eradication therapy. (a, c) Low magnification of gastric tumour specimen, (b, d) high magnification of surface epithelium. Patients were 71 years female (a, b) and 82 years male (c, d).

and the endoscopic findings. As shown in Table 3, relative high levels of PG I and a high I/II ratio were found in patients with endoscopic changes compared to those in the patients with no-changes; however, this is not statistically significant.

Relationship between serum gastrin level and endoscopic changes

Further to this, we studied the alteration of serum gastrin levels. Fasting sera were collected before and after eradication therapy. As shown in Table 4, a decrease of the serum gastrin level was not so obvious after 1 month of eradication therapy. We could not find a difference in the level of gastrin between patients with indistinct tumour appearance and those with no change.

Expressions of Ki-67 and ssDNA in tumour cells

We examined the cell kinetics in these lesions using immunohistochemical staining with the use of tumour specimens at posteradication. We could not detect any

Table 4. Changes in serum gastrin levels after eradication therapy

		Gastrin level (pg/mL, mean ± s.d.)	
	Number	Before eradication	After eradication
Successful eradication			
Indistinct	10	322.1 ± 356.0	296.1 ± 314.3
No change	16	309.9 ± 271.1	211.0 ± 173.1
Failed eradication	7	176.8 ± 162.5	245.6 ± 279.5

Table 5. Expressions of Ki-67 and ssDNA in gastric tumour cells

	Number	Ki-67 LI (%)	ssDNA LI (%) (deep area dominant)
Successful eradication†			
Indistinct	6	17.5 ± 16.3	46.2 ± 13.2 **(3/6)***
No change	14	15.4 ± 12.9	45.9 ± 20.8 **(0/14)***
Failed eradication†	7	9.0 ± 5.5	40.3 ± 27.6 (0/7)

LI, labelling index; ssDNA, single-stranded deoxyribonucleic acid.
* $P < 0.05$, chi-square test.
†Mean ± s.d.

difference in the Ki-67 labelling index, which is a marker for cell proliferation, or the ssDNA labelling index, which is a marker for cell apoptosis (Table 5).[11] However, in cases where there were indistinct tumour appearance after eradication, ssDNA expression was more frequently detected from deeper within the tumour at posteradication (Figure 4, Table 5). In other specimens, ssDNA expression was uniformly detected, and no lesions showed luminal side-dominant pattern in ssDNA expression.

DISCUSSION

In the present study, we demonstrated the direct effect of *H. pylori* eradication therapy on the morphological appearance in gastric adenomas and carcinomas. The

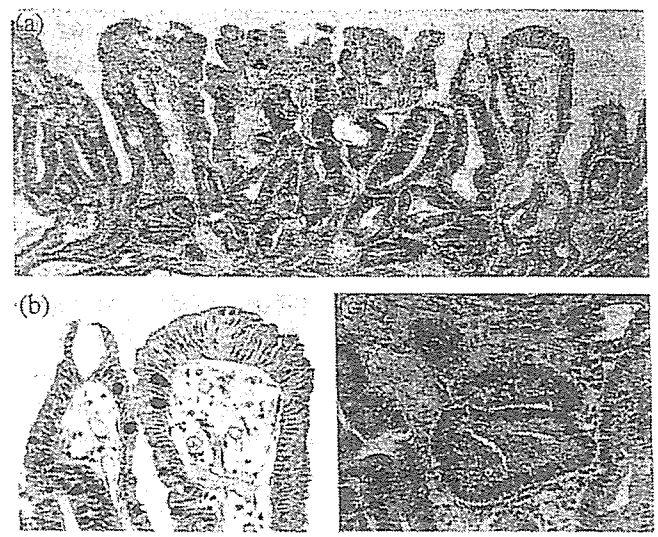

Figure 4. Expression of single-stranded DNA (ssDNA) in adenoma cells. Immunohistochemical analysis was performed as described in Methods. (a) Low magnification of gastric tumour specimen, (b) high magnification of the surface area, (c) high magnification of the deep area. Patient was 67 years male.

typical changes after eradication were (i) a flattened aspect to the elevated lesion and indistinct border of tumour lesion when viewed at endoscopy and (ii) the appearance of a normal columnar epithelium over the neoplastic lesion. Uemura *et al.* previously demonstrated the low incidence of a second cancer development by the eradication therapy in patients who underwent endoscopic mucosal resection of the gastric cancer.[7] In addition, we have published data demonstrating a low Ki-67 labelling index in those gastric cancer cells without *H. pylori* infection.[8] These results are indicative of the promoting effect of *H. pylori* on the growth of gastric cancer cells.

We found that the gastric tumour had flattened and showed indistinct feature after a short period and this result completely agrees with the previous findings. It is of interest that the main morphological change revealed by endoscopic observation was that it had flattened and this was only in the elevated lesions, regardless of the tumour's histology. No morphological change was found in the cases that had depressed features. This indicates that *H. pylori* eradication may inhibit the upward (expansive) growth of the gastric tumour. We have also found that most gastric cancers discovered after successful eradication therapy were of the flat, depressed type (under submission). This phenomenon also agrees with our hypothesis.

The mechanism of the tumour promoting effect of *H. pylori* is still unknown. In the *in vitro* studies, *H. pylori* itself was found to have the effect of modifying the expressions of several genes in gastric carcinoma cells.[12] And in the *in vivo* studies, *H. pylori* has been proved to modify directly the state of apoptosis or the cell cycle regulatory system including p27 expression.[13, 14] Recent evidence has clarified the direct mechanism of the translocation of harmful proteins (Cag A) from *H. pylori* to the host cell followed by specific intracellular signalling.[15, 16] Semino-Mora *et al.* recently demonstrated the presence of *H. pylori*-derived toxic proteins and mRNAs in gastric tumour cells *in vivo*.[17] However, their theory is still controversial, and, until now, it has been believed that *H. pylori* cannot exist on the surface of gastric carcinoma cells. Indeed, no evidence has demonstrated *H. pylori*-induced signalling, including CagA phosphorylation, in the human gastric carcinoma cells *in vivo*.

Thus, it is likely that *H. pylori* indirectly influences tumour cell growth by regulating the inflammatory reaction around the tumour tissue. Several cytokines have been reported to be induced by *H. pylori* infection[18] and some of them, such as interleukin-1 and hepatocyte growth factor, may act as growth factors for tumour cells.[19] In the present cases all were confirmed to have *H. pylori*-induced chronic gastritis in the background mucosa. Ohkusa *et al.* demonstrated that, after eradication, gastric inflammation had decreased by 1–3 months.[20] We found that ssDNA expression was mainly detected in the deeper area of the gastric tumour in three lesions at posteradication, and all three showed indistinct appearance. In other specimens, ssDNA expression was uniformly detected, and no lesions showed luminal side-dominant pattern in ssDNA expression. These suggest the importance of the growth inhibitory signals from the mucosal side (as opposed to those from the luminal side). This indicates the importance of gastric inflammation in the gastric mucosa rather than *H. pylori* itself on the luminal side. In this study, ssDNA expression was examined only in lesions after eradication, this should be examined at pre- and post-eradication and should be compared in the next step.

Gastrin is known to be an important gut-related hormone and a growth factor for gastric cancer cells[21, 22] and gastric tumour cells have been shown to contain its receptor. Reports have indicated that, after eradication therapy of *H. pylori*, a decreased level of several cytokines such as interleukin (IL)-1, IL-2, tumour necrosis factor-α and interferon-γ, in the gastric mucosa as well as increased acid output results in the decreased level of serum gastrin.[23, 24] However, our results showed that the decrease of the gastrin level is not so obvious after eradication, and alteration of the tumour lesion was not correlated with the serum gastrin level. In our protocol, the observation period is short and that may be a reason for the incomplete depression of the gastrin level. It is unlikely that our new findings of the morphological changes were induced by a gastrin-related system.

It was a surprising finding that a normal columnar epithelium appeared over the tumour tissue after successful eradication therapy. The reason for the alteration is still unknown but we can suggest two possibilities. First, *H. pylori* may directly affect the differentiation of gastric epithelial cells and its eradication could modify this effect although we could find little evidence to support this possibility. Secondly, the appearance of normal epithelium was induced as a regenerative change against injured tumour tissue.

After eradication therapy, it seems likely that gastric acid output increases in patients with atrophic gastritis.[25] In the cases we examined, most patients showed atrophic changes in the corpus suggesting low acid output and recovery after eradication therapy. This may lead to surface injury of the tumour lesion and thus induce regenerative changes. Indeed, we found surface erosion on the tumour lesion in four cases after eradication therapy (data not shown). We also confirmed that the mucosal injury by gastric biopsy before endoscopic resection did not correlate with the appearance of normal foveolar epithelium.

Recently, it has been a topic of discussion as to whether eradication therapy of *H. pylori* influences the reduction of gastric carcinogenesis or not. Previously published data indicated a reduced rate of second cancer discovery in patients who received an endoscopic mucosal resection for the first cancer.[7] Recently, a Chinese group has published data that conflict with previous findings.[26] They demonstrated, with a randomized-controlled trial, that *H. pylori* eradication eliminated cancer incidence in patients with no precancerous lesions upon presentation compared with infected subjects. There was a concurrent 37% relative decrease in cancer incidence in the overall population, but this difference did not reach a level of statistical significance.[26] The only way to study the degree of gastric carcinogenensis is through endoscopic discovery. If eradication therapy itself has an influence on the morphological change of the gastric tumour, this therapy must have an influence on cancer discovery rate. In the present study, we demonstrated the flattened and indistinct appearance of the gastric tumour after eradication even after a short time. Generally, the morphological feature of elevation is the most important characteristic required to find out the gastric neoplasms. Even if the true incidence of cancer was not affected by eradication, the incidence of cancer discovery would be decreased by successful eradication therapy in cases where there is an elevated tumour feature. Moreover, the appearance of normal foveolar epithelium must make it difficult to detect the gastric cancer by endoscopic observation. This must contribute to the reduction in the rate of cancer discovery after successful eradication therapy.

Taken together, this is the first report that has described the typical morphological changes of gastric adenoma or carcinoma tissue over a short period. However, the question still remains as to why only a part of the tumour tissue showed these alterations. It should be clarified as to what is the typical appearance of a gastric tumour that has been affected by *H. pylori* eradication therapy. Moreover, it should also be discussed as to whether eradication therapy can truly diminish the occurrence of gastric cancer and reduce the gastric cancer induced mortality rate of the population.

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Mucin Phenotype and Background Mucosa of Intramucosal Differentiated-Type Adenocarcinoma of the Stomach

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Key Words

Gastric carcinoma · Mucin phenotype · Background mucosa · Human gastric mucin · HIK1083 · Small intestinal mucinous antigen · MUC2 · Pepsinogen

Abstract

Objectives: Gastric carcinomas have been divided into differentiated (intestinal) and undifferentiated (diffuse) types. Recently, classification studies based on mucin expression have revealed that some differentiated-type carcinomas are of a gastric phenotype. In this study, we investigated the clinicopathological features of differentiated-type adenocarcinomas and evaluated the background mucosa of the stomach based on mucin expression by the tumors. ***Methods:*** Seventy-six intramucosal differentiated-type adenocarcinomas of the stomach were evaluated macroscopically and histologically. The mucin expression of tumor cells was examined by immunohistochemical staining with monoclonal antibodies against human gastric mucin (45M1), class III mucin (HIK1083), small intestinal mucinous antigen (SIMA-4D3), and MUC2 (Ccp58). Tumors were classified by phenotype as gastric (G-type), intestinal (I-type), mixed (M-type), or null (N-type). Not only the clinicopathological features but also the background mucosa of the stomach of G-type and I-type carcinomas were compared histologically and serologically. ***Results:*** Seventeen tumors (22.4%) were classified as G-type, 31 (40.8%) as I-type, 22 (28.9%) as M-type, and 6 (7.9%) as N-type. The frequencies of elevated type tumors and papillary adenocarcinomas and the ratio of moderately/well-differentiated adenocarcinomas were higher in G-type than in I-type carcinomas. The scores for glandular atrophy and intestinal metaplasia were higher and the scores for chronic inflammation, polymorphonuclear neutrophil activity, and the density of *Helicobacter pylori* were lower in G-type than in I-type tumors. The serum level of pepsinogen I and the pepsinogen I/II ratio were significantly lower in G-type than in I-type tumors. ***Conclusions:*** G-type carcinoma is the predominant phenotype of papillary adenocarcinoma. The background mucosa of G-type carcinoma is associated with glandular atrophy and intestinal metaplasia, whereas that of I-type carcinoma is associated with active and chronic inflammation induced by *H. pylori* infection.

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Introduction

Gastric carcinomas have been divided histologically into intestinal and diffuse types based on the glandular formations described by Lauren [1], and these two types correspond to the differentiated and undifferentiated types described by Nakamura et al. [2]. The histogenesis and progression of these carcinomas are known to be different [3–5], but the reasons for the differences are unknown. Recently, a new classification of gastric carcinomas based on mucin expression was proposed. Gastric carcinomas were classified as gastric or intestinal phenotype on the basis of mucin expression by surface mucous cells, glandular mucous cells, and intestinal columnar and goblet cells [6–8]. The differentiated-type tumor, which accounts for the majority of gastric carcinomas, was thought to display a predominantly intestinal phenotype because it is preceded by a precancerous stage that is characterized by the sequential steps of atrophic gastritis, intestinal metaplasia, dysplasia, and intramucosal carcinoma [9–12]. However, it has become clear, through mucin-histochemical or immunohistochemical studies, that some cases of differentiated-type adenocarcinoma arise from gastric mucosa without intestinal metaplasia and display gastric phenotypes [13–16].

Helicobacter pylori is known to induce histological gastritis, and long-term infection results in glandular atrophy and intestinal metaplasia [17]. A positive correlation between *H. pylori* infection and development of gastric carcinoma, especially differentiated-type adenocarcinoma, has been found from epidemiological studies [18–20] and the Mongolian gerbil experimental model [21–23]. *H. pylori* is now considered a risk factor for gastric carcinoma [24], but the relation between mucin expression by gastric carcinoma and *H. pylori*-induced histological gastritis has not been determined. In this study, we investigated the clinicopathological features of differentiated-type gastric adenocarcinomas and evaluated the background mucosa using the mucin expression classification system.

Materials and Methods

Subjects

Endoscopic mucosal resection was performed in 354 patients with intramucosal differentiated-type adenocarcinoma of the stomach treated at Hiroshima University Hospital between 1995 and 1999. We randomly selected 73 cases and 76 lesions for histological examination of the background mucosa. Macroscopic and histological evaluations of tumors were based on the classifications established by the Japanese Research Society for Gastric Cancer [25].

Immunohistochemical Staining

We investigated mucin expression of the gastric carcinomas by immunohistochemical staining using monoclonal antibodies against human gastric mucin (HGM) (45M1, Novocastra, Newcastle, UK), class III mucin (HIK1083, KANTO Reagents, Tokyo, Japan), small intestinal mucinous antigen (SIMA-4D3, Novocastra), and MUC2 (Ccp58, Novocastra). HGM, which is identical to MUC5AC, is expressed by mucous cells on the surface of the gastric epithelium and by goblet cells of the fetal and pre-cancerous colon, but it is not expressed in normal colon [26, 27]. The monoclonal antibody HIK1083 binds specifically to α-linked N-acetylglucosamine, which is one of the specific sugar residues found in class III mucin [28, 29]. Both HGM (fig. 1a) and class III mucin (fig. 1b) were designated as gastric phenotype markers. SIMA is an oncofetal glycoprotein antigen that is present in the small intestine and in cancers of both the stomach and large bowel but is not present in normal stomach and normal adult large bowel. The antibody recognizes goblet cells as well as the extracellular mucinous antigen [30, 31]. MUC2 is a 550-kDa glycoprotein that is regarded as the principal secretory mucin in the colorectum and is specific for goblet cells [32, 33]. Furthermore, it is colocalized with goblet cells in both normal and malignant tissues [34]. MUC2 is considered useful for detecting intestinal goblet cells. MUC2 (fig. 1c) and SIMA (fig. 1d) were designated as intestinal phenotype markers.

Immunohistochemical analysis was done on formalin-fixed, paraffin-embedded tissues. Paraffin blocks containing the tumor tissues were cut into 4-micrometer-thick sections. Immunohistochemical staining was done by the immunoperoxidase technique after microwave pretreatment. The sections were incubated with the primary antibody: 45M1 at 1:50 dilution, HIK1083 at 1:100 dilution, SIMA-4D3 at 1:100 dilution, or Ccp58 at 1:100 dilution. We applied the avidin-biotin complex immunostaining method, using a labeled streptavidin-biotin kit (Dako, Carpinteria, Calif., USA). Labeling was developed with a diaminobenzidine-hydrogen peroxidase substrate (DAB; Wako, Osaka, Japan). The tissue was then counterstained lightly with Mayer's hematoxylin.

Phenotyping for Mucin

The results of immunostaining were considered positive if more than 10% of tumor cells were stained for each marker. According to the results, tumors were classified into four phenotypes: gastric phenotype (G-type), intestinal phenotype (I-type), mixed phenotype (M-type), and null phenotype (N-type). Tumors were considered G-type if gastric phenotype marker (45M1 and/or HIK1083) was positive and intestinal phenotype markers (Ccp58 and SIMA-4D3) were negative, whereas I-type tumors expressed only intestinal phenotype marker. If staining was positive for both gastric and intestinal phenotype markers, the phenotype was defined as M-type, whereas N-type tumors stained negatively for all markers.

Assessment of Gastritis and H. pylori *Infection*

Four biopsy specimens (two from the lesser curvature of the antrum and one each from the anterior and posterior wall of the corpus) were obtained from each patient. Four-micrometer sections were stained with hematoxylin and eosin for histological examination and with Giemsa stain for *H. pylori* identification. The degree of gastritis (glandular atrophy, intestinal metaplasia, chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori*) was scored on a scale of 0 to 3 according to the updated Sydney System [35]. Two experts (M.I. and K.H.) assessed the histological

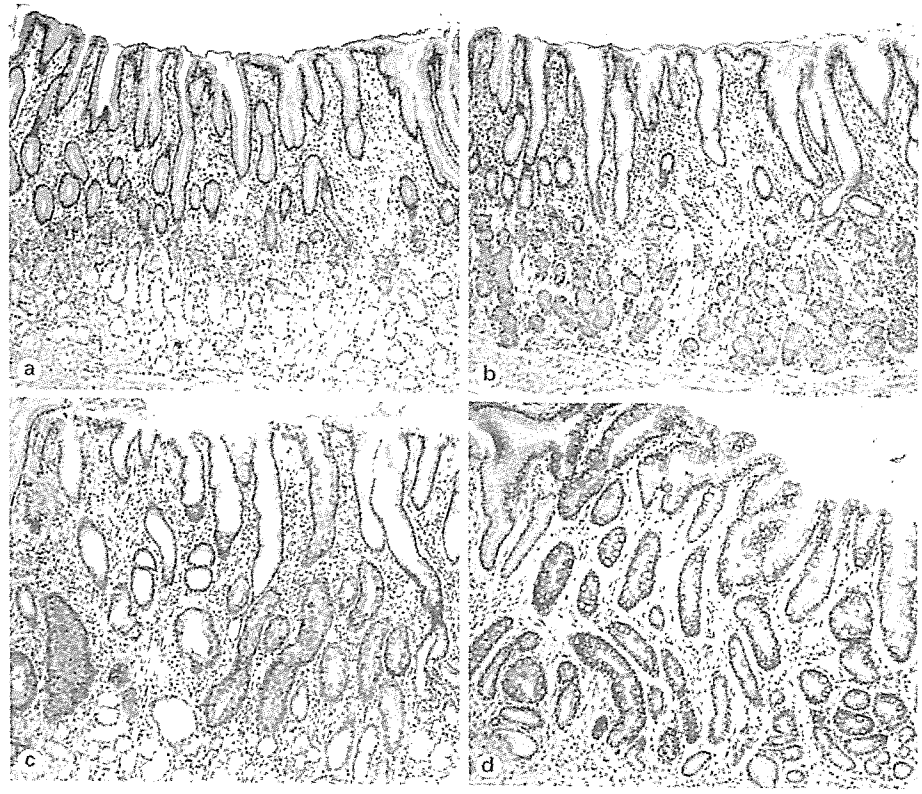


Fig. 1. Immunohistochemical staining results for gastric and intestinal mucins in noncancerous lesions. **a** Human gastric mucin (45M1) immunostaining is observed in the cytoplasm of surface epithelium cells. **b** Class III mucin (HIK1083) immunostaining is observed in the cytoplasm of pyloric gland cells. **c**, **d** Small intestinal mucinous antigen (**c**, SIMA-4D3) and MUC2 (**d**, Ccp58) immunostaining are observed in goblet cells of intestinal metaplasia. Original magnification ×40.

gastritis independently without clinical information on the patients. *H. pylori* infection was identified not only with Giemsa stain but also with anti-*H. pylori* antibody and by ^{13}C-urea breath test [36].

Measurement of Serum Pepsinogen and Gastrin

Fasting serum was collected from each patient upon entry into the study. The sample was centrifuged immediately at 4°C and stored at −20°C until use. The serum concentrations of pepsinogens (PGs) and gastrin were determined by a modified radioimmunoassay [37].

Statistical Analyses

Results are reported as the mean ± SE. We used the χ^2 test, the Fisher exact probability test, or the Mann-Whitney U test where appropriate. Results were considered statistically significant when p values were less than 0.05. All statistical analyses were conducted with the Statistical Analysis System (SAS Institute Inc., Cary, N.C., USA).

Results

Clinicopathological Features and Mucin Phenotypes of Gastric Carcinomas

Representative pictures of immunohistochemical stains of the G-type and I-type gastric carcinomas are shown in figure 2. Of the 76 intramucosal differentiated-type adenocarcinomas, 17 (22.4%) were classified as G-type, 31 (40.8%) as I-type, 22 (28.9%) as M-type, and 6 (7.9%) as N-type. The clinicopathological features of the G- and I-type gastric carcinomas are given in table 1. The proportion of female patients with G-type carcinoma was greater than the proportion of female patients with I-type carcinoma, but the difference was not significant. There was no difference between the two phenotypes in age, location, or size of tumors. Macroscopically, 13 of the 17 (76.5%) G-type carcinomas were elevated tumors, whereas 10 of the 31 (32.3%) I-type carcinomas were elevated ($p = 0.003$). Histologically, 4 (23.5%) of the G-type tumors were diagnosed as papillary adenocarcinomas, and all of the I-type tumors were diagnosed as tubular adenocarcinomas ($p = 0.005$). Among the tubular adenocarcinomas, the proportion of moderately differentiated adenocarcinomas (tub2) was significantly higher for G-type (69.2%) than for I-type (32.3%) tumors ($p = 0.024$).

Relation between Mucin Expression and Histological Gastritis in Background Mucosa

We compared the degree of histological gastritis in background mucosa between G-type and I-type carcinomas. We scored glandular atrophy, intestinal metaplasia, chronic inflammation, polymorphonuclear neutrophil ac-

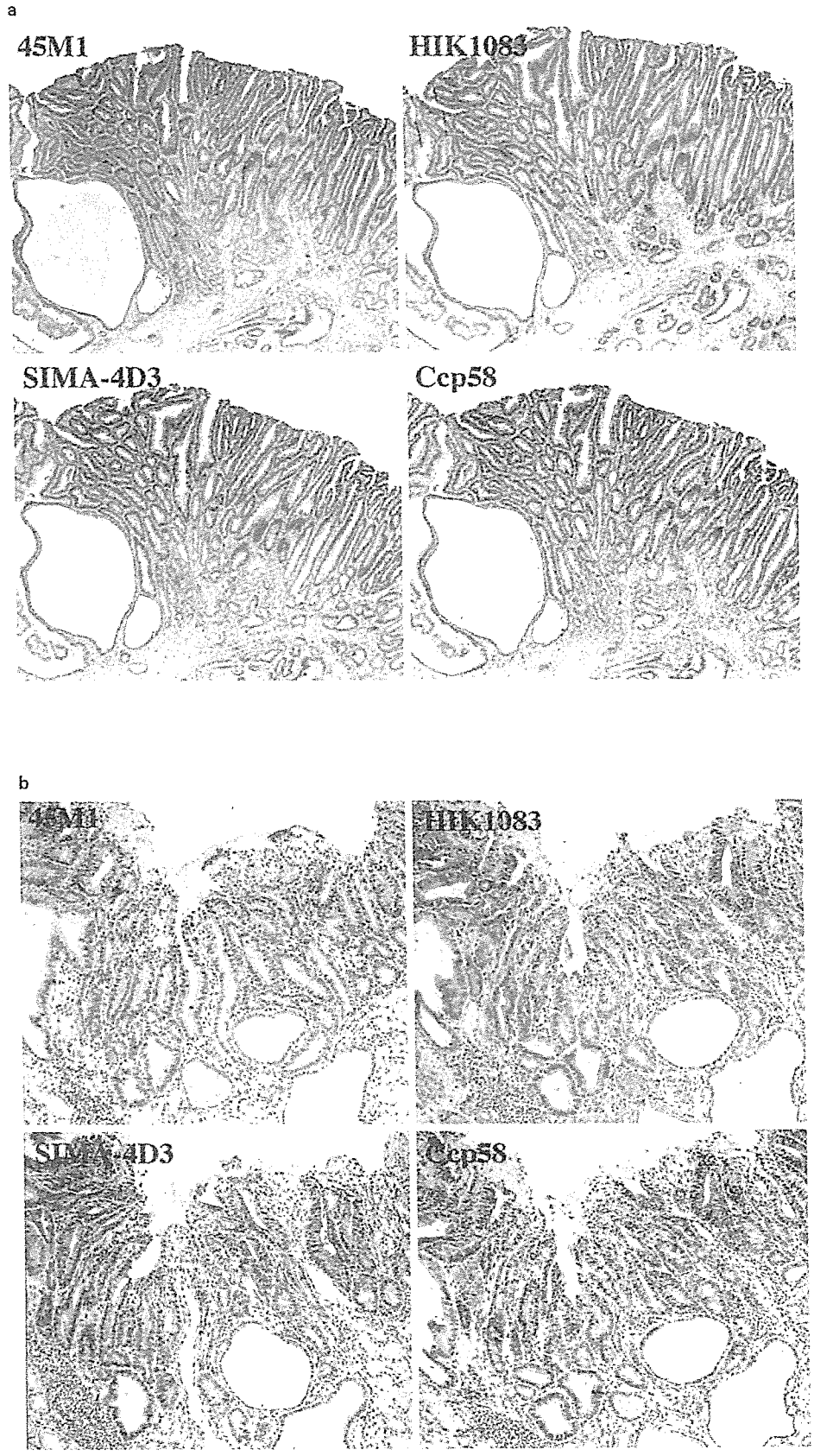


Fig. 2. Representative pictures of gastric phenotype (G-type) and intestinal phenotype (I-type) carcinomas. **a** In G-type carcinoma, human gastric mucin (45M1) staining is positive in the cytoplasm of cells in upper carcinoma glands, and class III mucin (HIK1083) staining is positive in the cytoplasm of cells in deeper carcinoma glands. Small intestinal mucinous antigen (SIMA-4D3) and MUC2 (Ccp58) are negative. Original magnification ×40. **b** In I-type carcinoma, 45M1 and HIK1083 are negative and SIMA-4D3 and Ccp58 are positive in the cytoplasm of carcinoma glands. Original magnification ×100.

tivity, and density of *H. pylori* in the antrum and corpus mucosa on a scale of 0 to 3 according to the updated Sydney System. We also examined non-neoplastic mucosa adjacent to the tumor and defined it as tumor periphery. Comparison of the mean score of each item in the antrum, corpus, and tumor periphery is shown in figure 3. The mean scores for glandular atrophy and intestinal metaplasia in all backgrounds tended to be higher in G-type than I-type carcinoma. The mean scores of chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori* were higher in I-type than in G-type carcinoma, especially at the antrum and the tumor periphery; there was a significant difference between the scores for chronic inflammation in the antrum and the tumor periphery. *H. pylori* infection was confirmed in 72 of 73 (98.6%) patients, and only 1 patient had negative results in all tests (Giemsa stain, anti-*H. pylori* antibody, and ^{13}C-urea breath test). This *H. pylori*-negative tumor was a well differentiated adenocarcinoma expressing both gastric and intestinal mucin (M-type).

Comparison of Serum Levels of PG I, PG II, and Gastrin between G-Type and I-Type Carcinomas

We examined the serum levels of PGs to estimate glandular atrophy objectively. A reduction of serum PG levels shows the existence of atrophic gastritis, and the magnitude of the reduction reflects the severity of the gastric mucosal atrophy [38]. The serum level of PG I in I-type cases (57.9 ± 6.2 ng/ml) was significantly higher than that in G-type cases (29.6 ± 7.9 ng/ml; p = 0.012), and the PG I/PGII ratio (2.5 ± 0.2, I-type; 1.4 ± 0.3, G-type; p = 0.011) was significantly higher in I-type cases. There was no significant difference in PG II levels between phenotypes. The serum gastrin level was significantly higher in G-type (253.4 ± 67.1 pg/ml) than in I-type (111.9 ± 10.6 pg/ml; p = 0.019) cases (fig. 4).

Table 1. Clinicopathological features of gastric mucin phenotype (G-type) carcinomas and intestinal mucin phenotype (I-type) carcinomas

	G-type (n = 17)	I-type (n = 31)	p value
Males, %	12 (70.6)	28 (90.3)	0.079
Females, %	5 (29.4)	3 (9.7)	
Age, years (mean ± SE)	63.0 ± 12.5	65.1 ± 9.7	0.597
Location			
U, %	1 (5.9)	5 (16.1)	0.573
M, %	8 (47.1)	14 (45.2)	
L, %	8 (47.1)	12 (38.7)	
Size, mm (mean ± SE)	15.5 ± 8.9	16.2 ± 11.1	0.940
Macroscopic type			
Elevated, %	13 (76.5)	10 (32.3)	0.003
Depressed, %	4 (23.5)	21 (67.7)	
Histology			
Papillary, %	4 (23.5)	0 (0)	0.005
Tubular, %	13 (76.5)	31 (100)	
tub1, %	4 (30.8)	21 (67.7)	0.024
tub2, %	9 (69.2)	10 (32.3)	

SE = Standard error; U, M, and L = upper, middle, lower third of the stomach; tub1 and tub2 = well and moderately differentiated tubular adenocarcinoma.

Discussion

Recently, it was proposed that gastric carcinomas can be classified into three mucin phenotypes [15, 39, 40]. The investigators found that 20 to 40% of early gastric carcinomas were of the G-type, but undifferentiated-type carcinomas and submucosal-invasive carcinomas were included in their studies. In the present study, we investigated differentiated-type adenocarcinomas limited to within the mucosal layer and classified them by immunohistochemical methods using four antibodies (45M1, HIK1083, 4D3, and Ccp58).

Previous studies, which investigated mucin expression of gastric adenocarcinomas, used HGM and class III mucin for gastric mucin markers [39–42]. 45M1 is an antibody against HGM. To detect class III mucin, paradoxical concanavalin A staining (PCS) has been used as described by Katsuyama and Spicer [15, 39–41, 43]. We used the monoclonal antibody HIK1083 because Nakamura et al. [29] reported its immunostaining and PCS to have similar specificities for mucins in glandular mucous cells and that it is a useful screening tool for class III mucin in normal, metaplastic, and carcinoma tissues. To detect intestinal mucin expression, we performed immunostaining of MUC2 (Ccp58), which is a core peptide of intestinal goblet-cell mucin, and sialo-syl-Tn (4D3) as a marker for small intestinal mucin antigen. Previous studies used CD10 as a marker for small intestine [39, 41], but it recognizes the brush border on the luminal surface of epithelial cells [44]. Takahashi et al. [42] also used these four antibodies (45M1, HIK1083, 4D3, and Ccp58) and classified 102 early gastric adenocarcinomas according mucin expression.

By immunostaining, expression of gastric mucin was observed in 39 of the 76 (51.3%) tumors, and 17 of these

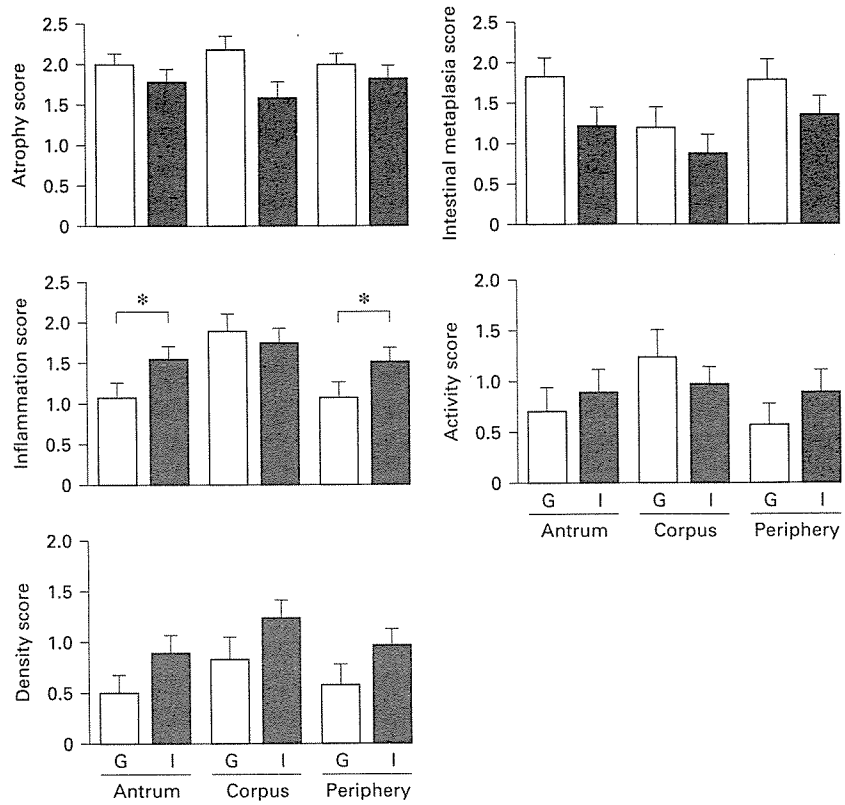
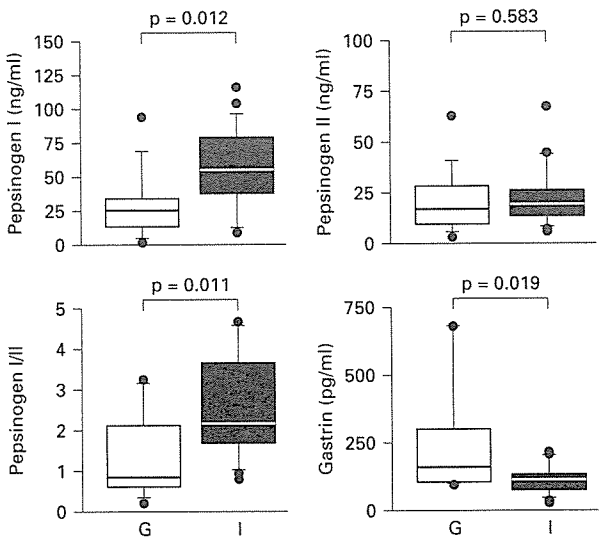


Fig. 3. Relation between mucin expression and histological gastritis in background mucosa. In G-type (G: □) and I-type (I: ■) carcinomas, the degree of gastritis (glandular atrophy, intestinal metaplasia, chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori*) was scored in the antrum, corpus, and tumor periphery. Data are shown as the mean ± SE. There is a significant difference between G-type and I-type carcinomas in the scores for chronic inflammation in the antrum and tumor periphery (* $p < 0.05$).



(22.4%) were classified as G-type carcinomas. Koseki et al. [39] investigated mucin expression by differentiated-type adenocarcinomas with definite submucosal invasion and found that the G-type was more predominant in papillary than in tubular adenocarcinoma. Our finding that there was a significant difference in the frequency of papillary adenocarcinoma between G-type (23.5%) and I-type (0%) carcinomas is consistent with theirs. Moreover, we found that the proportion of moderately differentiated

Fig. 4. Comparisons of serum levels of pepsinogen I, pepsinogen II and gastrin, and the ratio of pepsinogen I to II, between G-type (G: □) and I-type (I: ■) carcinomas. The midline of the box indicates the median value of all samples, and 50 and 90% of samples are included within the box and the bars, respectively. The serum level of pepsinogen I and the pepsinogen I/II ratio are significantly higher in I-type than in G-type carcinomas. Serum gastrin level is significantly higher in G-type than in I-type carcinomas.

adenocarcinomas in the G-type carcinomas (69.2%) was higher than in the I-type carcinomas (30.8%). Saito et al. [40] proposed that small differentiated-type adenocarcinoma with gastric mucin expression transforms into undifferentiated-type adenocarcinoma during progression of the tumor and Tatematsu et al. [14] found that gastric-type mucin was expressed more frequently in poorly differentiated adenocarcinomas than in papillary or tubular adenocarcinomas. Koseki et al. [39] also showed that G-type carcinomas are associated with lymphatic invasion and lymph node metastasis. These findings suggest that some undifferentiated-type adenocarcinomas may be derived from differentiated-type adenocarcinomas and that gastric mucin expression in tumor cells may predict the malignant potential of the tumor [45].

There are a few studies that found an association between mucin expression by gastric adenocarcinomas and their background mucosa, but these studies investigated only the presence of or subtypes (complete/incomplete type) of intestinal metaplasia [15, 41, 42]. To evaluate the degree of histological gastritis, we scored not only intestinal metaplasia but also other markers (glandular atrophy, chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori*) using the updated Sydney System. With regard to intestinal metaplasia and glandular atrophy, Yao et al. [46] noted that carcinomas arising in gastric hyperplastic foveolar polyps displayed the gastric phenotype, and Egashira et al. [15] reported that intestinal metaplasia was absent or only slight in the background mucosa of G-type differentiated-type adenocarcinomas. Therefore, we speculated that the background mucosa of G-type carcinoma is not associated with glandular atrophy or intestinal metaplasia. Unexpectedly, the scores for glandular atrophy and intestinal metaplasia were higher in G-type than in I-type carcinomas at each sample site (antrum, corpus, and tumor periphery). To confirm these histological results, we determined serum levels of PG and gastrin. The PG I level and the PG I/PG II ratio were significantly lower, and serum gastrin levels were significantly higher in the G-type than in the I-type cases, indicating that glandular atrophy developed in the background mucosa of G-type carcinomas. However, the findings do not mean that the carcinogenesis of G-type tumors occurred in gastric mucosa with glandular atrophy and intestinal metaplasia because there is a long time period between gastric carcinogenesis and endoscopic discovery of the tumor. The mean scores of chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori* were higher in I-type than in G-type carcinomas, especially at the tumor periphery. This means that there was more active gastritis induced by *H. pylori* present at the background mucosa of I-type in comparison to G-type carcinomas. We recently reported that *H. pylori* infection influences the tumor growth of gastric carcinoma [47]. Particularly with respect to tumor growth, I-type carcinoma may be associated with *H. pylori* infection. It is likely that G-type carcinomas become endoscopically detectable at the late phase of atrophic gastritis.

Tahara et al. [48] suggested that differentiated- and undifferentiated-type gastric carcinomas may be caused by distinct genetic alterations, but differences in genetic alterations between G-type and I-type differentiated-type adenocarcinomas are not well known. Endo et al. [49] reported that the mutator pathway, characterized by microsatellite instability, plays an important role in the tumorigenesis of G-type carcinomas, and the suppressor pathway, represented by p53 alteration, may participate in the tumorigenesis of I-type carcinomas. Koseki et al. [39] indicated that the abnormal expression of E-cadherin is correlated with G-type carcinoma and the nuclear and cytoplasmic accumulation of β-catenin is correlated with I-type carcinoma. Further studies that investigate the relation between mucin expression and genetic alteration may provide us with new knowledge about the genetic pathways of gastric carcinoma.

In conclusion, we found that expression of mucins by gastric adenocarcinoma is associated with histological gastritis of the background mucosa. The background mucosa of G-type carcinoma, which is the predominant papillary adenocarcinoma phenotype, is associated with glandular atrophy and intestinal metaplasia, whereas that of I-type carcinoma is associated with active and chronic inflammation induced by *H. pylori* infection. Molecular mechanisms contributing to mucin expression remain unknown. It is of great interest whether the organ microenvironment and tumor-host interaction influence mucin production and the morphology of gastric carcinoma.

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GASTROENTEROLOGY

Chromosomal and microsatellite instability in sporadic gastric cancer

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Abstract

Background: Gastric cancer can progress through two pathways of genomic instability: chromosomal (CIN) and microsatellite instability (MSI). It is hypothesized that these two pathways are not always independent and that some tumors show overlap between these two mechanisms.

Methods: A total of 98 sporadic gastric cancers were classified based on their MSI status, using microsatellite assay with BAT26. Evidence for CIN was investigated by identifying loss of heterozygosity (LOH) events on chromosome arms, 5q, 10p, 17p, 17q, and 18q, which are regions harboring tumor suppressor genes that are significant in gastric cancer development.

Results: Twelve tumors (12%) showed high-frequency MSI (MSI-H). Overall, 43 of the tumors (44%) had at least one LOH event, with most frequent chromosomal losses observed on 10p and 18q (30%, respectively), followed by 5q (21%), 17p (14%), and 17q (12%). Interestingly, overlap was observed between CIN and MSI pathways. Of 43 cancers with LOH events, four (9%) were also MSI-H. It was also found that 48% of cancers without MSI-H had no LOH events identified, comprising a subgroup of tumors that were not representative of either of these two pathways of genomic instability.

Conclusion: These results suggest that molecular mechanisms of genomic instability are not necessarily independent and may not be fully defined by either the MSI or CIN pathways in sporadic gastric cancers.

Key words: chromosomal instability, gastric cancer, genomic instability, microsatellite instability.

INTRODUCTION

Gastric cancer is the second most frequent malignant tumor in the world and contributes to significant cancer mortality, particularly in Asia.[1] Gastric carcinogenesis is characterized by the successive accumulation of mutations in genes controlling epithelial cell growth and differentiation.[2,3] The term 'genomic instability' describes conditions involving widespread loss of DNA integrity. The development of genomic instability is an important event in the multistep progression of gastric carcinogenesis. Two apparently independent pathways of genomic instability have been identified.[4,5] The first and more common pathway is characterized by the sequential inactivation of tumor suppressor genes, such as *APC* (chromosome 5q), *p53* (chromosome 17p), and *DCC* (chromosome 18q), and activation of oncogenes.[6] Tumors generated through this 'suppressor' pathway

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display chromosome instability (CIN), which means frequent allelic losses and genetic mutations. The mechanism driving the process of CIN is not fully understood. The second pathway is characteristic of tumors from patients with hereditary non-polyposis colorectal cancer (HNPCC), an autosomal-dominant condition that accounts for approximately 10% of all colorectal cancers. The hallmark of this 'mutator' pathway is widespread microsattelite instability (MSI), which is characterized by the accumulation of somatic alterations in the length of simple repeated nucleotide sequences called 'microsatellites'. The high-frequency MSI (MSI-H) found in tumors from patients with HNPCC result from defects in the DNA mismatch repair (MMR) system that are caused by germ-line mutations of the MMR genes, such as *hMSH2* and *hMLH1*.[7] High-frequency MSI has been identified in approximately 15% of sporadic gastric cancers.[8,9] In these cases, mutations of *hMSH2* and *hMLH1* are rarely found. Recent studies found that *hMLH1* inactivation by promoter hypermethylation produces the MSI-H phenotype in sporadic gastric cancers and is responsible for most sporadic gastric cancers with MSI-H.[10] These tumors accumulate slippage-induced frameshift mutations in the coding regions of target genes, such as *TGFβRII*, *BAX*, *hMSH3* and *hMSH6*.[11]

Gastric cancers originating by the suppressor and the mutator pathways differ in several pathological features. Tumors with MSI-H may be more likely to arise in the distal stomach, and may have less lymph node metastasis, compared with tumors without MSI-H.[12] Furthermore, patients with MSI-H tumors have a more favorable survival than do gastric cancer patients without MSI-H.[12]

Although these two mechanisms of genomic instability can be distinguished from one another, evidence suggests that there might be some degree of overlap. For instance, loss of heterozygosity (LOH) is occasionally a mechanism by which the wild-type allele of *hMLH1* is inactivated in some MSI-H tumors.[13] It is also possible that gastric cancers are initiated by mechanisms not involving CIN or MSI. For instance, epigenetic modification by the hypermethylation of the promoter regions of tumor suppressor genes may play an important role in the development of many gastric cancers.[10]

To date, no study has determined the extent of overlap between the CIN and MSI pathways in sporadic gastric cancers. In addition, available data do not fully address the question of whether every gastric cancer bears genetic alterations related to one of these two mechanisms of genomic instability. We hypothesized that some tumors show overlap between these two mechanisms and that some tumors do not show evidence for involvement of either of these mutational pathways. We therefore classified sporadic gastric cancers based on their CIN and MSI status.

METHODS

Patients

Ninety-eight sporadic gastric cancer patients were enrolled at the Hiroshima University Hospital between 1996 and 2000. None of the patients had HNPCC. For each patient, both cancerous and normal tissues were available.

Histological examination

Four-micrometer sections were prepared from formalin-fixed and paraffin-embedded specimens. The sections were stained with hematoxylin and eosin (HE) for histological examination. Gastric cancers were classified into intestinal type and diffuse type as defined by Lauren.[14,15] Depth of invasion was classified into two groups: early stage (mucosa and submucosa) and advanced stage (muscularis propria or deeper). Presence of lymph node metastasis was also examined. To analyze the relationship between tumor location and genetic alterations, the stomach was divided into three parts: the upper, the middle, and the lower parts.

DNA extraction

Ten-micrometer-thick tissue sections were placed on a glass slide and stained with HE. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Cancerous and normal tissues on the slides were scraped up with sterile needles, separately, using a microdissection technique.[6] The DNA was extracted from the tissues with 20 μL of extraction buffer (100 mmol/L Tris-HCl; 2 mmol/L ethylene diamine tetraacetic acid (EDTA), pH 8.0; 400 μL/mL of proteinase K) at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K and then 2 μL of these extracts was used for each polymerase chain reaction (PCR) amplification.

Microsatellite assay

Each tumor was evaluated for MSI by microsatellite assay with BAT26. The microsatellite assay was performed as described elsewhere.[9,16] Briefly, each 15 μL reaction mixture containing 10–20 ng of genomic DNA, 6.7 mmol/L Tris-HCl (pH 8.8), 6.7 mmol/L EDTA, 6.7 mmol/L $MgCl_2$, 0.33 μmol/L of labeled primer with [γ-^{32}P]dATP, 0.175 μmol/L unlabeled primer, 1.5 mmol/L of each deoxynucleotide triphosphate, and 0.75 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ, USA) was amplified for 40 cycles as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and strand elongation at 72°C for 30 s. The PCR products were electrophoresed on 6% polyacrylamide–8 mol/L urea–32% formamide gels and autoradiographed overnight at −80°C on Fuji RX film. Tumors with a shift at the BAT26 locus were classified as MSI-H.[17]

Seven sets of microsatellite loci that are tightly linked to tumor suppressor genes were used to identify significant allelic losses in the gastric cancers. The DNA was amplified by PCR at microsatellite loci linked to the *APC* locus on 5q21 (D5S505), possible tumor suppres-

sor/senescence gene locus on 10p15 (D10S501 and D10S602), *p53* locus on 17p13 (TP53), *BRCA1* locus on 17q21 (D17S855), and *DCC* locus on 18q21 (D18S58 and D18S61).[6,17] Assessment of LOH was assigned when a tumor allele showed at least 50% reduction in the relative intensity of one allele in cancerous tissue compared with the matched normal DNA.

Statistical analysis

The mean age of the cases was compared using Student's *t*-test. Fisher's exact probability test was used for comparisons of other clinicopathological parameters. A value of $P < 0.05$ was regarded as significant.

RESULTS

The male-to-female ratio of the patients was 70/28, and the mean age was 63.1 years (range: 33–84 years). Histologically, 65 patients (66%) had intestinal-type gastric cancer and 33 patients (34%) had diffuse-type cancer. Fifty-nine cases (60%) were early stage and 39 (40%) were advanced stage.

Twelve of the 98 cancers (12%) showed evidence of MSI-H (Fig. 1; Table 1[18]). Clinicopathologically, female patients had a significantly higher frequency of tumors with MSI-H, compared with male patients (7/28, 25%; *vs* 5/70, 7%; $P = 0.022$). In advanced gastric cancers, lymph node metastasis tended to be infrequent in tumors with MSI-H, compared with tumors without MSI-H (3/8, 38%; *vs* 23/32, 72%; $P = 0.082$). No other clinical features including age, tumor location, histological subtype and stage, had any significant differences between tumors with and without MSI-H.

We identified 43 tumors (44%) with LOH at one or more of the seven loci examined (Fig. 2). Overall, the frequency of LOH in tumors with any LOH event was most common on 10p and 18q (17 of 57 [30%] informative cases, respectively), followed by 5q (6 of 29, 21%), 17p (9 of 63, 14%), and 17q (6 of 51, 12%; Table 2).

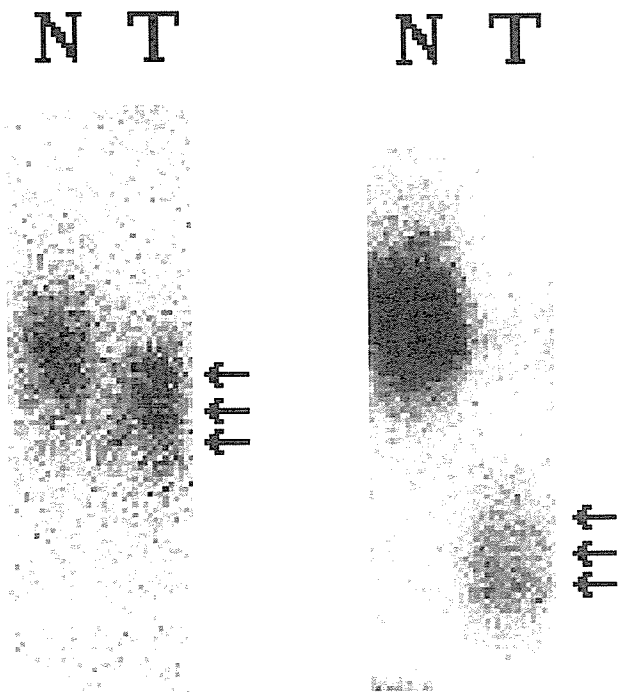

Figure 1 Representative examples of microsatellite instability at BAT26. N, normal; T, tumor.

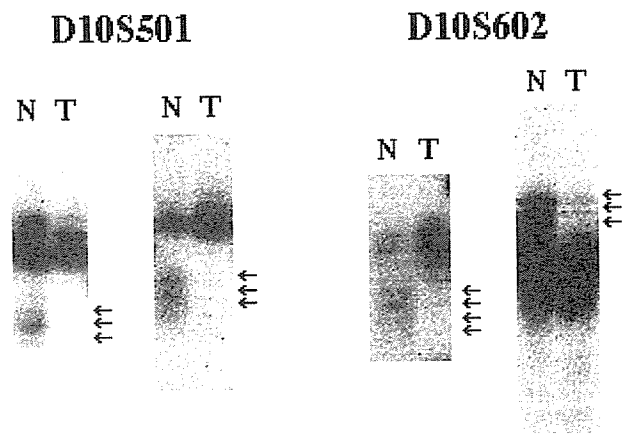

Figure 2 Representative examples of loss of heterozygosity. N, normal; T, tumor. Microsatellite loci are listed on the lanes.

Table 1 Frequency of MSI-H and LOH(+) and clinicopathological findings in sporadic gastric cancer patients

	MSI-H		LOH(+/–)	
Characteristics†	Frequency *n* (%)	*P*	Frequency *n* (%)	*P*
Age (years)		NS		NS
≤60	4/32 (13)		19/32 (59)	
>60	8/66 (12)		26/66 (39)	
Sex		NS		NS
Male	5/70 (7)		32/70 (46)	
Female	7/28 (25)		13/28 (46)	
Tumor location		NS		NS
Lower	7/53 (13)		24/53 (45)	
Middle	4/28 (14)		13/28 (46)	
Upper	1/17 (6)		8/17 (47)	
Stage		NS		NS
Early	5/60 (8)		26/60 (43)	
Advanced	7/38 (18)		19/38 (50)	
Histologic subtype		NS		NS
Intestinal	8/65 (12)		32/65 (49)	
Diffuse	4/33 (12)		13/33 (39)	

MSI-H, high-frequency microsatellite instability; LOH, loss of heterozygosity.

†Classified according to the Japanese Classification of Gastric Cancer as outlined by the Japanese Gastric Cancer Association (1998).[18]