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Advanced Method for Evaluation of Gastric Cancer Risk By Serum Markers: Determination of True Low-Risk Subjects for Gastric Neoplasm

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Helicobacter pylori, risk management, gastric cancer, pepsinogen, gastrin.

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Abstract

Background: Patients with negative anti-*Helicobacter pylori* antibody titer and high pepsinogen (PG) level (group A) are regarded as having a low risk for gastric cancer. However, gastric cancer cases are occasionally observed in this group. We aimed to elucidate the clinical features of gastric neoplasm in group A patients and reviewed advanced methods for mass screening.

Materials and Methods: A total of 271 gastric epithelial neoplasm patients were enrolled. We classified them according to the *H. pylori*-PG system and determined the number of patients in each group. After excluding true *H. pylori*-negative cases from group A (group A′), we examined the differences between group A′ and group non-A.

Results: Group A included 30 (11%) patients, and only three of these were true negative for *H. pylori*. All patients in group A′ (n = 27) exhibited endoscopic atrophy in the gastric corpus. Serologically, these patients showed low gastrin, low PG II and high PG I/II ratio, indicative of post-eradication. Histologically, 24 (89%) of these had little inflammation, and 26 (96%) were negative for *H. pylori* by immunohistochemistry. No difference was observed in the incidence of metachronous gastric tumors between group A′ and group non-A. The discriminant function using gastrin and PGs could distinguish these 27 patients from true *H. pylori*-negative controls with 85% sensitivity and 84% specificity.

Conclusions: Group A included a certain number of patients with atrophic gastritis who were potentially at risk of gastric neoplasm development. Although evaluation of corpus atrophy is necessary for the identification of these patients, the discriminant function may be useful.

In Japan, the incidence of gastric cancer is the highest among developed countries and is the second cause of cancer-related death, although its associated mortality has continued to decrease in recent decades [1,2]. To decrease cancer-related deaths in Japan, early detection of gastric neoplasm by an effective mass screening system and early treatment are very important. Recently, endoscopic submucosal dissection (ESD) for early gastric cancer is widely performed in Japan, and a lot of gastric neoplasms generally become indication for the endoscopic resection.

A number of epidemiologic studies have indicated that a significant relationship exists between *Helicobacter pylori* infection and gastric cancer development [3,4]. To date, many basic and clinical studies have indicated that *H. pylori* infection is an important and crucial factor for gastric cancer development [5,6]. Indeed, we have recently reported that the incidence of true *H. pylori*-negative gastric cancer is quite low [7]. Therefore, for gastric cancer screening, it is quite important to evaluate the status of *H. pylori* infection in each person.

Atrophic gastritis induced by *H. pylori* infection is another important risk factor associated with gastric cancer [8, 9]. Gastric atrophy in the gastric corpus is strongly associated with gastric cancer development, particularly intestinal-type cancers. Histologic evaluation is necessary to determine the grade of atrophy, although this method

is invasive. For the application of a mass screening system, a more objective and easy method should be considered. Miki et al.[10] developed a serum screening system that involved the evaluation of pepsinogen (PG) levels, which are known to reflect the status of gastric inflammation including corpus atrophy. A previous study demonstrated that a combination panel using serum anti-*H. pylori* antibody titers and serum PG levels (called the "ABC system") was effective for evaluating the individual risk for gastric cancer [11]. Several cities have already introduced this system as a mass screening program for gastric cancer [12].

In the ABC system, patients with negative anti-*H. pylori* antibody titers and high PG levels are classified into "group A," and are regarded as having a very low risk for gastric cancer [11,12]. To increase the efficiency of a mass screening system, it is quite important to identify "no risk" subjects and exclude them from mass screening. However, in clinical practice, gastric neoplasm is occasionally identified in patients in group A. The false-negative evaluation of gastric cancer risk must be prevented. In this study, we aimed to clarify the true risk for gastric epithelial neoplasm in patients classified as group A and retrospectively examined the clinicopathologic features of gastric neoplasms in group A. We also examined advanced methods for identifying the high-risk patients mixed into group A in a mass screening system for gastric cancer.

Methods

Patients

Of 1087 patients with gastric neoplasms (early gastric cancer and adenoma) who were treated with ESD at Hiroshima University Hospital between April 2002 and May 2010, we analyzed 373 patients with a prior evaluation of serum anti-*H. pylori* antibody titers and serum PG levels who were followed-up for more than 1 year without recurrence within 1 year in this study. We enrolled patients with gastric adenoma, because they were clinically diagnosed as having potent early gastric cancer with differentiated type, and regarded as an indication for endoscopic resection. We excluded patients with previous gastric surgical history, local recurrence of gastric neoplasm, gastric mucosa-associated lymphoid tissue lymphoma, Barrett's adenocarcinoma, severe renal dysfunction, previous *H. pylori* eradication therapy, and administration of proton pump inhibitor. We defined Barrett's adenocarcinoma as that endoscopically connected with Barrett's esophagus. Patients who had undergone additional resection of the stomach or gastric tube construction after ESD were also excluded. Typical case with EBV-related cancer [13] or hereditary cancer [14] case was not included. Patient with autoimmune gastritis [15] was also excluded. Finally, 271 patients (200 male, 71 female; mean age, 66.9 years) were enrolled in this study. Patients were followed-up by annual endoscopic examination in our hospital, and the average observation period was 40.4 (range 12.2–107) months. We also registered 213 subjects (132 male, 81 female; mean age, 57.1 years) as true *H. pylori*-negative controls; these subjects had no histologic atrophy of the gastric gland, no histologic inflammation of the gastric mucosa, and no histologic *H. pylori* infection or had no endoscopic gastric atrophy and negative anti-*H. pylori* antibody titers. In addition, we used the urea breath test (Otsuka, Tokushima, Japan) and rapid urease test (Pylori-Tek; Serim Research, IN, USA) for diagnosis of *H. pylori* infection. The protocol was approved by the Ethics Committee of Hiroshima University Hospital.

Evaluation of Endoscopic and Histologic Gastritis

Specimens obtained using ESD were fixed with buffered formalin and stained with hematoxylin and eosin. Gastritis scores in non-neoplastic mucosa obtained from the same region of gastric neoplasm and being far enough from it were independently evaluated by two specialists (MI and TB) using the updated Sydney system [16]. Endoscopic evaluation of atrophic gastritis was determined according to the criteria of Kimura and Takemoto [17]. Pathologic diagnosis of each neoplasm was judged according to the criteria of the Japanese Classification of Gastric Carcinoma [18].

Evaluation of Serum Markers

Fasting sera were collected and stored at −80 °C until use. Serum anti-*H. pylori* antibody titers (E-plate; Eiken, Japan), serum PG levels (LZ test; Eiken, Tokyo, Japan), and serum gastrin levels (Gastrin RIA Kit II; Dainabot, Tokyo, Japan) were evaluated [19]. If the antibody titer was >10 IU/L, the patients were considered *H. pylori*-positive. PG I ≤ 70 ng/mL and PG I/II≤3 were regarded as PG-positive, indicative of gastric mucosal atrophy [10]. We classified the patients into four groups, group A (*Hp*(−), PG(−)),group B (*Hp*(+), PG(−)), group C (*Hp*(+), PG(+)), and group D (*Hp*(−), PG(+)), according to the ABC method, and investigated the patients in group A.

Immunohistochemistry

We determined the presence of *H. pylori* infection using immunohistochemical staining with a polyclonal rabbit

anti-*H. pylori* antibody (Dako, Tokyo, Japan) as previously described [20]. Sections of fixed tissues (4 μm) were deparaffinized and rehydrated. After heat-induced epitope retrieval (95 °C, 20 minutes) in citrate buffer (pH 6.0), endogenous peroxidase was quenched with 0.3% H_2O_2 in methanol for 10 minutes, followed by rinsing with phosphate-buffered saline (PBS, pH 7.2). Non-specific binding was blocked with PBS containing 5% skim milk for 20 minutes. The sections were rinsed with PBS and incubated with primary antibodies overnight at 4 °C. We used the labeled streptavidin-biotin method (Dako, LSAB2 System-HRP, Japan), and diaminobenzidine-hydrogen peroxidase was used for color development. The tissues were finally counterstained lightly with hematoxylin.

Statistics

Statistical analyses for comparing categorical data were performed using the χ^2-test and Fisher's exact test, and the Wilcoxon rank sum test was used for numerical data, as appropriate. The cumulative incidence rate of metachronous gastric tumors was evaluated using Kaplan–Meier analysis. We used multivariate logistic regression for discriminant function. A p value of <.05 was considered significant. The JMP statistical software (SAS Institute Inc., Cary, NC, USA) was used for all calculations.

Results

Classification of Patients Using Anti-*H. pylori* Antibody Titers and Pepsinogen Levels

We evaluated the serum markers (anti-*H. pylori* antibody and PGs) and classified patients into four groups (A, B, C, and D) as previously described [21]. Of 271 patients, 30 (11.1%) were classified into group A, and 71, 153, and 17 were classified into group B, group C, and group D, respectively (Table 1). We confirmed that only three cases in group A were true negative for *H. pylori* as we described previously [7]. The remaining 27 patients (group A′) were considered those with previous *H. pylori* infection or whose anti-*H. pylori* antibody titers were false negative.

Table 1 The number of patients in each group classified with *Hp*-PG system

	Total	A *Hp* (−) PG (−)	B *Hp* (+) PG (−)	C *Hp* (+) PG (+)	D *Hp* (−) PG (+)
No. of patients	271	30[a]	71	153	17
%	100	11.1	26.2	56.5	6.3

Hp: *H. pylori*, PG: pepsinogen, *Hp* (+): anti-*Hp* antibody titer>10, PG (+): PG I ≤ 70 and PG I/II≤3.
[a]Including three true *Hp*-negative cases.

Comparison of Clinicopathologic Information Between group A' and Non-A

We compared the clinicopathologic features in group A′, regarded as low-risk group, with those in group non-A, regarded as high-risk groups (Table 2). Gastric neoplasms in group A′ tended to occur in the upper third of the stomach. The prevalence of depressed-type tumors was significantly higher in group A′ than in group non-A (p = .004). All patients in group A′ had endoscopic atrophy in the gastric corpus. Although no difference was observed in the extension of endoscopic

Table 2 The difference in clinicopathologic information between group A' and non-A

Factors	Group A' n = 27	Group non-A n = 241	p value
Age (years)			
Mean (range)	69.8 (49–88)	66.7 (35–84)	.10
Sex (%)			
Male	20 (74.1)	178 (73.9)	1.00
Female	7 (25.9)	63 (26.1)	
Location (%)			
Upper third	6 (22.2)	23 (9.5)	.05
Middle third	4 (14.8)	63 (26.1)	
Lower third	17 (63.0)	155 (64.3)	
Gross type (%)			
Elevated	5 (18.5)	115 (47.7)	.004
Depressed	22 (81.5)	126 (52.3)	
Synchronous multiple tumor (%)			
Negative	24 (88.9)	205 (85.1)	.78
Positive	3 (11.1)	36 (14.9)	
Histology (%)			
Intestinal	25 (92.6)	224 (92.9)	1.00
Diffuse	2 (7.4)	17 (7.1)	
Depth of invasion (%)			
Mucosa	24 (88.9)	217 (90.0)	.74
Submucosa	3 (11.1)	24 (10.0)	
Extension of gastric atrophy (%)			
C-1	0 (0)	0 (0)	.94
C-2	0 (0)	1 (0.4)	
C-3	2 (7.4)	18 (7.5)	
O-1	6 (22.2)	65 (27.0)	
O-2	17 (63.0)	132 (54.8)	
O-3	2 (7.4)	25 (10.4)	
Gastrin (pg/mL)	122.8	255.2	.005
Pepsinogen I (ng/mL)	46.6	42.1	.03
Pepsinogen II (ng/mL)	10.8	21.2	<.0001
Pepsinogen I/II	4.7	2.1	<.0001

gastric atrophy between the two groups, serum markers for gastric mucosal atrophy, gastrin, and PGs, were significantly different. In group A′, the serum levels of PG I and the PG I/II ratio were significantly higher, and the PG II level was significantly lower than in group non-A ($p < .05$). In addition, the mean serum gastrin level in group A′ was lower than that in group non-A ($p = .005$). No differences were observed with regard to age, sex, presence of synchronous tumor, and tumor depth/histology between the two groups.

Histologic and Serologic Analysis of Gastritis in Group A' Patients

Patients in group A′ had the following features: all of them had endoscopic gastric atrophy, depressed-type tumor was frequent, serum gastrin and PG II levels were low, and the PG I/II ratio was high. These features were similar to those of gastric cancer patients diagnosed after *H. pylori* eradication therapy [22,23]. However, previous history of eradication therapy was not confirmed in any patients as far as we carefully interviewed. Therefore, we examined the resected specimens histologically to characterize patients in group A′ on the basis of the gastric mucosa findings. We evaluated the grades of histologic gastritis in non-neoplastic mucosa (Fig. 1 A, B) and immunohistochemically evaluated the status of *H. pylori* infection (Fig. 1 C, D) in all 27 patients. We confirmed the presence of atrophic change in all 27 patients, however, active gastritis was absent in 24 (89%) patients. Most of patients in group A′ had histologic atrophic gastritis without active inflammation or *H. pylori* infection. *H. pylori* immunoreactivity was positive only in 1 (4%) patient. The distributions of PG I levels and PG I/II ratios are shown in Fig. 2. Twenty-four of 27 patients had high PG I/II (>3) ratios and low PG I (≤70 ng/mL) levels. Only one patient showed posive-*H. pylori* immunoreactivity, and the serum level of PG I and PG I/II ratio was plotted in a different area (high PG I and low PG I/II) (Fig. 2).

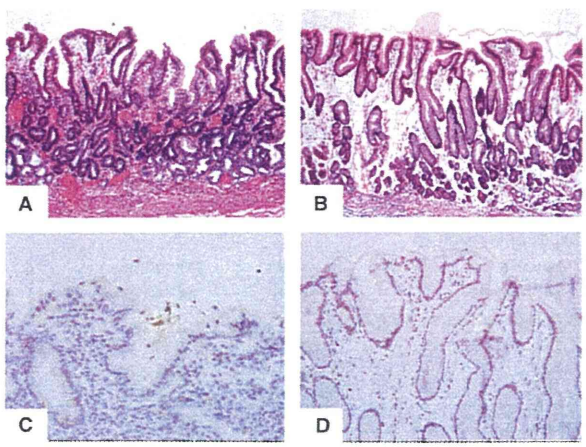


Figure 1 Histologic examination of gastric mucosa in group A′. Histologic atrophy and inflammation were evaluated using HE staining, and *H. pylori* infection was evaluated using immunohistochemistry with anti-*H. pylori* antibodies. (A) active gastritis, (B) no inflammation, (C) positive-*H. pylori* immunoreactivity, (D) negative-*H. pylori* immunoreactivity.

Kaplan–Meier Analysis of the Cumulative Incidence Rate of Metachronous Gastric Tumors in Groups A' and Non-A

Next, we examined the prevalence of metachronous gastric tumor development in a cohort study. As shown in Fig. 3, no difference was observed in the cumulative incidence rate of metachronous gastric tumors between groups A′ and non-A. Three patients developed a metachronous tumor in group A′. One of them had histologic active gastritis without *H. pylori* immunoreactivity, and the others did not have active gastritis. When we examined the prevalence again after excluding three patients who had histologic active gastritis, no difference was also observed between groups A′ and non-A (data not shown).

Identification of Patients with Atrophic Gastritis in Group A

It is necessary to distinguish patients with atrophic gastritis in group A from those who are true negative for *H. pylori* using serum markers. After *H. pylori* eradication

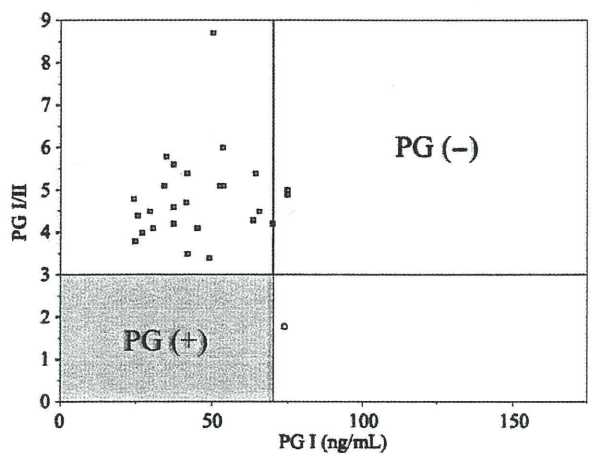
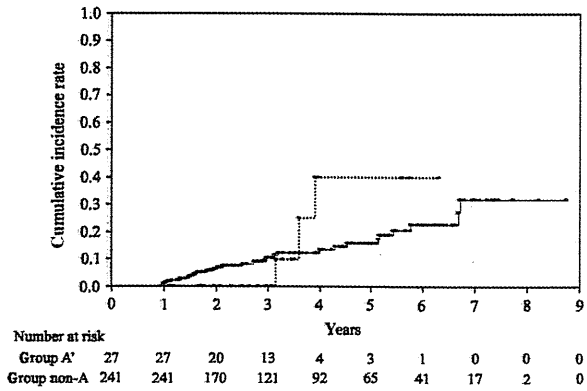


Figure 2 Serum pepsinogen levels in patients in group A′. Most patients had high PG I/II ratios (>3) and low PG I levels (≤70 ng/mL). square: negative-*H. pylori* immunoreactivity; circle: positive-*H. pylori* immunoreactivity.



Figur 3 Kaplan–Meier analysis of the cumulative incidence rate of metachronous gastric tumors in groups A′ and non-A. No difference was noted in the incidence of metachronous gastric tumors between groups A′ (dotted line) and non-A (solid line).

therapy, the levels of serum markers for gastric mucosal atrophy, gastrin, and PGs become similar, but not equal to those in patients who were true negative for *H. pylori* [22]. The differences in the levels of serum markers for gastric mucosal atrophy between patients in group A′ and true *H. pylori*-negative controls are shown in Table 3. Therefore, we investigated discriminant functions using these serum markers as parameters to distinguish between the two patient groups. Sex (male: 0, female: 1) and age were included as parameters in the analysis. Using the function, we classified patients with a probability (*P* value) greater than the cutoff as belonging to group A′. We examined appropriate sensitivity and specificity of the functions by setting various cutoffs of *P* value. As shown in Table 4, the discriminant functions using sex, age, and a serum marker could not be used because the specificity was less than approximately 70% on condition that the sensitivity was set to more than 80%. When a combination of PGs was used in addition to sex and age, the discriminant function could distinguish between the patient groups with 85% sensitivity and 75% specificity at most. Moreover, when gastrin was added to the parameters and sex, age, gastrin, PG I, and PG II were selected as parameters, the function representing the best results were obtained. When the cutoff was set to obtain the best sensitivity on condition that both the sensitivity and specificity were over 80%, the discriminant functions could distinguish patients with 85.2% sensitivity and 84.0% specificity.

Table 3 The differences in serum markers for gastric mucosal atrophy between patients in group A′ and true *Hp*-negative controls

Factors	Group A′ n = 27	True *Hp*-negative n = 213	*p* value
Gastrin (pg/mL)	122.8	92.6	.04
Pepsinogen I (ng/mL)	46.6	69.9	.001
Pepsinogen II (ng/mL)	10.8	13.2	.13
Pepsinogen I/II	4.7	5.6	.002

Hp: H. pylori.

Discussion

It is important to introduce an efficient and cost-effective practical mass screening method for gastric cancer. To detect gastric cancer in the early stages, mass screening with radiography examination has been performed since 1960s in Japan. Recently, as *H. pylor* infection, one of the main causes of gastric cancer, has become less frequent, it has become inefficient to screen all people using an imaging technique. To identify patients at a high risk for gastric cancer and strictly monitor them, a serum screening system using anti-*H. pylori* antibody titers and PG levels may be effective and beneficial [24]. The ABC classification system was established by Miki and Inoue [24,25], and its clinical benefit was confirmed in previous studies [26]. In this system, patients in group A (*Hp*(−), PG(−)) were regarded as true negative for *H. pylori*, and therefore,

Table 4 Results of multivariate logistic regression for patients with atrophic gastritis in group A

Parameter (Function)	Cutoff *P* value[a]	Sensitivity (%)	Specificity (%)	Accuracy (%)
sex, age, G (S = −8.763 − 1.240*sex* + 0.0930*age* + 0.0103*G*)	.115	81.5	68.1	69.6
sex, age, PG I (S = −5.574 − 1.298*sex* + 0.114*age* − 0.0619*PGI*)	.1	81.5	72.3	73.3
sex, age, PG II (S = −6.338 − 1.034*sex* + 0.0919*age* − 0.113*PGII*)	.07	81.5	56.8	59.6
sex, age, PG I/II (S = −3.979 − 1.013*sex* + 0.0794*age* − 0.565*PG I/II*)	.1	81.5	65.7	67.5
sex, age, PG I, PG II (S = −5.249 − 1.425*sex* + 0.108*age* − 0.0977*PG I* + 0.178*PGII*)	.09	85.2	75.1	76.3
sex, age, G, PG I, PG II (S = −7.363 − 1.758*sex* + 0.125*age* + 0.0148*G* − 0.0998*PGI* + 0.155*PGII*)	.1	85.2	84.0	84.2

G: gastrin, PG: pepsinogen.
[a]*P* = 1/{1 + exp(−S)}.

these patients were recommended to be excluded from mass screening.

Surprisingly, however, we found that 11% of patients treated for gastric epithelial neoplasms with ESD were classified into group A, and most of them had a high risk for gastric cancer with endoscopic corpus atrophy. Although 27 patients in group A′ were negative for anti-*H. pylori* antibody, we confirmed the presence of endoscopic atrophy and histologic atrophic change in all 27 patients. However, active gastritis was absent in 24 (89%) patients, and *H. pylori* immunoreactivity was positive only in 1 (4%) patient. We further evaluated histologic gastritis of non-neoplastic gastric mucosa in group D patients who were also negative for anti-*H. pylori* antibody. Of 17 patients in group D, eight patients had moderate mononuclear cell infiltration. However, in group A′, all 24 patients without active gastritis showed none or mild mononuclear cell infiltration, which was quite similar to that in patients with previous successful eradication therapy. Our results suggest that patients in group A′ are not true *H. pylori*-negative case but have previous *H. pylori* infection. The distributions of PG I levels and PG I/II ratios supported this hypothesis. Although their anti-*H. pylori* antibody titer may have been false negative, all our results suggested that the majority of them had clinical features similar to those of patients who had undergone eradication therapy. These patients may have received unexpected *H. pylori* eradication because they did not have a history of *H. pylori* eradication therapy as far as we carefully interviewed. This could be because antibiotics such as penicillin, macrolide, and quinolone are commonly used for other diseases in Japan. On the other hand, it may be caused by misunderstanding of patients concerning previous eradication therapy or by insufficient explanation from the chief physician. With regard to serum markers for gastric mucosal atrophy, these patients mostly had high PG I/II ratios and low PG I levels (so called group α) [26]. Yanaoka et al. [26] reported that people with this classification had the lowest risk of gastric cancer. However, we found that a part of these patients had a similar potential for generating metachronous cancer in a cohort study.

We identified a certain number of high-risk patients for gastric epithelial neoplasm in group A, even though the risk in this group is expected to be particularly low. It is clinically important to identify the high-risk patients mixed into group A and exclude them from this group to develop an effective examination for gastric cancer. To this end, one method may involve performing an imaging examination using radiography or endoscopy at least once during the lifetime of all people in group A, because we confirmed that all gastric epithelial neoplasm patients in group A except for true *H. pylori*-negative patients exhibited atrophy of the gastric mucosa. However, this is not a realistic method with respect to patient safety, staffing, and economic benefit. In this study, we suggested that the discriminant function using serum markers might be useful for distinguishing patients from true *H. pylori*-negative controls. The discriminant function can be calculated easily from serologic data in a cost-effective manner that requires only low staffing. We consider it suitable for enabling mass screening of gastric cancer.

Sex, age, gastrin, and PGs were selected as parameters, and various combinations of these were investigated for the discriminant function. Although previous reports indicated that neither age nor sex affected basal gastrin and pepsinogen concentrations in *H. pylori*-negative subjects [27,28], discriminant function using sex and age produced better results than when these parameters were not used. When the function for mass screening is used, the sensitivity must be sufficiently high to reduce all false negative results. The specificity should also be as high as possible. As a result, the function using all parameters, including sex, age, gastrin, and PGs produced the best results. We could distinguish patients in group A′ from true *H. pylori*-negative controls with 85% sensitivity and 84% specificity when the cutoff of the calculated value using the function was set on condition that both the sensitivity and the specificity were over 80% and the sensitivity became as high as possible. Although the number of patients in group A′ was not enough for multivariate logistic regression, this approach showed the high potential of the discriminant function for distinguishing high-risk patients (as well as patients after *H. pylori* eradication therapy) from true *H. pylori*-negative subjects. However, in this study, true *H. pylori*-negative controls were selected from patients who visited Hiroshima University hospital for some treatment and they were not healthy regional residents. We did not investigate the differences in the clinical characteristics including smoking, alcohol intake, and so on, between group A′ and true *H. pylori*-negative controls, which would affect the condition of gastric mucosa and serum markers. Therefore, it is necessary to analyze more cases to investigate the utility of the function in the general population.

As discussed above, a certain number of high-risk patients in group A could develop gastric epithelial neoplasm. However, many papers have already reported that people in group A rarely develop gastric cancer [11,12,24,25]. Ohata et al. [9] reported that none of 4655 normal male individuals in group A who could be followed-up for at least 10 years had developed gastric cancer. This discrepancy may be because of the age of

the subjects. The mean age of people in group A was 48.3 years in Ohata's study and 69.8 years in our study. Therefore, gastric cancer may appear when patients are followed-up into old age. When young generations undergo unexpected *H. pylori* eradication, the gastric mucosal atrophy should be relatively mild. Therefore, they may not be at a high risk for gastric cancer development.

Recently, Japanese multicenter trials proved that *H. pylori* eradication therapy could reduce the incidence of metachronous gastric cancers after endoscopic resection for early gastric cancer [29]. In this study, no difference was noted in the incidence of metachronous gastric tumors between gastric tumor patients in group A′ and non-A. Although group A is generally regarded as low-risk group for gastric cancer, patients in group A′ may have a high risk of subsequent gastric tumor development. Because we clarified that most of the gastric tumor patients in group A were those with previous *H. pylori* infection, this result may indicate that *H. pylori* eradication therapy cannot always reduce the incidence of gastric tumor. Many of patients in group A′ had severe atrophic gastritis. Therefore, the incidence of gastric tumor in group A′ might be relatively high. Patients with a history of gastric tumors have a high risk of subsequent gastric tumor development [8,29] and evaluating cancer risk using ABC system is not suitable for these patients.

Use of the ABC system to classify people into low-, intermediate-, and high-risk groups for gastric cancer is very effective, but the inclusion of high-risk individuals as well as patients after *H. pylori* eradication therapy in group A is a key problem. To resolve this problem, new methods such as the discriminant function using serum markers for identifying patients are needed.

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Research Article

Characteristics of Metachronous Gastric Tumors after Endoscopic Submucosal Dissection for Gastric Intraepithelial Neoplasms

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Background. Recently, endoscopic submucosal dissection (ESD) has become a standard treatment method for early gastric cancer and concurrent stomach preservation. However, metachronous recurrences have become a major problem. We evaluated the incidence and clinicopathologic features of and examined the risk factors for metachronous gastric tumors. *Methods.* A total of 357 patients who underwent ESD for gastric tumors (245 early gastric cancers and 112 adenomas) and were followed up for more than 12 months without recurrence within the first 12 months were enrolled. We investigated the incidence and clinicopathologic features of metachronous tumors after ESD. We also analyzed the potential risk factors for metachronous tumors using the Kaplan-Meier method and Cox's proportional hazards model. *Results.* The annual incidence of metachronous tumors after ESD was 2.4%. The median period until discovery after initial ESD was 26.0 months, and the median observation period was 52.6 months. Male patients developed metachronous tumors more frequently ($P = 0.04$), and the hazard ratio of female to male patients was 0.36 (95% confidence interval: 0.11–0.89). *Conclusions.* Patients with a previous history of gastric tumors have a high risk of subsequent gastric tumor development and male patients should be carefully followed up after ESD for gastric tumor.

1. Introduction

Gastric cancer is the second most frequent cause of cancer death, and the incidence of gastric cancer among developed countries is the highest in Japan [1]. A number of epidemiological studies have indicated that *Helicobacter pylori* (*H. pylori*) infection is significantly related to gastric cancer development [2–4]. Approximately 10–20% of gastric cancer patients develop multiple synchronous and metachronous cancers [5–8]. To detect early gastric cancer (EGC) after treatment, surveillance procedures should be carefully adhered to.

In recent years, endoscopic submucosal dissection (ESD) for EGC has been widely performed in Japan. With this method, stomach preservation and maintenance of the patients' quality of life are possible [9–12]. However, this approach has been associated with an increase in the risk of gastric cancer recurrence, especially metachronous multiple cancers. The cumulative 3-year incidence of metachronous multiple gastric cancer after partial gastrectomy for EGC was reported to be 1.9% [13]. Previous studies also reported that the annual incidence of metachronous multiple gastric cancer after ESD for EGC was 2.6–3.5% [7, 14, 15]. However, the median observation periods of these studies are short (less than 3 years) and there is no study using gastric tumor including adenoma, which generally becomes indication for ESD in Japan, because the pathological finding after ESD occasionally shows adenocarcinoma even though preoperative biopsy showed adenoma [16].

In the present study, we evaluated the incidence and clinicopathologic features of metachronous multiple tumors that developed during long-term observation and investigated whether we could predict the occurrence of such tumors on the basis of the patient and tumor features during initial ESD.

2. Materials and Methods

2.1. Patients. We enrolled 1,087 consecutive patients with gastric tumors (766 EGCs and 321 adenomas) who underwent ESD at Hiroshima University Hospital between April 2002 and May 2010. We excluded patients with 28 previous gastric surgical histories, 11 local gastric tumor recurrences, 6 gastric mucosa-associated lymphoid tissue lymphoma, 3 Barrett's adenocarcinoma, and 611 patients who had not been followed-up for more than 12 months. Sixty patients who underwent gastric surgery after ESD and 11 patients who underwent *H. pylori* eradication therapy were also excluded. A final total of 357 patients (273 male, 84 female; mean age: 67.4 years) were enrolled in this study, including 245 EGC patients and 112 adenoma patients. Three hundred and thirty-five patients (94%) were resected as curative resection according to the Japanese gastric cancer treatment guidelines [17] and others were observed without additional surgical resection. The median observation period was 52.6 months (range: 12.2–113.4 months). Three hundred and twelve patients (88%) were followed up by annual endoscopic examination in our hospital. We defined a metachronous tumor as a new tumor that developed in another region at least 12 months after ESD.

The protocol was approved by the Ethics Committee of Hiroshima University Hospital (number 669).

2.2. Evaluation of Clinicopathologic Features. We investigated the incidence of metachronous tumors in 357 patients using the Kaplan-Meier method and retrospectively investigated the clinicopathologic features associated with metachronous tumors, including patient age and gender, tumor size, location, gross type, extension of gastric mucosal atrophy, presence of synchronous multiple tumors, histology, and depth. We also evaluated the outcomes of metachronous tumors after ESD.

In patients with synchronous multiple tumors, we chose as the main lesion a tumor that had the highest malignant potential as determined by a malignancy, diffuse type, or increased size or depth. Tumor location and macroscopic types of gastric tumors were classified according to the Japanese Classification of Gastric Carcinoma (JCGC) [18]. In this study, type 0-I (protruded) and type 0-IIa (superficial elevated) were grouped together as "elevated," while type 0-IIc (superficial depressed) and type 0-IIa+IIc (elevated with central depression) were grouped together as "depressed." Endoscopic evaluations of atrophic gastritis were determined according to the criteria of the Kimura and Takemoto classification [19]. The pathological diagnosis of each tumor was also judged according to the JCGC criteria [18]. In this study, we included adenoma among the intestinal-type tumors.

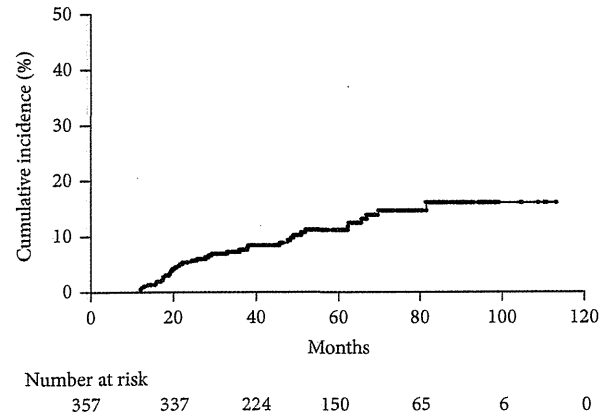

FIGURE 1: Kaplan-Meier curve of the cumulative incidence of metachronous tumors after ESD for gastric tumors.

2.3. Evaluation of Serum Markers. We evaluated the levels of serum gastrin (Gastrin RIA Kit II; Dainabot Co., Ltd., Osaka, Japan) and serum pepsinogen (LZ test; Eiken Chemical Co., Ltd., Tokyo, Japan) instead of performing histological evaluation of the gastric mucosa. We could evaluate fasting serum gastrin and pepsinogen levels in 281 of the 357 patients.

2.4. Statistics. The cumulative incidence of metachronous gastric tumors was evaluated using the Kaplan-Meier method. To analyze potential risk factors for metachronous tumors, we performed univariate analysis using the Kaplan-Meier method, log-rank test, and Cox's proportional hazards modeling. A P value of <0.05 was considered significant. The JMP statistical software package (SAS Institute Inc., Cary, NC, USA) was used for all calculations.

3. Results

3.1. Kaplan-Meier Analysis of the Cumulative Incidence of Metachronous Gastric Tumors. We investigated the incidence of metachronous gastric tumors after ESD in 357 patients with gastric tumors using the Kaplan-Meier method (Figure 1). Thirty-nine patients developed metachronous tumors (24 EGCs and 15 adenomas), and the median period until discovery after initial ESD was 26.0 months (range: 12.2–81.8 months). According to the investigation of initial/metachronous tumor, 5 patients had adenoma/adenoma, 2 patients had adenoma/adenocarcinoma, 10 patients had adenocarcinoma/adenoma, and 22 patients had adenocarcinoma/adenocarcinoma, respectively. The cumulative incidence curve of metachronous gastric tumors revealed a gradual increase and an incidence of 2.4% per year. When we excluded cases in which the initial or second tumors were adenoma from the 357 patients, the incidence of metachronous EGC was 2.0% per year (n = 236, data not shown). There was no difference in the incidence of metachronous gastric tumor between adenoma and adenocarcinoma of initial treatment.

3.2. Clinicopathologic Characteristics of Metachronous Gastric Tumors after ESD. We investigated the clinicopathologic characteristics of the tumors and patients at the time of second tumor discovery in the above-mentioned 39 patients who developed metachronous tumors (Table 1). The average age was 70.3 years, and 35 (90%) of the patients were male. The average tumor size was 11.1 mm (range: 3–20 mm) in diameter. Of 39 lesions, 10 (26%), 12 (31%), and 17 (44%) lesions developed in the upper, middle, and lower third of the stomach, respectively. When we compared the second tumors and initial tumors with regard to development location, the second tumors more frequently developed in the upper third of the stomach ($P = 0.0002$). Eighteen (46%) lesions were diagnosed as elevated type and the others were of depressed type. Almost all patients had severe gastric mucosal atrophy, which is termed as open-type according to the Kimura-Takemoto classification. Only 3 (8%) patients had developed multiple tumors at the time of second tumor detection. According to the pathological evaluation, 37 (95%) patients developed intestinal-type tumors and the others developed diffuse-type tumors. Five cases (13%) developed submucosal invasive gastric cancers. No advanced gastric cancers occurred. All intramucosal tumors were curatively resected by ESD. Four patients with submucosal gastric cancers underwent additional resection of the stomach, and 1 patient was followed up without surgery. There were no gastric cancer deaths during follow-up period.

3.3. Analysis of Risk Factors for Metachronous Gastric Tumors after ESD. According to the univariate analysis performed using the Kaplan-Meier method and log-rank test, only gender significantly affected the incidence of metachronous tumors. The incidence of metachronous tumors was greater among male patients than among female patients ($P = 0.04$, Figure 2). As shown in Table 2, the hazard ratio of female to male patients was 0.36 (95% confidence interval: 0.11–0.89), and no other factors affected the incidence in the univariate analysis according to Cox's proportional hazards model.

Table 1: Clinicopathologic characteristics associated with metachronous gastric tumors.

Factors	No. of patients
Total patients	39
Age (years)	
Mean (range)	70.3 (50–88)
Gender	
Male	35 (90%)
Female	4 (10%)
Tumor size (mm)	
Mean (range)	11.1 (3–20)
Location	
Upper third	10 (26%)
Middle third	12 (31%)
Lower third	17 (44%)
Gross type†	
Elevated	18 (46%)
Depressed	21 (54%)
Gastric mucosal atrophy	
Closed	1 (3%)
Open	38 (97%)
Synchronous tumor	
Negative	36 (92%)
Positive	3 (8%)
Histology	
Intestinal	37 (95%)
Diffuse	2 (5%)
Depth	
Mucosa	34 (87%)
Submucosa	5 (13%)

†Elevated: 0-I and 0-IIa; depressed: 0-IIc and 0-IIa+IIc.

4. Discussion

In Japan, ESD has been standardized as a local treatment for EGC with no risk of lymph node (LN) metastasis. According to the Japanese gastric cancer treatment guidelines [17], ESD is indicated as a standard treatment for differentiated-type adenocarcinomas without ulcerative findings (UL(+)), with a depth of invasion clinically diagnosed as T1a and a diameter of ≤2 cm (absolute indication). Tumors that are clinically diagnosed as T1a and are (a) of the differentiated type, UL(−), but >2 cm in diameter; (b) of the differentiated type, UL(+), and ≤3 cm in diameter; or (c) of the undifferentiated type, UL(−), and ≤2 cm in diameter have a very low possibility of LN metastasis, and ESD for these tumors is regarded as an investigational treatment (expanded indication). Additionally, resection of differentiated-type adenocarcinomas with submucosal invasion of <500 μm and a diameter of ≤3 cm is considered curative. Some reports have supported the validity of these indications [20–22]. Furthermore, risk factors for LN metastasis of submucosal invasive gastric cancer or undifferentiated type EGC have been reported [23–29]. We have been able to perform resection in difficult-to-treat cases such as those with ulceration because of advances in the ESD technique and device [30, 31]. ESD may have a potential that the criteria for curative endoscopic resection (ER) is increasingly expanded in the future. It is commonly known that gastric cancers often recur metachronously, and the risk of metachronous multiple tumors after ESD is thought to be higher than that after gastrectomy [7, 13–15].

Our data revealed that the annual incidence of metachronous gastric tumors was 2.4%, which is almost equal to the previously reported incidence [7, 14, 15]. The median interval period to the detection of a second tumor after initial ESD was 26.0 months (range: 12.2–81.8 months), and Kaplan-Meier curve seemed to reach a plateau after 80 months. Kobayashi et al. [32] reported that the median interval between the discovery of metachronous cancer and the initial ER was 3.2 years in patients who were followed up for 3.0 to 19.6 years (median: 5.0 years), and no metachronous cancers were detected in patients who were followed up for more than 10 years. These data suggest that metachronous

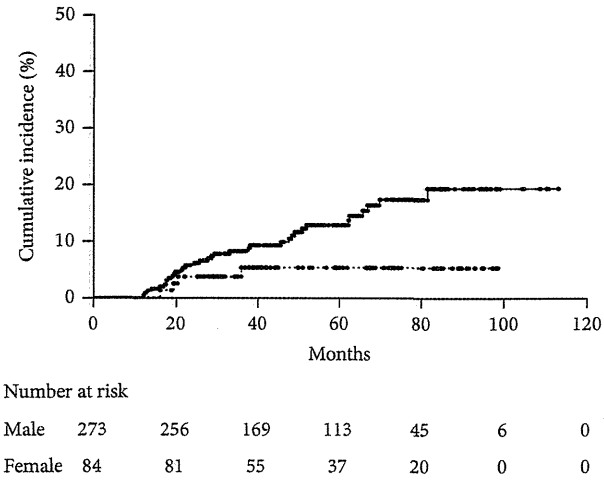

FIGURE 2: Cumulative incidence of metachronous tumors after ESD in male and female patients. The incidence of metachronous tumors was significantly greater among male patients (solid line) than among female patients (dotted line; $P = 0.04$).

gastric tumors may develop around 3 years after ESD and that the incidence may gradually decrease after ESD. However, it is necessary to investigate more cases because of too few patients followed up during long term. Since the annual incidence of gastric cancer in *H. pylori*-positive patients was reported to be 0.38–0.5% [4, 33], after ESD, patients have a higher risk of developing gastric cancer.

We investigated the clinicopathologic characteristics of metachronous gastric tumors and revealed that second lesions tended to develop in the upper third of the stomach. Kato et al. [15] reported that many synchronous gastric cancers after ESD that had been missed by the preoperative endoscopic examination were located in the upper third of the stomach. Since it is difficult to detect tumors in this region, we might be able to detect them by more frequent and careful endoscopic examinations after ESD.

Our data showed that only gender significantly affected the metachronous tumor incidence. Some reports indicated that male patients more frequently developed metachronous gastric cancer after surgery or ER [13, 15, 32]. It is commonly known that the incidence of gastric cancer is higher in male than in female. It has also been reported that differences in smoking rates and salt intake between male and female affect the incidence [34, 35]. Patient age and the presence of synchronous multiple gastric cancers at the time of the initial ER have been reported to significantly affect the incidence of metachronous gastric cancer [36], and antral atrophy was significantly associated with incidence in a previous multivariate analysis [14]. However, these factors did not significantly affect the results of this study. The fact that patients with synchronous tumors are susceptible to metachronous tumors implies that gene mutations or gastric mucosal conditions may be causes of metachronous tumors. A few reports indicated that microsatellite instability (MSI) was a factor that affected the development of both synchronous and metachronous multiple gastric cancers, and

TABLE 2: Analysis of risk factors for metachronous gastric tumors according to Cox's proportional hazards model.

Factors	No.	Univariate analysis	
		Hazard ratio	95% CI
Age (1-year increment)		1.00	0.97–1.04
Gender			
Male	272	1	
Female	85	0.36	0.11–0.89
Tumor size (increment of 1 mm)		1.01	0.98–1.03
Location			
Upper third	38	1	
Middle third	91	1.68	0.55–7.29
Lower third	228	1.02	0.35–4.32
Gross type			
Elevated	161	1	
Depressed	196	1.07	0.57–2.04
Gastric mucosal atrophy			
Closed	30	1	
Open	327	1.20	0.43–4.99
Synchronous tumor			
Negative	307	1	
Positive	50	1.26	0.47–2.79
Histology			
Intestinal	331	1	
Diffuse	26	0.84	0.20–2.33
Depth			
Mucosa	326	1	
Submucosa	31	1.05	0.25–2.92
Serum gastrin			
≤100 pg/mL	94	1	
>100 pg/mL	187	0.58	0.28–1.21
Serum pepsinogen†			
Negative	111	1	
Positive	170	1.23	0.59–2.75

CI: confidence interval.
†Serum pepsinogen-positive: PG I ≤ 70 ng/mL and PG I/II ≤ 3.

the frequency of MSI was found to be significantly higher in patients with metachronous gastric cancers than in those with single gastric cancers [37, 38]. Although we could not evaluate the histological condition of the gastric mucosa, we investigated serum gastrin and pepsinogen levels instead. Serum gastrin levels of patients with severe gastric mucosal atrophy are higher than those of patients with mild or no gastric mucosal atrophy because severe atrophy reduces the secretion of gastric acid [39, 40]. Serum levels of pepsinogen (PG) I and PG II and the PG I/II ratio vary according to gastric mucosal atrophy and inflammation [40–42]. Cases with PG I ≤ 70 ng/mL and PG I/II ≤ 3 were regarded as PG positive, indicative of gastric mucosal atrophy [41]. It was thought that gastric mucosal condition seldom affected the metachronous tumor incidence because serum gastrin and pepsinogen levels did not affect the incidence. In this study, we excluded patients who received *H. pylori* eradication therapy to avoid the influence of *H. pylori* eradication on the development of

metachronous tumor. Eradication of *H. pylori* infection was reported to reduce the risk of gastric cancer development [33, 43]. Recently, an open-label, randomized controlled trial showed that *H. pylori* eradication prevented the development of metachronous cancer after ER for EGC patients during a 3-year follow-up period [7], and therefore, eradication therapy is recommended after ER for EGC in Japan [44]. As a result, metachronous gastric cancer detection after *H. pylori* eradication will increase. Furthermore, some reports suggested that macroscopic/biological features of gastric tumors could change after *H. pylori* eradication [45–47]. In the near future, it will be necessary to investigate predictive factors of metachronous gastric tumors after ESD for gastric tumors in patients who have undergone *H. pylori* eradication.

5. Conclusions

Patients with a previous history of gastric tumors have an increased risk of subsequent gastric tumor development and male patients should be carefully followed up after ESD for gastric tumor.

Conflict of Interests

No author has conflict of interests or financial arrangements that could potentially influence the described research.

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ORIGINAL ARTICLE

High Expression of Gastrin Receptor Protein in Injured Mucosa of *Helicobacter pylori*-Positive Gastritis

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Abstract
Background and Aim Gastrin is a growth factor for the gastric epithelial cells. However, it is unknown how gastric receptor (GR) expression is regulated in the gastric mucosa. We studied GR expression using a newly raised antibody and investigated the relationship between GR expression and gastritis.
Methods Gastric receptor expression in 63 human gastric mucosa was studied. *Helicobacter pylori* infection and histological gastritis status were evaluated in gastric biopsy samples. In gastric ulcer cases, additional biopsy specimens were taken from injured mucosa. Fasting sera were collected and serum gastrin level evaluated. MKN-28 cells were cultured at various pH conditions, and the change in GR expression was determined.
Results Gastric receptor expression was detected in the foveolar epithelium of the gastric mucosa, and its expression was stronger in patients infected with *H. pylori*. In particular, higher expression was detected in regenerating injured mucosa. There was no association between gastritis score/serum gastrin level and GR expression in *H. pylori*-positive cases. In MKN-28 cells, GR protein expression was lower in neutral conditions than in acidic or alkaline conditions.
Conclusion Gastric mucosal injury with *H. pylori* infection destroys the pH barrier on the foveolar epithelium and may induce GR expression through pH changes.

Keywords Gastrin · Gastrin receptor · Gastritis · *Helicobacter pylori* · Gastric acid

Abbreviations

GR	Gastrin receptor
PPI	Proton pump inhibitor
H. pylori	*Helicobacter pylori*

Introduction

Human gastrin is a multifunctional gastro-intestinal hormone that stimulates acid secretion and promotes cell growth [1, 2]. The serum level of gastrin increases with increasing grade of atrophic changes in the body during *Helicobacter pylori* infection [3]. Consequently, the serum gastrin level is regarded as an important biomarker for evaluating the status of gastric inflammation [4]. Gastrin is also an important growth factor that plays a significant role in the carcinogenesis of gastro-intestinal malignant tumors [5].

The mechanism behind the effects of gastrin on cell proliferation is still unclear. Previous reports have suggested that gastrin stimulates the secretion of Reg protein from the endocrine cells of the gastric mucosa, which in turn acts as a growth factor in gastric epithelial cells [6, 7]. Alternatively, the importance of the gastrin–gastrin receptor (GR) system in malignant cells has been demonstrated,

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suggesting an autocrine growth mechanism in these cells [8, 9]. One major problem encountered by researchers in this field is the limited number of methodologies available for examining the expression of the gastrin receptor, which is identical to that of the cholecystokinin B receptor, CCKBR, in the clinical setting. The GR gene was cloned by Lee et al. [10] and found to possess an open reading frame encoding 447 amino acids with seven transmembrane domains. The expression of GR mRNA in the gastric mucosa has been demonstrated with reverse-transcription (RT) PCR [11], and immunohistochemistry studies have demonstrated GR protein in the gastric mucosa as well as in cancer cells [12–14]. In a previous study we used an I^{125}-labeled gastrin binding assay to show that GR is present in the human gastric corpus [15]. However, it is important to recognize that the results of these different studies are identical, likely due to the varying reactivity of each antibody and the methods used.

In a previous study, we raised a new polyclonal antibody (OK-524) that reacts with human GR in paraffin-embedded materials and demonstrated the expression of GR in human gastric carcinoma tissue using immunohistochemistry [16]. *H. pylori* infection is known to be a major cause of gastritis and gastric carcinogenesis, especially in Japanese patients [17], possibly by inducing chronic inflammation followed by atrophic gastritis and intestinal metaplasia, which are important risk factors for gastric cancer [18]. In the study reported here, we examined the expression of GR in non-neoplastic human gastric epithelial cells with or without *H. pylori*-induced gastritis and discuss the role of the gastrin–GR system during the pre-malignant state. We also examined the factors that induce GR expression in gastric epithelial cells.

Patients and Methods

Patients

A total of 63 Japanese patients (43 men, 20 women; mean age 60.5 years) with dyspepsia and 12 patients (12 men; mean age 61.6 years) with peptic ulcer were enrolled in this study. The 12 patients with gastric ulcer were treated with proton pump inhibitor (PPI). Patients with a history of gastrectomy, pernicious anemia, severe hepatic/renal dysfunction, or eradication therapy were excluded. All patients received a routine endoscopic examination, and gastric biopsies were performed from the gastric antrum and the gastric corpus. In the 12 patients with gastric ulcers, an additional gastric biopsy was performed in regenerating mucosas (peripheral area around injured gastric mucosa). The grade of gastritis was determined using the updated Sydney system [19].

H. pylori status was evaluated by histological examination using biopsy specimens and by serum antibody titer against *H. pylori* (E-plate, Eiken, Japan) [20]. If any of two tests was positive, the patient was regarded as *H. pylori*-positive. We confirmed the absence of histological active gastritis in biopsy specimens from *H. pylori*-negative patients. Fasting sera were collected from patients and stored at −20 °C until use. The serum gastrin level was also determined by radioimmunoassay [21]. The ethics committee of Hiroshima University hospital approved the protocol.

Cell Culture

A human gastric cancer cell line, MKN-28, provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan), was routinely cultured in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10 % fetal bovine serum. The pH of the conditioned medium was controlled at various pH ranging from 6.0 to 8.0. Following incubation under these different conditions for 24 h, whole-cell extracts were used for Western blotting.

Western Blotting

A 20-μg sample was dissolved in a sample buffer with 2-mercaptoethanol without boiling and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 12.5 % polyacrylamide gel. The proteins were then blotted onto Immobilon (Millipore, Billerica, MA) in a transfer buffer and subjected to immunoreactions. The antibody against GR was diluted with phosphate buffered salin [PBS; final immunoglobulin G (IgG) concentration 1.0 μg/ml] and used as a primary antibody. A peroxidase-conjugated anti-rabbit IgG goat antibody (diluted 1/500) was used as a secondary antibody.

Immunohistochemistry

Sections of formalin-fixed paraffin-embedded tissues (thickness 4 μm) were used for the immunohistochemical studies. After deparaffinization and hydration, internal peroxidase activity was blocked by incubation with 0.3 % H_2O_2 in methanol for 15 min, and the sections were incubated with 5 % skimmed milk/PBS for 30 min before being reacted with our newly raised antibody against GR (OK-524; diluted with PBS to a final concentration of 1.0 μg/ml) overnight at room temperature in a moist box [16]. After the reaction, the LSB-2 system (DAKO, Kyoto, Japan) was used for further reactions. Antigen retrieval was performed with a microwave treatment before the section was reacted with OK-524.

Statistical Analysis

Statistical analysis was performed using the t test and χ^2 test with StatView (SAS Institute, Cary, NC). A value of $P < 0.05$ was considered to indicate significance.

Results

Expression of the Gastrin Receptor in Human Gastric Mucosa

We carried out the immunohistochemistry studies using our newly raised antibody against GR (OK524). Positive signals were detected in the parietal cells of the gastric mucosa, which disappeared using absorbed antibody, as reported previously [16]. A clear positive signal was also detected in the foveolar epithelium of the gastric mucosa in both the gastric corpus and the antrum, especially during hyperplastic/regenerative changes after mucosal injury by endoscopic resection (Fig. 1). We confirmed GR expression in parietal cells irrespective of *H. pylori* infection, which was used as internal control.

Relationship Between GR Expression and *H. pylori* Infection, Gastritis Score, or Gastrin Level

The relationship between the grade of gastritis and GR expression in biopsy specimens was examined. Gastritis grade was determined using the updated Sydney system in 28 *H. pylori*-positive (23 men; mean age 65.2 years.) and 35 *H. pylori*-negative patients (20 men; mean age 56.8 years). Endoscopic examination revealed no tumor or active ulcer at entry. As a result, we found that GR expression was significantly increased in *H. pylori*-positive patients (Table 1). A total of 112 *H. pylori*-positive specimens (4 specimens for each patient) were investigated to clarify the relationship between gastritis grade and GR expression. As shown in Table 2, we were unable to find any relationship between the degree of gastritis and GR expression, nor between serum gastrin level and GR expression (Table 3).

High Expression of GR in Regenerating Mucosa of Gastric Ulcer Patients

In the 12 *H. pylori*-positive patients with gastric ulcers, we took an additional biopsy sample from the regenerating mucosa tissue around the gastric ulcers. Examination of

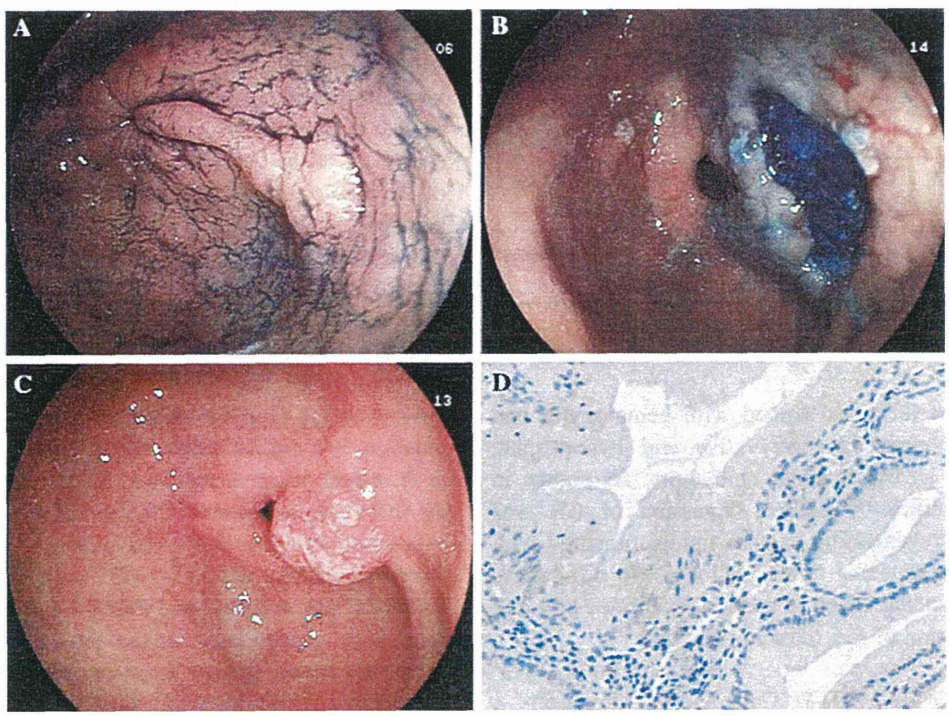

Fig. 1 Immunohistochemical staining of the gastrin receptor (GR) in the human gastric mucosa. Mucosal adenocarcinoma was discovered in the gastric antrum of the 73-year-old male (**a**), and endoscopic mucosal resection was performed (**b**). After 6 months, a hyperplastic polyp was identified on the ulcer scar (**c**). Immunohistochemical staining revealed that high GR expression in the foveolar epithelium with hyperplastic changes after endoscopic resection (**d**)