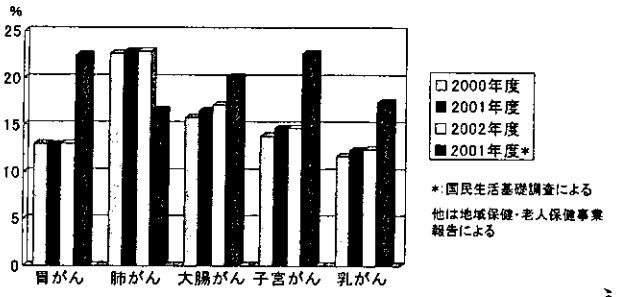

図1　部位別がん検診の受診率

表1　がん検診の受診率（1997年）

がん検診の種類	胃がん	肺がん	大腸がん	子宮がん	乳がん
地域集団における受診率*1	13.8%	22.4%	14.6%	15.2%	12.7%
地域集団における受診者数*	4,272,814	7,061,535	4,872,954	3,766,047	3,228,771
職域集団における推定受診率**	31.2%	9.8%	16.6%	32.0%	27.6%
職域集団における推定受診者数**	11,821,429	3,729,600	6,281,173	6,343,703	5,488,505
地域・職域集団の推定受診者数	16,093,814	10,791,535	11,153,954	10,109,750	8,717,276
地域・職域集団の推定受診者の受診率	25.3%	17.0%	17.5%	24.3%	20.9%

*：厚生省大臣官房統計情報部「平成9年度老人保健事業報告」[3]
**：労働省大臣官房政策調査部「平成9年労働者健康状況調査報告」[4]と同年の性・年齢階級別就業者数から40歳以上について（子宮がんと乳がんは30歳以上について）推定

5つの回答選択肢から複数回答を可として○をつけてもらう調査が実施された。胃、肺、大腸は40歳以上の男女について、子宮と乳房は30歳以上の女性についての受診者数を01年の当該年齢人口で除し、同じ表1に示す。

受診率は、胃がん検診で約22%、肺がん検診で約17%、大腸がん検診で約20%、子宮がん検診で約23%、乳がん検診で約17%となっていた。肺がん検診を除く他の4つのがん検診では国民生活基礎調査[2]による受診率の方が地域保健・老人保健事業報告[1]による受診率よりも高値であり、それは職域での受診者も受診したと回答しているためと解釈される。

地域保健・老人保健事業報告による受診率よりも低値となった肺がん検診については、胸部X線検査を受けていても肺がん検診と認識していない国民が多く存在するためであろうと思われる。今後、この設問はがん検診の受診の有無を質問するのではなく、がん検診のスクリーニング検査の受診の有無を質問するように改変されるべきであろう。

地域と職域を併せたがん検診の受診率を明らかにするために、97年度の老人保健事業報告と97年の労働者健康状況調査報告[4]を用いて推計した結果[5]を表1に示す。胃がんが25・3%と最も高く、続いて子宮がん24・3%、乳がん20・9%、大腸がん17・5%、肺がん17・0%の順であった。年が異なるが01年の国民生活基礎調査[2]と似かよった大きさの受診率であった。国民のおおよそ20～25%が各部位のがん検診を毎年受診しているというのが現状である。

精密検査受診率の低さは大きな問題

00年度の地域保健・老人保健事業報告[1]資料において、要精検者のうち結果別人員の報告が「異常認めず」「がんであった者」「がんの疑いのある者」「がん以外の疾患であった者」の合計の人数の割合である精検受診率は、胃がん76・5%、肺がん77・5%、大腸がん59・2%、子宮がん67・7%、乳がん78・6%であった。[1,5]

大腸がん検診の精検受診率が低いのは、大腸内視鏡検査や注腸X線検査といった精密検査の方法が受診者にとって負担の重いものであることと、その検査を提供できる医療機関が地域によっては限定され、処理能が十分でないこと、逐年検診受診者で毎年便潜血陽性の者が負担の重い精密検査の受診を忌避していること等が影響していると考えられるが、40%以上もの要精検者が精密検査を受診していないことは、検診のやりっぱなしであり、大きな問題である。

02年度の日本対がん協会の資料[6]によれば、大腸がん検診の精検受診率は地域の住民検診で71・8%、職域検診で41・9%と、職域の方が地域よりもさらに低値となっていた。大腸がん検診の精検受診率については、真剣に対策を講ずる必要があると考える。

受診率向上は未受診者対策から

国民のおおよそ20～25%が各部位のがん検診を毎年受診しているという現状に対して、「健康日本21」では受診者の5割増加を目指している。受診者が増加することは、精密検査の受診が伴えば2次予防が期待できるので、基本的には良いことであるが、どのようにすれば実現可能であろうか。

その前に、そもそも欧米での現状はどうなっているのだろうか。米国のCancer Control Planetのホームページには、例えば、00年のデータとして乳がんのスクリーニングであるマンモグラフィーを過去2年以内に受けたことのある50歳以上の女性の割合が州別に示されている。その数値は最低のワイオミングで70・9%であり、最高のデラウエアではなんと90・3%であった。

ここには参考となることが2点ある。この統計は全米各州における標本調査であり、人口規模にもよるが数百から数千の個人を対象に、スクリーニングそのものの受診の有無ではなくスクリーニング検査であるマンモグラフィーの受診の有無を質問していることと、過去1年間ではなく過去2年間について尋ね

ていることである。

具体的なスクリーニング検査の受診の有無を尋ねることで回答がより明確になり、国民生活基礎調査の中で肺がん検診受診者が過小評価されていることへの対応ともなり得、このような工夫を通してより正確な受診率の推定が必要であると考える。臨床におけるスクリーニング検査受診有の者を加算すれば、わが国のがん検診に用いられるスクリーニング検査の受診率はもっと高いのかもしれない。

また、わが国よりも罹患率の高い米国の乳がんにおいてすら、スクリーニングは過去2年以内に1回以上を受診ありとしていることである。これは12～33カ月ごとのマンモグラフィーによるスクリーニングが乳がんの死亡率を減少させていたというHumphrey LLらによる根拠[7]に基づいた判断である。わが国におけるがん検診は基本的に逐年検診として設計されているが、適正な受診間隔の検討も今後行っていく必要がある。

地域保健の現場では、胃がんや大腸がんによって不幸な転帰をとられた方の胃がん検診や大腸がん検診の受診歴を調べてみると、全く受診したことがなかったという例によく遭遇する。また、糖尿病で医療機関を長期受診している患者さんから、症状発見の進行胃がんが診断され、カルテをよく調べてみると、糖尿病についてはきちんと医療を受けていたのに、胃の検査を一度も受けたことがないといった事例も時に聞く。

そのようなことを考えると、やみくもに受診率向上を模索するのではなく、全くがん検診を受診したことのない未受診者のがん検診受診を勧めることを通した受診率向上を考えていく必要があるのではないかと考える。すなわち、がん検診を単独の保健サービスとして位置づけるのではなく、医療におけるスクリーニング検査の受診も含めて、トータルのがん対策として見直していくことも意味があるのではないかとも思う次第である。

参考文献

1 平成12～14年度地域保健・老人保健事業報告、厚生省大臣官房統計情報、厚生統計協会、2002～2004。

2 平成13年国民生活基礎調査、厚生省大臣官房統計情報、厚生統計協会、2003。

3 平成9年度老人保健報告、厚生省大臣官房統計情報、厚生統計協会、1999。

4 平成9年労働者健康状況調査報告、労働省大臣官房政策調査部、労働省大臣官房政策調査部第二課、1998。

5 渡邊能行、森田益次：わが国のがん検診の実施現状。住民検診・職域検診・人間ドックのためのがん検診計画ハンドブック（三木一正、渡邊能行編）。南江堂、27、2004

6 平成14（2002）年度がん検診の実施状況、日本対がん協会、2003

7 Humphrey LL, et al: Screening for Breast Cancer. Systematic Evidence Review No. 15 (Prepared by the Oregon Health & Science University Evidence-based Practice Center under Contract No. 290-97-0018). Rockville, MD: Agency for Healthcare Research and Quality. September 2002. (Available on the AHRQ Web site at: www.ahrq.gov/clinic/serfiles.htm).

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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

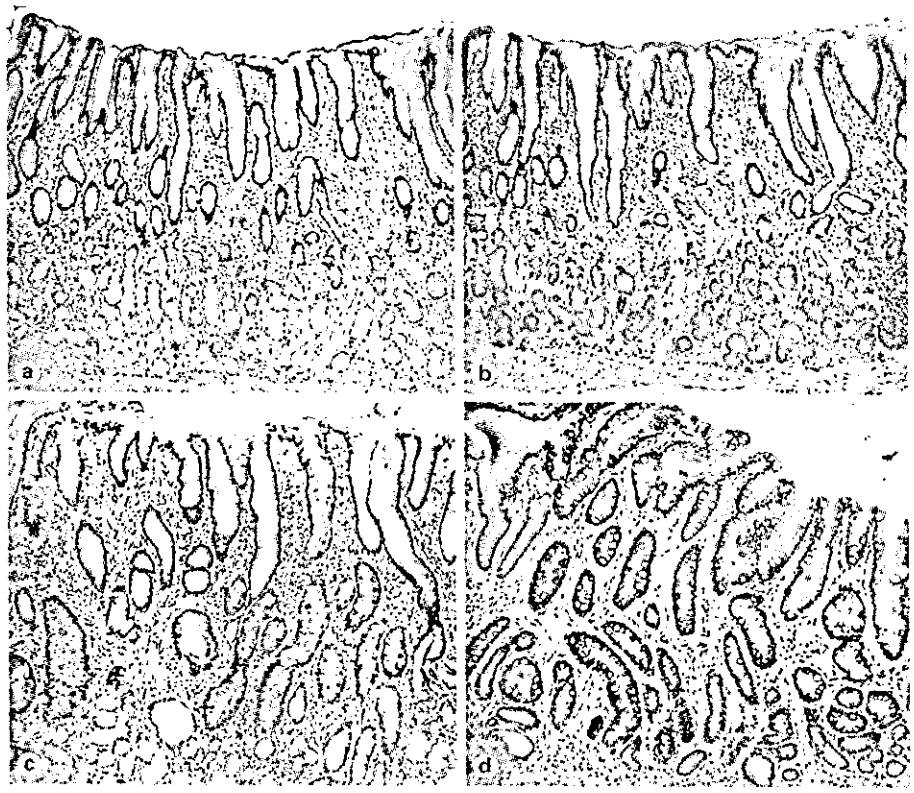


Fig. 1. Immunohistochemical staining results for gastric and intestinal mucins in noncancerous lesions. **a** Human gastric mucin (45M1) immunostaining is observed in the cytoplasm of surface epithelium cells. **b** Class III mucin (HIK1083) immunostaining is observed in the cytoplasm of pyloric gland 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-

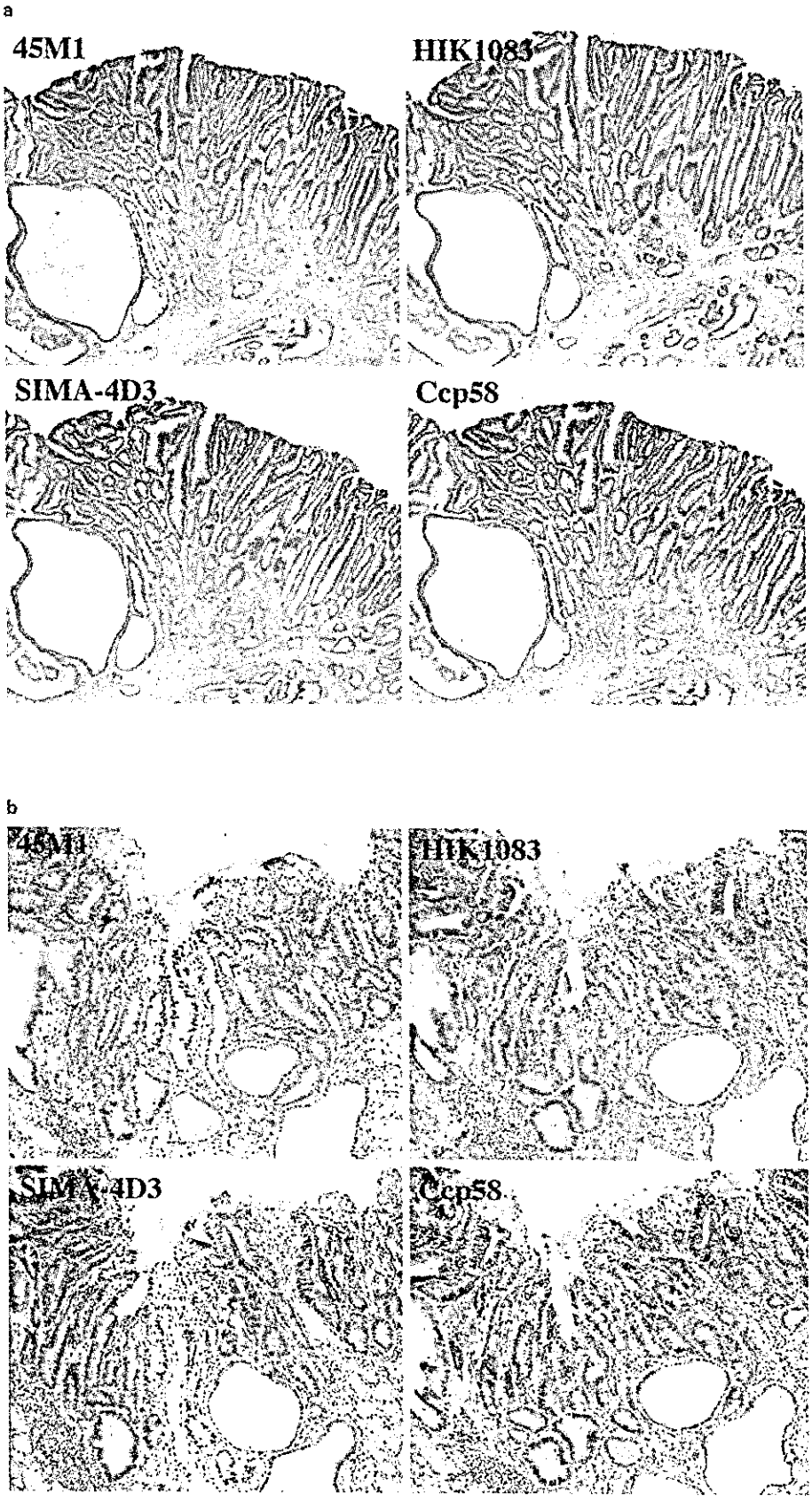


Fig. 2. Representative pictures of gastric phenotype (G-type) and intestinal phenotype (I-type) carcinomas. **a** In G-type carcinoma, human gastric mucin (45M1) staining is positive in the cytoplasm of cells in upper carcinoma glands, and class III mucin (HIK1083) staining is positive in the cytoplasm 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

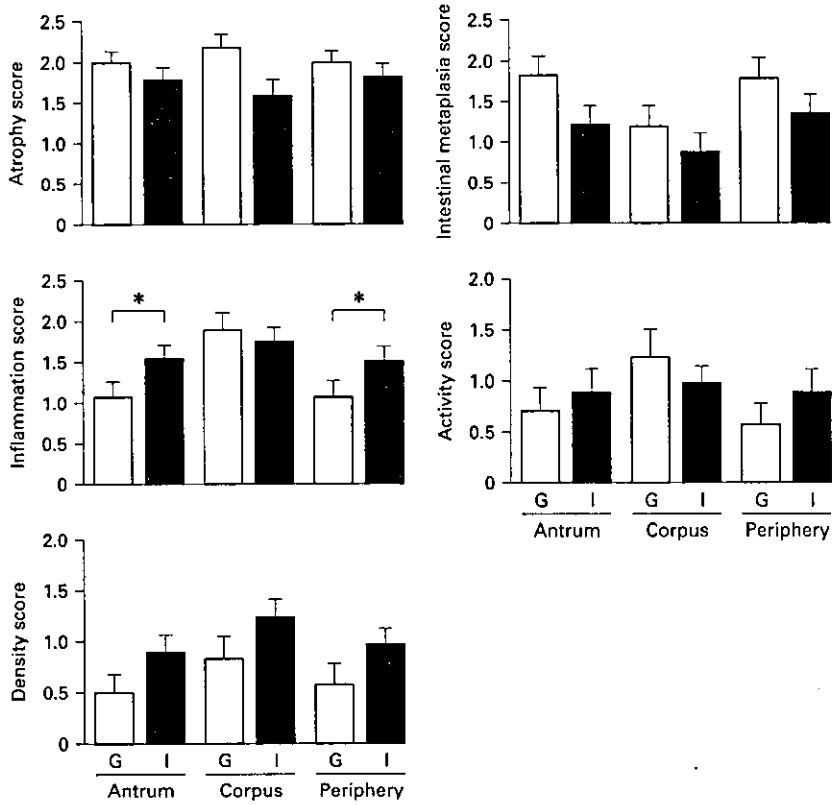
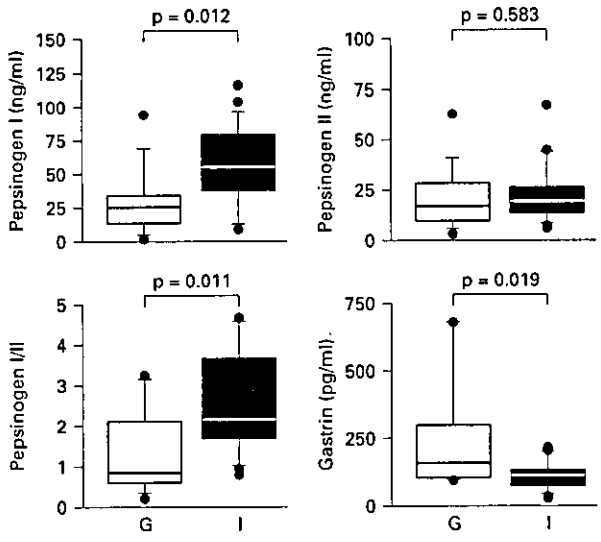


Fig. 3. Relation between mucin expression and histological gastritis in background mucosa. In G-type (G: □) and I-type (I: ■) carcinomas, the degree of gastritis (glandular atrophy, intestinal metaplasia, chronic inflammation, polymorphonuclear neutrophil activity, and density of *H. pylori*) was scored 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.

Acknowledgments

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References

1 Lauren P: The two histological main types of gastric carcinoma: Diffuse type and so-called intestinal type carcinoma. An attempt at a histological classification. Acta Pathol Microbiol Scand 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 Nakamura K: Special features of intestinal metaplasia and its relation to early gastric carcinoma in man: Observation by a method in which leucine aminopeptidase activity is used. J Natl Cancer Inst 1978;61:693–701.

4 Nakamura K, Sugano H, Takagi K, Kumakura K: Histogenesis of cancer of the stomach, with special reference to light and electron microscopic and statistical studies of primary microcarcinoma of the stomach. Gan No Rinsho 1969;15:627–647.

5 Nagayo T: Microscopical cancer of the stomach: A study on histogenesis of gastric carcinoma. Int J Cancer 1975;16:52–60.

6 Fiocca R, Villani L, Tenti P, Solcia E, Cornaggia M, Frigerio B, Capella C: Characterization of 4 main cell types in gastric cancer: Foveolar, mucopeptic, intestinal columnar and goblet cells. A histopathologic, histochemical and ultrastructual study of early and advanced tumours. Pathol Res Pract 1987;182:308–325.

7 Sepulveda AR, Wu L, Ota H, Gutierrez O, Kim JG, Genta RM, Graham DY: Molecular identification of main cellular lineages as a tool for the classification of gastric cancer. Hum Pathol 2000;31:566–574.

8 Machado JC, Nogueira AM, Carneiro F, Reis CA, Sobrinho-Simoes M: Gastric carcinoma exhibits distinct types of cell differentiation: An immunohistochemical study of trefoil peptides (TFF1 and TFF2) and mucins (MUC1, MUC2, MUC5AC, and MUC6). J Pathol 2000;190:437–443.

9 Correa P: A human model of gastric carcinogenesis. Cancer Res 1988;48:3554–3560.

10 Correa P: Human gastric carcinogenesis: A multistep and multifactorial process-first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735–6740.

11 Correa P, Shiao YH: Phenotypic and genotypic events in gastric carcinogenesis. Cancer Res 1994;54:1941–1943.

12 Sipponen P, Kekki M, Siurala M: Atrophic chronic gastritis and intestinal metaplasia in gastric carcinoma: Comparison with a representative population sample. Cancer 1983;52: 1062–1068.

13 Sasaki I, Yao T, Nawata H, Tsuneyoshi M: Minute gastric carcinoma of differentiated type with special reference to the significance of intestinal metaplasia, proliferative zone, and p53 protein during tumor development. Cancer 1999;85:1719–1729.

14 Tatematsu M, Ichinose M, Miki K, Hasegawa R, Kato T, Ito N: Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pepsinogen immunohistochemistry and mucin histochemistry. Acta Pathol Jpn 1990;40:494–504.

15 Egashira Y, Shimoda T, Ikegami M: Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. Pathol Int 1999;49: 55–61.

16 Endoh Y, Tamura G, Watanabe H, Ajioka Y, Motoyama T: The common 18-base pair deletion at codons 418–423 of the E-cadherin gene in differentiated-type adenocarcinomas and intramucosal pre-cancerous lesions of the stomach with the features of gastric foveolar epithelium. J Pathol 1999;189:201–206.

17 Kawaguchi H, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection is the major risk factor for atrophic gastritis. Am J Gastroenterol 1996;91: 959–962.

18 Komoto K, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, Kajiyama G, Talley NJ: *Helicobacter pylori* infection and gastric neoplasia: Correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998;93:1271–1276.

19 Haruma K, Komoto K, Kamada T, Ito M, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection is a major risk factor for gastric carcinoma in young patients. Scand J Gastroenterol 2000;35:255–259.

20 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.

21 Sugiyama A, Murata F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M: *Helicobacter pylori* infection enhances N-methyl-N-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 1998;58:2067–2069.

22 Tatematsu M, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H Kaminishi M, Oohara T, Sugiyama A, Ikeno T: Induction of glandular stomach cancers in *Helicobacter pylori*-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N′-nitro-N-nitrosoguanidine in drinking water. Jpn J Cancer Res 1998;89:97–104.

23 Watanabe T, Tada M, Nagai H, Sasaki S, Nakano M: *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology 1998;115:642–648.

24 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Schistosomes, liver flukes, and *Helicobacter pylori*. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241.

25 Japanese Research Society for Gastric Cancer: The General Rules for Gastric Cancer Study, ed 13. Tokyo, Kinbara, 1999.

26 Bara J, Gautier R, Daher N, Zaghouani H, Decaens C: Monoclonal antibodies against oncofetal mucin M1 antigens associated with precancerous colonic mucosae. Cancer Res 1986; 46:3983–3989.

27 Bara J, Gautier R, Mouradian P, Decaens C, Daher N: Oncofetal mucin M1 epitope family: Characterization and expression during colonic carcinogenesis. Int J Cancer 1991;47: 304–310.

28 Ishihara K, Kurihara M, Goso Y, Urata T, Ota H, Katsuyama T, Hotta K: Peripheral α-linked N-acetylglucosamine on the carbohydrate moiety of mucin derived from mammalian gastric gland mucous cells: Epitope recognized by a newly characterized monoclonal antibody. Biochem J 1996;318:409–416.

29 Nakamura N, Ota H, Katsuyama T, Akamatsu T, Ishihara K, Kurihara M, Hotta K: Histochemical reactivity of normal, metaplastic, and neoplastic tissues to α-linked N-acetylglucosamine residue-specific monoclonal antibody HIK1083. J Histochem Cytochem 1998;46: 793–801.

30 Hertzog PJ, Robinson HC, Ma J, Mackay IR, Linnane AW: Oncofetal expression of the human intestinal mucin glycoprotein antigens in gastrointestinal epithelium defined by monoclonal antibodies. Int J Cancer 1991;48:355–363.

31 Pinczower GD, Williams RP, Gianello RD, Robinson HC, Preston BN, Linnane AW: Characterization of the tumour-associated carbohydrate epitope recognized by monoclonal antibody 4D3. Int J Cancer 1996;66:636–644.

32 Tytgat KM, Buller HA, Opdam FJ, Kim YS, Einerhand AW, Dekker J: Biosynthesis of human colon mucin: MUC2 is the prominent secretory mucin. Gastroenterology 1994;107: 1352–1363.

33 Ajioka Y, Watanabe H, Jass JR: MUC1 and MUC2 mucin in flat and polypoid colorectal adenomas. J Clin Pathol 1997;50:417–421.

34 Ajioka Y, Allison LJ, Jass JR: Significance of MUC1 and MUC2 mucin expression in colorectal cancer. J Clin Pathol 1996;49:560–564.

35 Dixon MF, Genta RM, Yardley JH, Correa P: Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–1181.

36 Chen X, Haruma K, Kamada T, Mihara M, Komoto K, Yoshihara M, Sumii K, Kajiyama G: Factors that affect results of the 13C urea breath test in Japanese patients. Helicobacter 2000;5:98–103.

37 Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Kitadai Y, Komoto K, Tanaka S, Kajiyama G: Correlation of ratio of serum pepsinogen I and II with prevalence of gastric cancer and adenoma in Japanese subjects. Am J Gastroenterol 1998;93:1090–1096.

38 Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI: Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology: A study in relatives of patients with pernicious anemia. Gastroenterology 1982;83:204–209.
39 Koseki K, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K: Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. Cancer 2000;89:724–732.
40 Saito A, Shimoda T, Nakanishi Y, Ochiai A, Toda G: Histologic heterogeneity and mucin phenotypic expression in early gastric cancer. Pathol Int 2001;51:165–171.
41 Kabashima A, Yao T, Sugimachi K, Tsuneyoshi M: Gastric or intestinal phenotypeic expression in the carcinomas and background mucosa of multiple early gastric carcinomas. Histopathology 2000;37:513–522.
42 Takahashi H, Endo T, Yamashita K, Arimura Y, Yamamoto H, Sasaki S, Itoh F, Hirata K, Imamura A, Kondo M, Sato T, Imai K: Mucin phenotype and microsatellite instability in early multiple gastric cancers. Int J Cancer 2002; 100:419–424.
43 Katsuyama T, Spicer SS: Histochemical differentiation of complex carbohydrates with variants of the concanavalin A-horseradish peroxidase method. J Histochem Cytochem 1978;26: 233–250.
44 Mcintosh GG, Lodge AJ, Watson P, Hall AG, Wood K, Anderson JJ, Angus B, Horne CH, Milton ID: NCL-CD10–270: A new monoclonal antibody recognizing CD10 in paraffin-embedded tissue. Am J Pathol 1999;154:77–82.
45 Tajima Y, Shimoda T, Nakanishi Y, Yokoyama N, Tanaka T, Shimizu K, Saito T, Kawamura M, Kusano M, Kumagai K: Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. Oncology 2001;61:212–220.
46 Yao T, Kajiwara M, Kuroiwa S, Iwashita A, Oya M, Kabashima A, Tsuneyoshi M: Malignant transformation of gastric hyperplastic polyps: Alternation of phenotypes, proliferative activity, and p53 expression. Hum Pathol 2002;34:1016–1022.
47 Sasaki A, Kitadai Y, Ito M, Sumii M, Tanaka S, Yoshihara M, Haruma K, Chayama K: *Helicobacter pylori* infection influences tumor growth of human gastric carcinomas. Scand J Gastroenterol 2003;38:153–158.
48 Tahara E: Molecular mechanism of stomach carcinogenesis. J Cancer Res Clin Oncol 1993; 119:265–272.
49 Endo Y, Sakata K, Tamura G, Ohmura K, Ajioka Y, Watanabe H, Motoyama T: Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach depend on the genetic pathways. J Pathol 2000; 191:257–263.

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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).

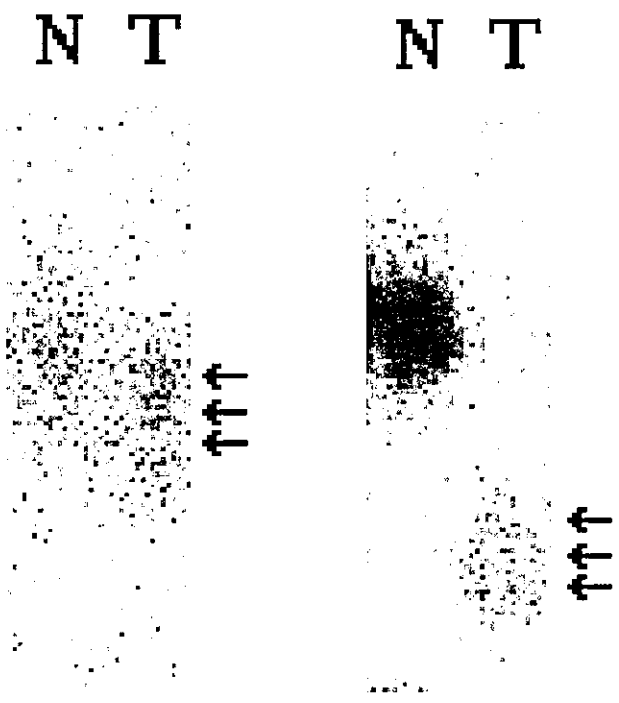

Figure 1 Representative examples of microsatellite instability at BAT26. N, normal; T, tumor.

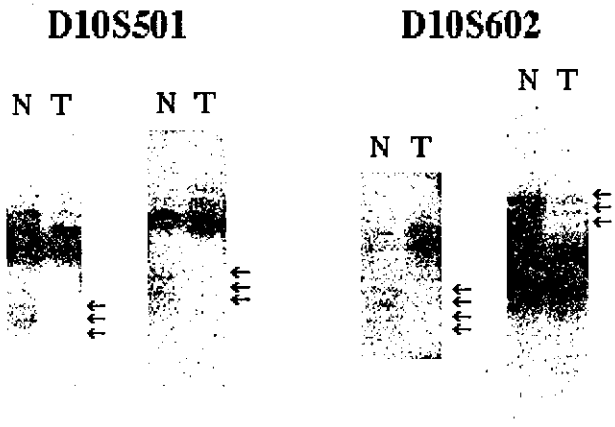

Figure 2 Representative examples of loss of heterozygosity. N, normal; T, tumor. Microsatellite loci are listed on the lanes.

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]

Table 2 LOH at specific chromosome loci in sporadic gastric cancers

Chromosome	Frequency of LOH *n* (%)
10p	17/57 (30)
18q	17/57 (30)
5q	6/29 (21)
17p	9/63 (14)
17q	6/51 (12)

LOH, loss of heterozygosity. Markers used were D5S505 (5q), D10S501, D10S602 (10p), TP53 (17p), D17S855 (17q), D18S58 and D18S61 (18q).

Table 3 Patterns of LOH and MSI-H for sporadic gastric cancers

	MSI-H	
	(–) *n* (%)	(+) *n* (%)
LOH (–)	47 (48)	8 (8)
LOH (+)	39 (40)	4 (4)

LOH, loss of heterozygosity; MSI-H, high-frequency microsatellite instability. Markers used were D5S505 (5q), D10S501, D10S602 (10p), TP53 (17p), D17S855 (17q), D18S58 and D18S61 (18q).

We next investigated the degree of overlap between tumors with LOH and those with MSI-H (Table 3). Of the 43 tumors with LOH, four (9%) were MSI-H. Of 86 tumors without MSI-H, 39 (45%) also contained an LOH event at one or more of the loci examined, as did four (33%) of the 12 MSI-H tumors. We found that 4% of all tumors were both LOH(+) and MSI-H(+), 8% were LOH(–) and MSI-H(+), whereas 40% of the tumors were LOH(+) and MSI-H(–). Interestingly, we identified a group of 48% of all gastric cancers examined that were LOH(–) and MSI-H(–), and therefore did not demonstrate signs for either of the two pathways of genomic instability.

DISCUSSION

This study indicates that CIN and MSI pathways are not always independent and that some tumors show overlap between these two mechanisms in sporadic gastric cancers. Our current understanding of gastric carcinogenesis suggests that at least two mechanisms are capable of producing the mutations that are required for a cell to demonstrate a malignant phenotype. These mechanisms include CIN, characterized in tumor DNA by the presence of multiple LOH events, and loss of MMR function, which is defined by MSI-H.

In the present study MSI-H was defined by the microsatellite assay with BAT26. BAT26 is a polyadenine tract localized in the fifth intron of the *hMSH2* gene.[19] The locus does not show important size variation between both alleles, nor between individuals in DNA from normal tissues and microsatellite stable tumors and cell lines, and was thus quasi-monomorphic. It has been indicated that a single test of BAT26 could identify the MSI-H cases defined by the National Cancer Institute (Bethesda consensus panel).[19-21]

To detect LOH, we used seven polymorphic markers mapped closely to key tumor suppressor genes that may be lost during gastric carcinogenesis. Detection of one or more of these loci was taken as evidence of loss of tumor suppressor activity by CIN. It is highly unlikely that a tumor could exhibit the widespread LOH that is characteristic of tumors arising in the setting of CIN without a single LOH event at the seven markers examined.

With obtained results, we classified the cancers into four subtypes; MSI-H(+)/LOH(–), MSI-H(+)/LOH(+), MSI-H(–)/LOH(–), and MSI-H(–)/LOH(+). The MSI-H(+)/LOH(–) tumors were found in 8% of the cancers analyzed. The MSI-H in the sporadic gastric cancers is caused by a defect in DNA MMR capability, which most commonly is achieved by hypermethylation of the *hMLH1* promoter.[10] Cancers associated with MMR defects may demonstrate amplifications and deletions of single alleles or chromosomes. In agreement with this, MSI-H and LOH events coincided in 4% of the cancers in the present study. The LOH events in MSI-H tumors may be caused by a general genomic instability that is typical for neoplasms.

Loss of heterozygosity was observed in 44% of the cancers examined. Tumors that contained LOH without MSI-H comprised 40% of the cancers. The presumed course of tumor progression in this subset involves accumulated allelic losses at tumor suppressor loci. In the literature, frequencies in gastric cancer range from 9 to 30% for LOH of the *APC* gene,[8,22] from 30 to 50% for LOH of the *DCC* gene,[23] and from 30 to 65% for LOH of the *p53* gene.[11,24] In the present study, LOH at the *APC*, *DCC* and *p53* gene loci was identified in 22%, 12%, and 14% of gastric cancers, respectively. Our data on the frequencies of LOH at the *APC* gene loci are consistent with those of previous studies. In contrast, LOH at the *DCC* and *p53* gene loci was relatively lower than those of previous studies. It has been reported that alterations of the *DCC* and *p53* genes were significantly more frequent in advanced-stage gastric cancers than in early stage cancers.[24,25] The present study included a relatively high frequency of early stage cases. This may be the reason why our data on the frequencies of LOH at the loci were relatively lower.

The most interesting subgroup identified was that containing approximately half of the sporadic gastric cancers, in which evidence of both MSI and LOH was lacking. These cancers may be associated with the transcriptional silencing of growth and differentiation genes by epigenetic alterations. Hypermethylation of the promoter region of tumor suppressor genes, which leads to loss of tumor suppressor function, has been observed in a variety of cancers. Even in normal tissues, methylation

was observed in an age-dependent manner.[10] Some tumors show the methylation phenotype, indicating simultaneous methylation of multiple loci. Genes associated with carcinogenesis, such as *APC*, *p16*, and *IGFII*, are silenced by their promoter methylation. Toyota *et al.* reported that approximately 40% of gastric cancers may be of the methylation phenotype, and suggest that methylation phenotype may be one of the major pathways that contribute to tumorigenesis in gastric cancers.[10] We did not examine the methylation status in the present cases. This will be addressed in a future examination.

The MSI-H tumors exhibited distinct clinicopathological features, including predominant antral location and a lower frequency of lymph node metastasis, compared with tumors without MSI-H.[12] These results strengthen the hypothesis that gastric cancers with MSI-H are clinicopathologically distinguishable from those without MSI-H. In the present study, we classified the cancers into four subtypes by LOH and MSI status. We did not find any significant differences in clinicopathological parameters, including patient age, sex, tumor location and histological subtype between these subtypes (data not shown). However, only a limited number of patients was examined in the present study, and to clarify the clinicopathological differences further examinations are necessary.

In conclusion, our data 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. Future studies that stratify gastric cancers on the basis of genetic and epigenetic changes may identify factors that contribute to one pathway or others.

REFERENCES

1 Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics 1998. *CA Cancer J. Clin.* 1998; **48**: 6–29.

2 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.* 1992; **52**: 6735–40.

3 Hiyama T, Yokozaki H, Kitadai Y *et al. In situ* mRNA hybridization technique for analysis of human telomerase RNA in gastric precancerous and cancerous lesions. *Jpn J. Cancer Res.* 1998; **89**: 1187–94.

4 Tahara E. Molecular mechanism of stomach carcinogenesis. *Cancer Res. Clin. Oncol.* 1993; **119**: 265–72.

5 Loeb LA. Microsatellite instability: marker of a mutator phenotype in cancer. *Cancer Res.* 1994; **54**: 5059–63.

6 Hiyama T, Yokozaki H, Shimamoto F *et al.* Frequent *p53* gene mutations in serrated adenomas of the colorectum. *J. Pathol.* 1998; **186**: 131–9.

7 Liu B, Parsons R, Papadopoulos N *et al.* Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat. Med.* 1996; **2**: 169–74.

8 Semba S, Yokozaki H, Yamamoto S, Yasui W, Tahara E. Microsatellite instability in precancerous lesions and adenocarcinomas of the stomach. *Cancer* 1996; 77: 1620–7.

9 Miyoshi M, Haruma K, Hiyama T *et al.* Microsatellite instability is a genetic marker for the development of multiple gastric cancers. *Int. J. Cancer* 2001; **95**: 350–3.

10 Toyota M, Ahuja N, Suzuki H *et al.* Aberrant methylation in gastric cancer associated with the CpG island methylation phenotype. *Cancer Res.* 1999; **59**: 5438–42.

11 Renault B, Calistri D, Buonsanti G, Nanni O, Amadori D, Ranzani GN. Microsatellite instability and mutations of *p53* and TGF-β *RII* genes in gastric cancer. *Hum. Genet.* 1996; **98**: 601–7.

12 Seruca R, Santos NR, David L *et al.* Sporadic gastric carcinomas with microsatellite instability display a particular clinicopathologic profile. *Int. J. Cancer* 1995; **64**: 32–6.

13 Hemminki A, Peltomaki P, Mecklin JP *et al.* Loss of the wild type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat. Genet.* 1994; **8**: 405–10.

14 Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma: an attempt at a histoclinical classification. *Acta Pathol. Microbiol. Scand.* 1965; **64**: 31–49.

15 Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma, 2nd English edition. *Gastric Cancer* 1998; **1**: 10–24.

16 Hiyama T, Haruma K, Kitadai Y *et al. Helicobacter pylori* eradication therapy for high-grade mucosa-associated lymphoid tissue lymphomas of the stomach with analysis of *p53* and K-*ras* alteration and microsatellite instability. *Int. J. Oncol.* 2001; **18**: 1207–12.

17 Hiyama T, Tanaka S, Shima H *et al.* Frequent loss of heterozygosity on chromosome 10p15, a putative telomerase repressor/senescence gene locus, in gastric cancer. *Oncol. Rep.* 2003; **10**: 1297–9.

18 Japanese Gastric Cancer Association. *Japanese Classification of Gastric Cancer.* Tokyo: Kanehara, 1998.

19 Zhou XP, Hoang JM, Li YJ *et al.* Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites. *Genes Chromosomes Cancer* 1998; **21**: 101–7.

20 Boland CR, Thibodeau SN, Hamilton SR *et al.* A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998; **58**: 5248–57.

21 Hoang J-M, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R. BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. *Cancer Res.* 1997; **57**: 300–3.

22 Powell SM, Cummings OW, Mullen JA *et al.* Characterization of the *APC* gene in sporadic gastric adenocarcinomas. *Oncogene* 1996; **12**: 1953–9.

23 Fang DC, Jass JR, Wang DX. Loss of heterozygosity and loss of expression of the *DCC* gene in gastric cancer. *J. Clin. Pathol.* 1998; **51**: 593–6.

24 Wu MS, Shun HP, Wang HP *et al.* Genetic alterations in gastric cancer: relation to histological subtypes, tumor stage, and *Helicobacter pylori* infection. *Gastroenterology* 1997; **112**: 1457–65.

25 Rajnakova A, Moochhala S, Goh PM, Ngoi S. Expression of nitric oxide synthase, cyclooxygenase, and p53 in different stages of human gastric cancer. *Cancer Lett.* 2001; **172**: 177–85.

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Digestive Endoscopy

Magnifying gastroendoscopy for diagnosis of histologic gastritis in the gastric antrum

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See related commentary on pages 251–252

Abstract

Background. We investigated the potential of magnifying endoscopy for diagnosis of histologic gastritis in the gastric antrum. In addition, we investigated whether magnifying endoscopy can be applied for evaluation of *Helicobacter pylori* eradication therapy.

Methods. We examined 176 Japanese patients including 53 with *H. pylori* eradication. We evaluated the antrum by magnifying observation and ordinary endoscopic findings, and compared these results. Biopsy specimens were taken from the sites observed.

Results. The magnified views were classified into four types. Histology of the biopsy specimens allowed us to match the four magnified views with normal mucosa with fundic glands, normal mucosa with pyloric glands, mucosa with gastritis and intestinal metaplasia/epithelial hyperplasia. The types of magnifying appearances were specific enough for the diagnosis of histologic gastritis (148 out of the 176 (82.4%) cases; sensitivity, 96.3%; specificity, 73.7%). We could accurately diagnose the histologic gastritis by magnifying endoscopy in 49 out of the 53 (92%) cases with *H. pylori* eradication, while only in 38% by ordinary endoscopy. The accuracy of diagnosis was statistically higher with the use of magnifying endoscopy than with ordinary endoscope ($P < 0.001$).

Conclusion. Magnifying gastroendoscopy is useful to judge the histologic gastritis, especially, in cases with *H. pylori* eradication.

Keywords: Eradication therapy; Gastritis; *Helicobacter pylori*; Magnifying endoscopy

1. Introduction

Gastric diseases including peptic ulcer and gastric cancer are firmly linked with chronic gastritis induced by *Helicobacter pylori* infection [1–4]. The correct diagnosis of histologic gastritis is difficult by endoscopic observation, making gastric biopsy necessary. Histologic gastritis is recognised endoscopically by the atrophic border [5]. Changes in the areae gastricae are also used diagnostically [6]. Chromoendoscopy has been useful for identifying intestinal metaplasia [7,8]. Although endoscopic attempts have been made to classify gastritis, no markers of inflammation activity have been defined. Sakaki et al. established a classification system for magnifying gastroendoscopy, but it has not been applied clinically because of difficulties in observation and the complexity of classification [9]. Recently, it has been reported that the regular arrangement of collecting venules (RAC) finding is useful for judging gastritis in the gastric corpus [10]. However, no endoscopic criteria have been established for determination of gastritis till date, especially in the antrum.

We reported previously that feature of the gastric folds in the corpus showed good correlation with the degrees of histologic gastritis, because pan-gastritis is common in Japanese patients [11]. Changes in these gastric folds indicate the presence of histologic gastritis also in the antrum. In patients less than 49 years of age, however, it is difficult to diagnose gastritis correctly, because atrophic change is not as obvious in these patients as it is in patients over 50 years of age [11]. In addition, eradication therapy for *H. pylori* makes correct diagnosis even more complicated. It is likely that active histologic gastritis improves for a short

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period after eradication of *H. pylori*, though it takes a relatively long time for atrophic change [12,13]. And it is still unknown which part is proper to detect the histological modification of eradication therapy.

Progress in magnifying endoscopy has made it possible to observe microstructures of the gastrointestinal mucosae. Magnifying observation has become essential for the clinical diagnosis of colorectal diseases [14,15]. In the present study, we tried observing the features of the gastric antrum directly using a new magnifying gastroscope. We analysed our endoscopic findings of the antrum in comparison to histologic findings. We established a new classification system for endoscopic findings to be used in the diagnosis of histologic gastritis. In addition, we examined the possibility of evaluating *H. pylori* eradication therapy under magnifying gastroendoscopy.

2. Patients and methods

2.1. Patients

We examined 176 Japanese patients (84 men and 92 women, mean age 57.0 years, range 21–84 years) with dyspepsia including 53 patients who underwent *H. pylori* eradication therapy more than 12 months prior to our study at Hiroshima University Hospital. *H. pylori* infection was evaluated on the basis of anti-*H. pylori* antibody titres (Pylori Stat Kit, Whittaker Bioproducts Inc., Walkersville, MD), histologic examination and ^{13}C urea breath test. Patients in whom any of these assays were positive were classified as *H. pylori*-positive. Those in whom all three were negative were considered *H. pylori*-negative. All patients were informed of the purpose of the study and agreed to magnifying examination and biopsy.

2.2. Gastroendoscopy

We used a magnifying videoendoscope (EG-450ZH and EG-450ZW5, Fujinon Co. Ltd., Saitama, Japan) with a zoom (80×) and TV monitor (38 cm). We evaluated the presence of ordinary endoscopic gastritis by the criteria of Sydney system [16] and with the findings of gastric fold [11]. After routine endoscopic examination without using the cap, indigocarmine dye was applied to the gastric antrum. The gastric antrum was widely observed and it was recorded in image filing system (nexus sif SD, Nexus Co. Ltd., Tokyo, Japan). At the time of endoscopy, magnifying observation was carried out in the greater curvature of the gastric antrum (approximately 5 cm from the pyloric ring). Two endoscopy specialists independently classified the ordinary and magnifying endoscopic findings without the patient information including the result of the eradication therapy. When we found the heterogeneity in the magnifying observation, we judged by the major appearance of magnifying endoscopy. A biopsy specimen with the use of standard forceps was obtained from the lesions that were observed, and histologic gastritis in haematoxylin and eosin (HE)-stained sections were estimated with the use of updated Sydney system [17]. The pathologist judged the histological finding without having information of clinical and endoscopic findings.

2.3. Statistical analysis

Statistical analysis was performed by χ^2-test with StatView software (SAS Institute Inc., Cary, NC). A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Classification of magnifying view in the gastric antrum

Total magnifying endoscopy findings of the 176 patients were classified into four types: pinpoint pits on the flat field (type 1, Fig. 1A); a regular, trabecular ridge pattern or a regular, flat granular pattern (type 2, Fig. 1B); mucosa consisting of irregular and coarse granular structure (type 3, Fig. 1C); and a prominent clubbing (villous) or papillary pattern (type 4, Fig. 1D). Kappa value of the classification by two endoscopists was calculated as 0.906 (95% confidence interval (95% CI): 0.846–0.966).

Representative histologic features corresponding to each of these four patterns are shown in Fig. 2. Normal mucosa with fundic and pyloric glands with no or mild inflammation were found to correspond to types 1 and 2 of magnifying endoscopy findings, respectively (Fig. 2A and B). A small part of the patient shows the histological feature of fundic glands even if we have collected the biopsy specimen from the gastric antrum. Active inflammation was detected in the lesion showing type 3 (Fig. 2C). Intestinal metaplasia corresponded with type 4 (Fig. 2D). In type 4 cases, without intestinal metaplasia, severe inflammation with epithelial hyperplasia was frequently observed.

3.2. Relation between endoscopic findings and histologic gastritis

The histological findings characterising each magnifying endoscopy type are summarised in Table 1. All type 1 sections consisted of normal mucosa with fundic glands.

Table 1
Relation between magnifying endoscopic and histologic findings

	Type 1 (n = 11)	Type 2 (n = 84)	Type 3 (n = 64)	Type 4 (n = 17)
Atrophy[a]	0 (0)	12 (14)	44 (69)	11 (65)
Mononuclear cell infiltration[a]	1 (9)	14 (17)	44 (69)	14 (82)
Neutrophil infiltration[b]	0 (0)	15 (18)	44 (69)	13 (76)
Intestinal metaplasia[b]	0 (0)	9 (11)	28 (44)	7 (41)

Data is presented as number of cases (%).
[a] Moderate or severe.
[b] Mild to severe.

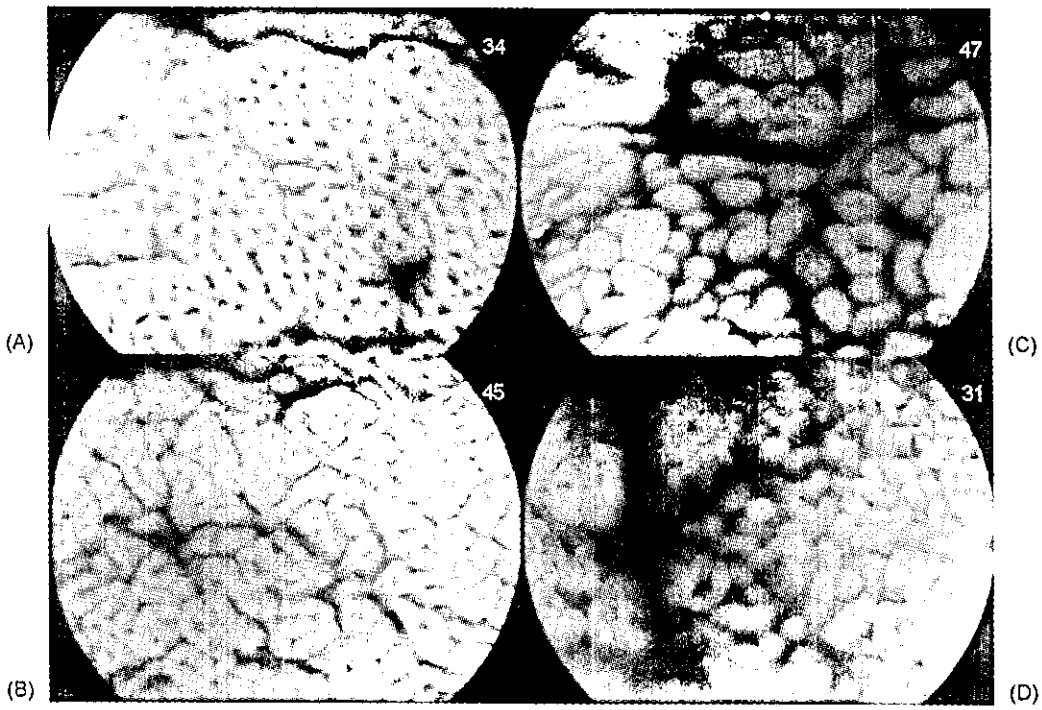

Fig. 1. The four types of magnifying endoscopy appearances. (A) Type 1 is pinpoint pits on the flat field. (B) Type 2 is a regular, trabecular ridge pattern or a regular, flat granular pattern. (C) Type 3 is mucosa consisting of irregular and coarse granular structure. (D) Type 4 is a prominent clubbing (villous) or papillary pattern (magnification 80×).

Type 2 sections included normal mucosa with pyloric glands. The histologic features of gastritis (mononuclear cell infiltration, neutrophil infiltration, atrophy and intestinal metaplasia) were more frequently observed in the type 3 and 4 sections than in the type 1 and 2 sections. Sixty-two out of the 64 (96.9%) type 3 sections showed histologic gastritis. In type 4 sections, no normal features were observed and intestinal metaplasia or severe inflammation was clearly seen.

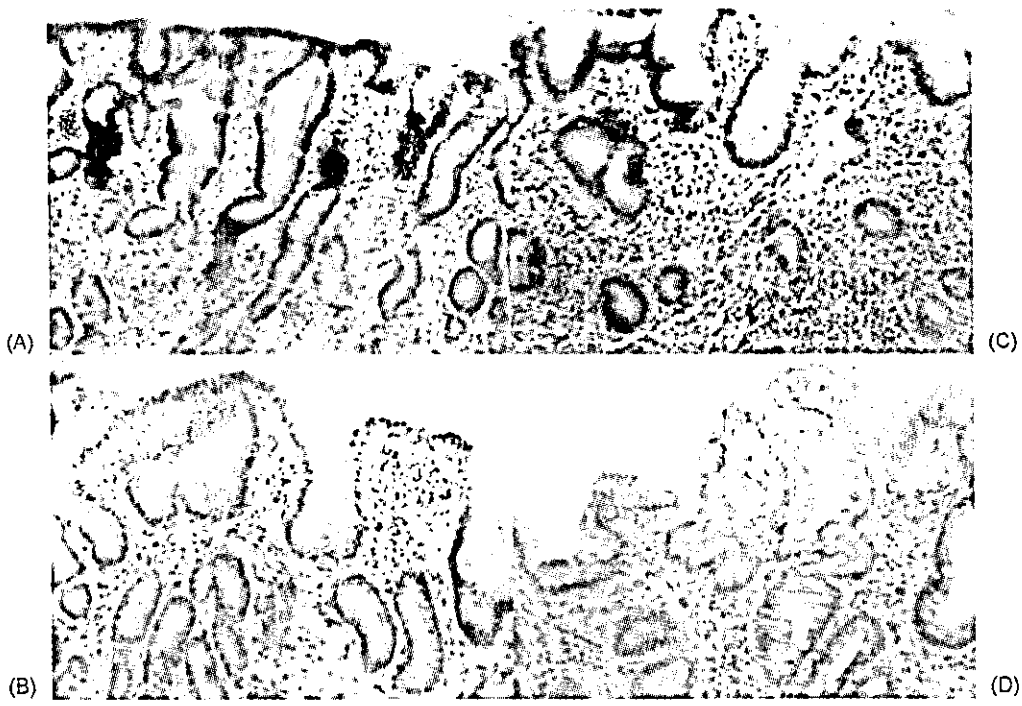

Fig. 2. Histologic features corresponding to the four magnifying appearances. (A) Type 1 represents normal fundic mucosa. (B) Type 2 represents normal pyloric mucosa. (C) Type 3 represents mucosa with active inflammation. (D) Type 4 represents intestinal metaplasia or severe inflammation with epithelial hyperplasia. Sections were stained with HE.

Table 2
Relationship between magnifying endoscopic findings and histologic gastritis

	Magnifying findings		Total
	Types 1 and 2	Types 3 and 4	
Histologic gastritis (−)	70	3	70
Histologic gastritis (+)	25	78	106
Total	95	81	176

Data is presented as number of cases.

3.3. Diagnosis of histologic gastritis

We regarded magnifying endoscopy type 1 and 2 images are gastritis-negative. Histologically, corresponding sections were normal, showing no neutrophil infiltration, no intestinal metaplasia and none or mild lymphocyte infiltration [18]. As shown in Table 2, the types of magnifying endoscopy were specific enough for diagnosis of histologic gastritis and its status in 148 out of the 176 (84.1%) cases. Sensitivity and specificity of diagnosis of histological gastritis by magnifying endoscopic examination were 96.3 and 73.7%, respectively.

Further, we evaluated the ordinary endoscopic findings in the same patients and compared the results with that of the magnifying endoscopy. One hundred and six cases were judged as "gastritis" and most of these were regarded as atrophic gastritis by Sydney system and by enlarged and swollen folds. With the use of ordinary endoscopy, we could diagnose the histologic gastritis correctly only in 107 out of the 176 (60.8%) cases (Table 3). Sensitivity and specificity of diagnosis of histologic gastritis by ordinary endoscopic examination were 66 and 52.9%, respectively. The accuracy was inferior to that of the magnifying endoscopy ($P = 0.006$).

3.4. Evaluation of gastritis in patients with H. pylori eradication therapy

Of the 53 patients who underwent *H. pylori* eradication therapy, 40 were considered to be treated successfully. In patients with successful eradication therapy, we confirmed the absence of active inflammation, whereas persistent active inflammation was detected in the patients with failed eradication therapy. Out of the 40 successful eradication therapies, 39 (98%) had mainly type 1 and 2 endoscopy findings (Table 4), whereas 10 out of the 13 patients, in whom *H. pylori* eradication treatment failed, had type 3 and 4 magnifying observation findings. In 49 out of the 53 (92%) patients, we could completely diagnose the status of histologic gastritis by magnifying endoscopy, while we could only diagnose only in 20 (38%) by ordinary endoscopy. The accuracy of diagnosis was statistically higher with the use of magnifying endoscopy than of ordinary endoscope ($P < 0.001$; Table 4). We have four patients in whom we could perform magnifying endoscope before and after eradication therapy. We confirmed that findings of magnifying endoscope improved from abnormal pattern (three of type 3 and one of type 4) to normal pattern of type 2 (data not shown), while the atrophic finding still remained by ordinary endoscopic observation.

Table 3
Relationship between ordinary endoscopic findings and histologic gastritis

	Endoscopic gastritis		Total
	−	+	
Histologic gastritis (−)	37	36	73
Histologic gastritis (+)	33	70	103
Total	70	106	176

Data is presented as number of cases.

Table 4
Diagnostic ability of gastritis in patients after *H. pylori* eradication

	H. pylori eradication		Accuracy (%)
	Success ($n = 40$)	Fail ($n = 13$)	
By magnifying endoscopy			
Types 1 and 2	39	1	49/53 (92.5)*
Types 3 and 4	3	10	
By ordinary endoscopy			
Gastritis (−)	14	5	20/53 (37.7)*
Gastritis (+)	28	6	

* $P < 0.001$, Yates' corrected χ^2-test.

4. Discussion

It is clinically important to diagnose the presence of histologic gastritis. Many gastric diseases are closely associated with the presence of histologic gastritis induced by *H. pylori* infection [1–3]. Notably, the presence of histologic gastritis with *H. pylori* infection is considered as a risk factor for gastric cancer [4,19,20]. Till date, diagnosis of gastritis has been dependent upon histologic examination by multiple biopsies and does not provide information for the entire stomach. Endoscopic examination for gastritis is considered a non-invasive examination and provides total observation of the stomach not as a "point" but as a "field". However, no objective clinical criteria have been established.

We were able to establish a four-grade classification system for magnifying endoscopy determination of histologic gastritis by matching magnifying examination appearances with histologic features. We focussed on the gastric antrum because the effect of heartbeats is less and few reports have mentioned the feature of magnifying endoscopy in the gastric antrum. We first clarified the normal appearance of the gastric mucosa. Type 1 features represent normal mucosa with fundic glands. The type 1 features represent the narrow and small orifices of the fundic glands, as also reported