ment and maintenance of tissue-specific gene expression patterns in mammals. Disruption of epigenetic regulation can lead to altered gene function and malignant cellular transformation[47]. Recent cancer epigenetic studies have revealed various alterations in the epigenetic machinery in GC, including DNA methylation, histone modifications, nucleosome positioning, noncoding RNAs and miRNAs[48-52]. Aberrant DNA methylation in the promoter CpG islands of genes results in inactivation of tumor suppressor and other tumor-related genes in cancer cells and is the most well-defined epigenetic hallmark in GC. Methylation of a large number of genes with different biological functions has been found to be correlated with the clinicopathological characteristics and prognosis in GC[48-52]. DNA methylation with its advantages as a biomarker for the detection of cancer in biopsy specimens and body fluids that can be obtained non-invasively, such as serum and gastric washes, may have a clinical application in GC. Detection of aberrant DNA methylation of genes, such as *CDH1*, *DAPK*, *GSTP1*, *p15*, *p16*, *RARβ*, *RASSF1A*, *RUNX3* and *TFPI2*, in the serum may be a useful biomarker for the detection of GC[50]. Studies of DNA methylation and histone modification using NGS technologies, such as whole-genome bisulfite sequencing and targeted bisulfite sequencing, will lead to new discoveries and improve our knowledge of the epigenomics of GC[11].

Association of the aberrant methylation of RASGRF1 with an epigenetic field defect and an increased risk of GC

Aberrant DNA methylation is implicated in the epigenetic field defect seen in GC. Thus, it is important to identify predictive biomarkers by screening for DNA methylation in the noncancerous background gastric mucosa of patients with GC. Using methylated-CpG island amplification coupled with CpG island microarray (MCAM) analysis, Takamaru *et al*[53] found 224 genes that were methylated in the noncancerous gastric mucosa of patients with GC. Among them, RASGRF1 methylation was significantly elevated in the gastric mucosa from patients with either intestinal- or diffuse-type GC, compared with the mucosa from healthy individuals. RASGRF1 methylation was independent of mucosal atrophy and could be used to distinguish both serum pepsinogen test-positive and -negative patients with GC from healthy individuals. Ectopic expression of RASGRF1 suppressed colony formation and Matrigel invasion by GC cells. RASGRF1 methylation appears to be significantly involved in the epigenetic field defect of the stomach and to be a useful biomarker to identify individuals at high risk for GC.

Association of aberrant methylation of miR-34b/c with an epigenetic field defect and an increased risk of GC

The silencing of miRNAs is often associated with CpG island hypermethylation. Thus, to identify epigenetically silenced miRNAs in GC, Suzuki *et al*[54] screened for miRNAs that were induced by treatment of GC cells with 5-aza-2'-deoxycytidine and 4-phenylbutyrate. Hypermethylation of the neighboring CpG island epigenetically silenced miR-34b and miR-34c. Methylation of the miR-34b/c CpG island was frequently observed in GC cell lines (13/13, 100%) but not in normal gastric mucosa from healthy *H. pylori*-negative individuals. Transfection of the precursors of miR-34b and miR-34c into GC cells suppressed growth and changed the gene expression profile. Methylation of miR-34b/c was found in a majority of primary GCs (83/118, 70%). Notably, analysis of the non-cancerous gastric mucosae from GC patients (n = 109) and healthy individuals (n = 85) revealed that methylation levels were higher in the gastric mucosae of patients with multiple GC lesions than in the mucosae from those patients with single GC and the mucosae from healthy *H. pylori*-positive individuals. These results suggest that miR-34b and miR-34c are novel tumor suppressors frequently silenced by DNA methylation in GC. Methylation of miR-34b/c appears to be significantly involved in an epigenetic field defect in the stomach and to be a useful biomarker to identify individuals at high risk for multiple GC.

Methylation of miR-34b/c in the mucosa of the noncancerous gastric body may be a useful biomarker for predicting the risk of metachronous GC

Metachronous GC can develop after endoscopic resection of GC and is not predictable based on the clinical characteristics alone. Aberrant DNA methylation in noncancerous gastric mucosa has been implicated in gastric carcinogenesis and may be a useful biomarker of GC risk. Suzuki *et al*[55] evaluated the clinical utility of DNA methylation as a biomarker of metachronous GC risk. Scheduled follow-up endoscopy was performed in 129 patients after curative endoscopic resection of early GC. Biopsy specimens were collected from noncancerous mucosa in the gastric antrum and body. A quantitative methylation analysis of miR-34b/c, SFRP1, SFRP2, SFRP5, DKK2 and DKK3 using bisulfite pyrosequencing was performed on the collected biopsy specimens. The utility of the methylation status for predicting the risk of developing metachronous GC was analyzed using Kaplan-Meier and Cox proportional hazards models. During the follow-up period, 17 patients (13%) developed metachronous GCs. The cumulative incidence of metachronous GC was significantly higher among patients with elevated miR-34b/c, SFRP2 and DKK2 methylation in the gastric body. Elevated methylation of miR-34b/c showed the most significant association with the risk of metachronous GC; the cumulative incidence of metachronous GC was much higher in the high miR-34b/c-methylation group than in the low methylation group. Multivariate analysis adjusted for age, sex, *H. pylori* status and pathological findings showed that miR-34b/c methylation in the gastric body was an independent predictor of metachronous GC risk. Methylation of miR-34b/c in the mucosa of the noncancerous gastric

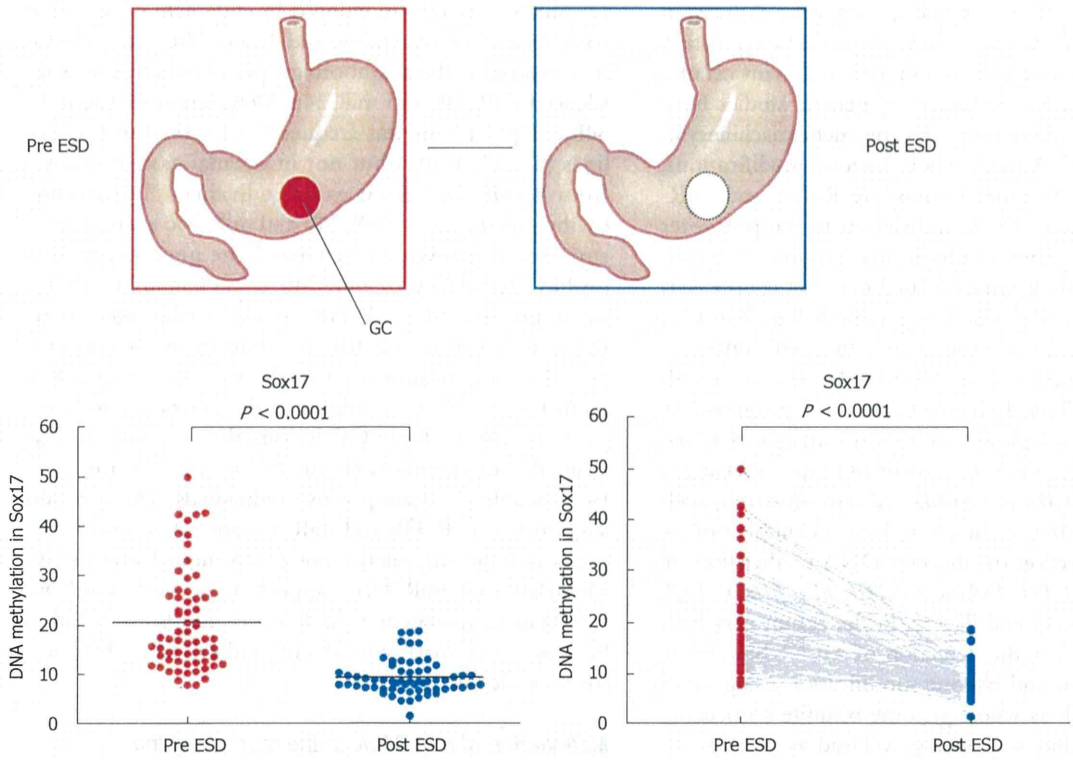

Figure 3 Methylation levels of Sox17 before and after endoscopic submucosal dissection. Methylation levels of Sox17 were analyzed by pyrosequencing using the DNA recovered from gastric washes before and after endoscopic submucosal dissection[57].

body may be a useful biomarker for predicting the risk of metachronous GC. Finally, NGS technologies may characterize an epigenetic field defect more clearly and highlight more useful biomarkers.

Sensitive and specific detection of early GC by DNA methylation analysis of gastric washes

Because many mucosal cells can be found in the gastric juice, the detection of molecular markers in the gastric juice was a possible noninvasive approach to detect GC. However, the use of gastric juice as a molecular diagnostic or predictive tool has been previously reported to be impractical because the DNA is easily degraded by gastric acidity. In this regard, Watanabe *et al*[56] have developed a new method for GC detection by DNA methylation in gastric washes but not in gastric juice. These authors analyzed 51 candidate genes in 7 GC cell lines and 24 GC samples (training set). They then selected 6 genes (*MINT25*, *RORA*, *GDNF*, *ADAM23*, *PRDM5* and *MLF1*) for further analyses. The methylation status of these genes was analyzed in a test set consisting of 131 GCs at various stages. The 6 candidate genes were validated in a different population of 40 primary GC samples and 113 noncancerous gastric mucosa samples. The 6 genes showed differential methylation in GC and normal mucosa in the training, test and validation sets. GDNF and MINT25 were the most sensitive molecular markers of early-stage GC, whereas PRDM5 and MLF1 were markers of a field defect. A close correlation between methylation levels in tumor biopsy samples and gastric washes was noted. MINT25 methylation showed the best sensitivity (90%) and specificity (96%), and it had the greatest area under the receiver operating characteristic curve (0.961) in terms of tumor detection in gastric washes. MINT25 methylation in gastric washes may be a sensitive and specific marker for the screening of GC.

Detection of early GC by DNA methylation analysis of Sox17 in gastric washes

Although minimally invasive treatment is widely accepted for early-stage GC, appropriate risk markers to detect residual cancer after endoscopic resection and the potential for recurrence are not available. To find candidate genes that might be markers for the detection of early GC, Oishi *et al*[57] performed methylated CpG island amplification microarray analysis on 12 gastric washes (from the pre- and post-endoscopic treatment of six patients). Among the candidate genes, the *Sox17* gene was selected for further analysis. The DNA methylation status of Sox17 was examined in a validation set consisting of 128 gastric wash samples (64 pre-treatment and 64 post-treatment) from cases of early GC. Sox17 showed significant differential methylation in the pre- and post-treatment gastric washes of early GC patients (Figure 3). Moreover, the treatment of GC cells that lacked Sox17 expression with the methyltransferase inhibitor 5-aza-2'-deoxycytidine restored the gene's expression. Additionally, the introduction of exogenous Sox17 into silenced

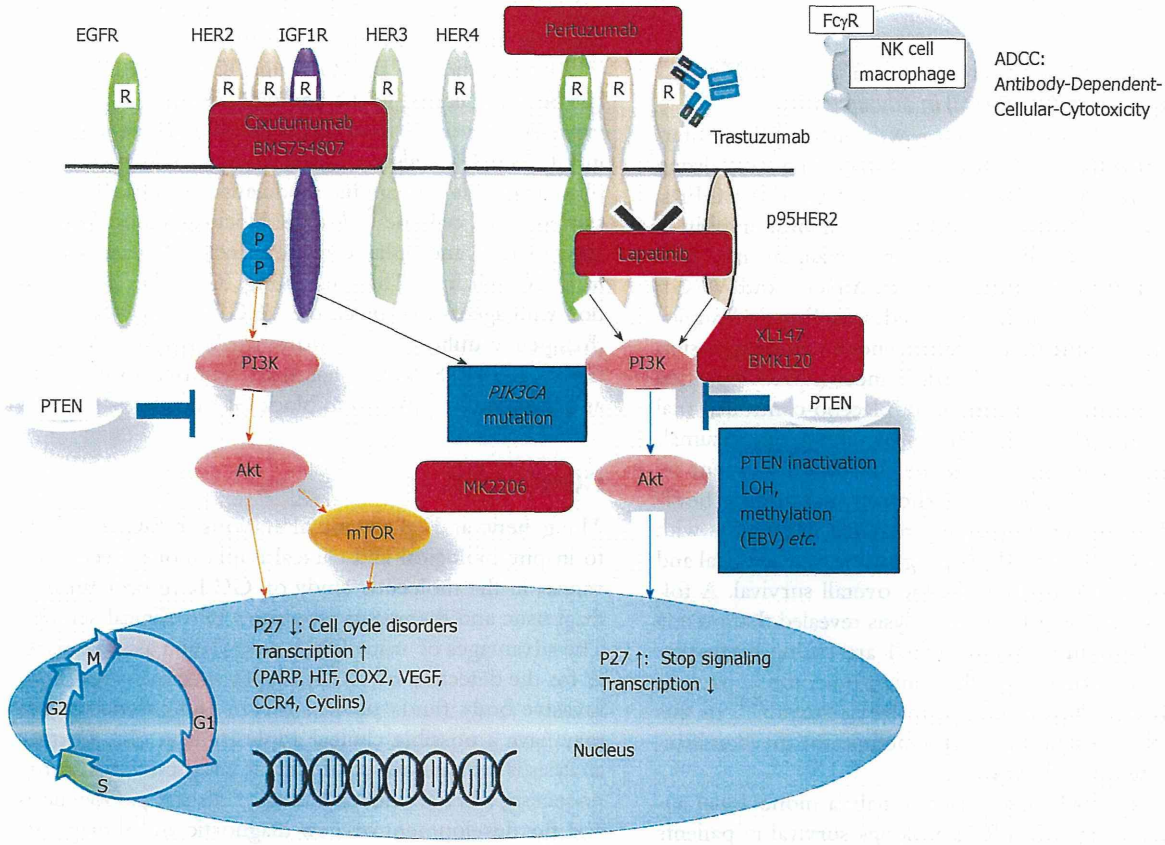

Figure 4 Human epidermal growth receptor family members, the PI3K/Akt pathway, and targeted drugs. HER: Human epidermal growth receptor; NK: Natural killer; IGF1R: α-insulin-like growth factor 1-receptor; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol-3-kinase; PTEN: Phosphatase and tensin homologue.

GC cells suppressed colony formation. The data suggest that the silencing of Sox17 occurs frequently in early GC and plays a key role in the disease. Gastric wash-based DNA methylation analysis could be useful for the early detection of recurrence following endoscopic resection in early GC patients. Interestingly, the usefulness of gastric wash-based molecular testing for antibiotic resistance in *H. pylori* has also been reported[58]. It will be interesting to analyze gastric washes using NGS.

Anti-HER2 antibody trastuzumab has led to an era of personalized therapy in GC

Trastuzumab is an antibody that targets the HER2 extracellular domain and induces antibody-dependent cellular cytotoxicity and inhibition of the HER2 downstream signals (Figure 4). In the ToGA study, standard chemotherapy regimens (capecitabine plus cisplatin or fluorouracil plus cisplatin) combined with trastuzumab resulted in a longer survival time than standard regimens without trastuzumab in patients with HER2-positive GC[59]. Thus, HER2 expression has become a major concern in GC[60]. HER2 overexpression is observed in 7%-34% of GC cases. Mechanisms of resistance to trastuzumab have been reported in breast cancer. There are various mechanisms underlying trastuzumab resistance, such as alterations of the HER2 structure or surroundings, dysregulation of HER2 downstream signal effectors and interaction of HER2 with other membrane receptors (Figure 4). The PI3K-Akt pathway is one of the main downstream signaling pathways of HER2. It is well known that PIK3CA mutations and PTEN inactivation cause over-activation of a downstream signal without activation of an upstream signal. The frequencies of PIK3CA mutations and PTEN inactivation in GC have been reported to be 4%-25% and 16%-77%, respectively. However, little is known about the association between HER2 expression and PI3K-Akt pathway alterations in GC. Sukawa *et al*[29] have found that HER2 overexpression was significantly correlated with pAkt expression in GC tissues. Furthermore, pAkt expression was correlated with poor prognosis. These results suggest that the PI3K-Akt pathway plays an important role in HER2-positive GC. Moreover, PIK3CA mutations and PTEN inactivation could affect the effectiveness of HER2-targeting therapy. Thus, it is necessary to clarify not only HER2 alterations but also PI3K-Akt pathway alterations to optimize HER2-targeting therapy in patients with GC. In this regard, NGS will be useful for the identification of complicated mechanisms of trastuzumab resistance in GC. The only approved targeted therapy for patients with advanced GC is trastuzumab. It is hoped that NGS will reveal a driver gene alteration that will make other targeted

therapies possible[13,61].

Monoclonal antibodies targeting VEGF (AVAGAST trial) and VEGFR-2 (REGARD trial) in advanced GC

Several vascular endothelial growth factor (VEGF)-targeted agents have been developed, including neutralizing monoclonal antibodies (MoAbs) to VEGF/VEGFRs, soluble VEGF receptors and tyrosine kinase inhibitors (TKIs). The anti-VEGF MoAb bevacizumab has been approved for colorectal cancers. VEGF and VEGF receptor-2 (VEGFR-2)-mediated signaling and angiogenesis contribute to the pathogenesis and progression of GC. The Avastin in Gastric Cancer (AVAGAST) trial was a multinational, randomized, placebo-controlled trial designed to evaluate the efficacy of adding bevacizumab to capecitabine-cisplatin in the first-line treatment of advanced GC[62]. The study showed that adding bevacizumab to the chemotherapy regimen in patients with advanced GC improved the progression-free survival and tumor response rate but not the overall survival. A following biomarker evaluation analysis revealed that plasma VEGF-A and tumor neuropilin-1 are strong biomarker candidates for predicting the clinical outcome in patients with advanced GC treated with bevacizumab[63]. In this regard, NGS will be a powerful method for the identification of predictive biomarkers.

To analyze whether ramucirumab, a monoclonal antibody targeting VEGFR-2, prolongs survival in patients with advanced GC, an international, randomized, double-blind, placebo-controlled, phase 3 trial was conducted in 29 countries[64]. In total, 355 patients with advanced gastric or gastro-esophageal junction adenocarcinoma and disease progression after first-line chemotherapy were randomly assigned (2:1) to receive best supportive care plus either ramucirumab 8 mg/kg (n = 238) or placebo (n = 117), intravenously once every 2 wk. The primary endpoint was overall survival. The median overall survival was 5.2 mo in the ramucirumab group and 3.8 mo in the placebo group (HR = 0.776, 95%CI: 0.603-0.998, P = 0.047). The survival benefit with ramucirumab remained unchanged after multivariate adjustment for other prognostic factors (multivariate HR = 0.774, 95%CI: 0.605-0.991, P = 0.042). Thus, ramucirumab is the first biological treatment given as a single drug that showed survival benefits in patients with advanced gastric or gastro-esophageal junction adenocarcinoma who progressed after first-line chemotherapy. The findings also validate VEGFR-2 signaling as an important therapeutic target in advanced GC.

Potential targeted drugs for GC

Using NGS to target a subset of druggable genes becomes a more effective way to discover therapeutic targets[13,14,61]. There are several potential targeted drugs, either MoAb or small-molecule TKIs, that are being investigated either in synergy with, or in place of, established treatments. These drugs include inhibitors of growth factors and their receptors [*i.e.*, VEGF, epidermal growth factor receptor, HER2, insulin-like growth factor 1 (IGF1) receptor, c-MET], MEK inhibitors and drugs targeting the Hedgehog pathway[65].

Dysregulation of the IGF1 and IGF2/IGF1R system has been implicated in the pathogenesis of GC[66-69]. The expression levels of both IGFs and IGF1R are increased in GC. IGF1R is also involved in angiogenesis and lymphangiogenesis through the modulation of VEGF expression in a GC cell line[70]. IGF1R blockade reduced tumor angiogenesis and enhanced the effects of bevacizumab in a GC cell line. Thus, targeting IGF1R in combination with agents that block the VEGF pathway may have therapeutic utility in GC. Moreover, targeting the novel miR-7/IGF1R/Snail axis has been reported to be useful as a therapeutic approach to block GC metastasis[71].

CONCLUSION

The genetic and epigenetic alterations in GCs continue to inspire biological and clinical implications. Recent advances in the molecular study of GC have brought new diagnostic and therapeutic strategies into clinical settings. The advantages of using DNA methylation as a biomarker for the detection of GC in biopsy specimens and non-invasive body fluids such as serum and gastric washes may have a possible clinical application in GC. Further analysis is required to gain a deeper insight into GC carcinogenesis, a better understanding of disease pathogenesis and the development of new diagnostic and therapeutic approaches targeting essential pathogenic alterations. In this regard, the rapid advances in NGS technologies will hopefully continue to reveal driver alterations of GC, further our understanding of gastric carcinogenesis and improve the therapy for each individual tumor. The characterization of genes that were discovered by NGS rather than by laboratory and clinical research is also necessary.

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GASTROENTEROLOGY

Serum *Helicobacter pylori* CagA antibody titer as a useful marker for advanced inflammation in the stomach in Japan

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Abstract

Background and Aim: Subjects infected with *Helicobacter pylori* containing *cagA* do not always induce serum CagA antibody. Our previous meta-analysis showed that serum CagA seropositivity was associated with gastric cancer even in East Asian countries. However, it remains unclear why serum CagA-positive status is associated with gastric cancer. In this study, we aimed to examine the relationship between anti-CagA antibody titer and the levels of pepsinogen (PG), and histological score.

Methods: Eighty-eight *H. pylori*-positive Japanese patients with gastritis were included. Serum CagA antibody titer, PG I, and PG II were evaluated by ELISA. Histological scores were evaluated according to Update Sydney System. CagA expression was examined by immunoblot.

Results: Seroprevalence of CagA antibody was found in 75.0%. Interestingly, serum CagA antibody titer was significantly correlated with PG I and PG II levels ($P = 0.003$ and 0.004, respectively). Serum CagA antibody titer was also significantly correlated with mucosal inflammation in the corpus ($P = 0.04$). On the other hand, bacterial density was not related with CagA antibody titer. CagA expression level of the strains was irrespective of the status of PG and serum CagA antibody.

Conclusions: Subjects with higher serum CagA antibody titer can be considered as high-risk population for the development of gastric cancer from the point of strong gastric inflammatory response even in Japan. Host recognition rather than bacterial colonization might be associated with the difference of serum CagA antibody titer.

Introduction

Helicobacter pylori is a spiral Gram-negative bacterium that infects more than half of the world's population.[1] *H. pylori* infection is now accepted to be linked to severe gastritis-associated diseases, including peptic ulcer and gastric cancer.[1] The infection remains latent in the majority of infected patients, only a minority of individuals with *H. pylori* infection ever develop it.[2] Uemura *et al.* reported that gastric cancer developed in approximately 3% of *H. pylori*-infected patients compared with none of the uninfected patients.[3] In addition to host, environmental, and dietary factors, the differences in the virulence of *H. pylori* strains are related with the varying outcomes of *H. pylori* infection.

The best-studied virulence factor of *H. pylori* is the CagA protein. CagA-producing strains are reported to be associated with severe clinical outcomes, especially in Western countries.[4–7] CagA is a highly immunogenic protein with a molecular weight between 120 and 140 kDa.[8,9] In 2003, Huang *et al.* performed meta-analysis of the association between CagA seropositivity and gastric cancer.[10] They concluded that the infection of CagA-positive strains increase the risk of gastric cancer. However, because they included studies from both Western and Asian countries, it was not clear whether an association between CagA seropositivity and gastric cancer really exists in East Asian countries. In East Asian countries, it is difficult to prove the importance of the *cagA* gene in clinical outcomes because almost all *H. pylori* strains possess the *cagA* gene. For example, we previously examined 491 Japanese strains from a region in the middle of Japan (Kyoto) and found that 96.3% of the strains were *cagA* gene-positive, irrespective of clinical outcomes;[11] similar results have been published for different regions in Japan[12–14] and other countries in East Asia.[15,16]

Interestingly, subjects infected with *cagA*-positive *H. pylori* do not always induce serum CagA antibody even in East Asian countries. For example, although most Japanese *H. pylori* possess *cagA*, serum CagA antibody is detected in only 53.7–81.1% of infected subjects in Japan.[17,18] This suggests that serum CagA antibody rather than the presence of *cagA* may be a more useful marker to detect the high-risk population for severe outcomes in East Asian countries. Intriguingly, we reported that CagA seropositivity was significantly associated with gastric cancer even in

East Asian countries in meta-analysis.[19] This suggests that anti-CagA antibody can be used as a biomarker for gastric cancer even in East Asian countries.

It remains unclear why not all subjects have serum CagA antibody in Japan. As described earlier, subjects with serum CagA antibody can be considered as a high-risk group for gastric cancer. Several factors such as bacterial factors and/or host recognition of CagA, and environmental factors may affect the difference of serum CagA antibody titer. In addition, it is not clear why serum CagA positive is associated with gastric cancer. In this study, we aimed to examine the relationship between anti-CagA antibody titer and the levels of pepsinogen (PG) and histological score.

Methods

Patients. Patients were considered to be *H. pylori*-infected when at least one of rapid urease test, culture, and microscopic examination showed positive results. Total of 88 *H. pylori*-positive Japanese patients with gastritis (29 males, 59 females, aged 22–87 years [mean, 58.4 years]) were recruited. Patients with drug allergies and those with serious complications, such as cardiac diseases, renal diseases, and hepatic diseases, were excluded from the study. Four biopsy samples (two from the antrum and two from the corpus) were endoscopically obtained from each patient and used for *H. pylori* culture and histopathological examination. Written informed consent was obtained from all participants, and the protocol was approved by the Ethics Committee of Oita University.

ELISA for serum CagA antibody titer and PG. Serum anti-CagA immunoglobulin G (IgG) antibody was measured by using a commercially available ELISA kit (Genesis Diagnostics Ltd, Cambridgeshire, UK). Equal and more than 6.25 U/mL were defined as positive based on the manufacturer's instructions. The level of the serum PG I and PG II were measured by PG ELISA kit (Eiken, Co. Ltd, Tokyo, Japan) according to the manufacturer's instructions.

Histological analysis. All biopsy materials were fixed in 10% buffered formalin for 24 h, then embedded in paraffin. Serial sections were stained with HE and with May–Giemsa stain. State of the gastric mucosa was evaluated according to the updated Sydney system.[20] The degree of inflammation, neutrophil activity, atrophy, intestinal metaplasia, and bacterial density were classified into four grades: 0, normal; 1, mild; 2, moderate; and 3, marked.

Isolation and genotyping of H. pylori. Antral biopsy specimens were obtained for isolation of *H. pylori* using standard culture methods.[11] *H. pylori* DNA was extracted from confluent plate cultures using a commercially available kit (QIAGEN, Valencia, CA, USA). The presence of *cagA* were determined by polymerase chain reaction (PCR) using primer pair cagTF; 5′-ACCCTAGTCGGTAATGGG-3′ and cagTR; 5′-GCTTTAGC TTCTGAYACYGC-3′ (Y = C or T) designed in the 3′ repeat region of *cagA*, as described previously.[21] The PCR conditions were initial denaturation for 5 min at 95°C, 35 amplification steps (95°C for 30 s, 56°C for 30 s, and 72°C for 30 s) and a final extension cycle of 7 min at 72°C, using Blend Taq DNA polymerase (TOYOBO, Osaka, Japan).

Immunoblot. Whole protein extracts from *H. pylori* isolates were obtained by suspending the bacteria in Laemmli sample buffer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and boiling this suspension at 100°C for 10 min. Immunoblotting was performed using standard methods. Two type of anti-CagA antibody (Abcom, Hong Kong; and Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as primary antibody at a 1:2000 dilution. Secondary antimouse or rabbit IgG was diluted 1:2000 (Jackson ImmunoResearch Lab, Inc., West Grove, PA, USA). Detection was performed using ECL Plus reagents (GE Healthcare, Buckinghamshire, UK). Protein concentrations were determined by the Lowry method and adjusted.

Statistical analysis. The univariate association was quantified by the chi-square test. Spearman rank coefficients (r) were determined to evaluate the association between anti-CagA antibody titer and the levels of PG, and histological score. A P value of less than 0.05 was accepted as statistically significant. The SPSS statistical software package version 19.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Association between serum CagA antibody titer and PG. Total of 88 patients with gastritis were examined their serum CagA antibody titer. Serum CagA antibody titer ranged from 0.3 to 137.1 U/mL, and average titer was 32.1 ± 33.4 U/mL. When equal and more than 6.25 U/mL was defined as positive based on the manufacturer's instructions, 66 (75.0%) patients were serum CagA antibody positive, and the remaining 22 were considered as negative. The average levels of PG I and II were 62.6 ± 37.0 (range 8.7–259.0) and 21.6 ± 12.6 (range 2.4–74.6) ng/mL, respectively. The PG I/II ratio ranged from 1.1 to 13.6 and average was 3.3 ± 1.9.

The comparison of age, gender, and PG level according to the status of CagA antibody was shown in Table 1. There was no difference of average age between serum CagA antibody positive and negative groups ($P = 0.49$). The percentage of male was significantly higher in serum CagA antibody negative group than positive group (54.5% *vs* 25.7%, $P = 0.01$). Among 59 female,

Table 1 The comparisons of age, gender and pepsinogen (PG) level according to the status of serum CagA antibody

	Serum CagA antibody positive	Serum CagA antibody negative	*P*
n	66	22	
Age (years)	57.9 ± 13.2	60.0 ± 11.6	0.49
Male	17 (25.7%)	12 (54.5%)	0.01
PG I	64.9 ± 38.8	58.8 ± 30.9	0.30
PG II	23.1 ± 13.2	17.6 ± 8.8	0.04
PG I/II	5.6 ± 21.8	3.5 ± 1.7	0.12

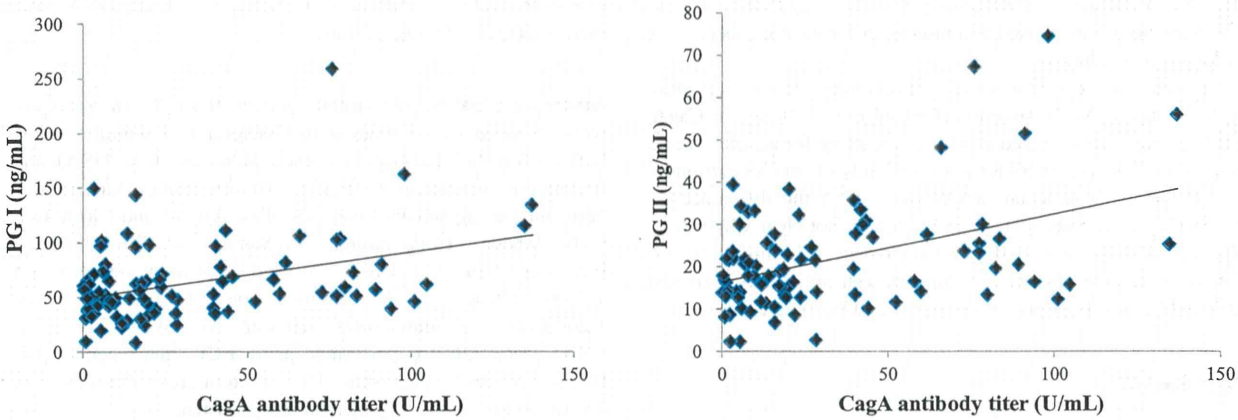

Figure 1 The correlation between serum CagA antibody titer and pepsinogen (PG) I and PG II.

49 (83.0%) showed serum CagA antibody positive. On the other hand, serum CagA antibody positive rate was 58.6% (17/29) in male. In fact, serum CagA antibody titer was significantly higher in female than male (38.6 ± 35.7 *vs* 18.6 ± 23.2 U/mL, $P = 0.003$). PG II level was significantly higher in serum CagA antibody positive group than negative group ($P = 0.04$). PG I level was also higher in serum CagA antibody positive than negative group; however, it was not statistically significant ($P = 0.30$). There was no difference of PG levels between male and female (data not shown).

The correlation between serum CagA antibody titers and PG levels was also examined (Fig. 1). Serum CagA antibody titer was significantly correlated with PG I level ($r = 0.30$, $P = 0.003$). In addition, serum CagA antibody titer was also correlated with PG II level ($r = 0.30$, $P = 0.004$). There was no correlation between serum CagA antibody titer and PG I/II ratio ($P = 0.77$). Even when only serum CagA antibody positive group was selected, serum CagA antibody titer was significantly correlated with PG I and PG II ($r = 0.40$, $P = 0.001$ for PG I; $r = 0.40$, $P = 0.001$ for PG II, respectively).

Association between serum CagA antibody titer and histological score. Next, the relationship between serum CagA antibody titer and histological score was examined. There were no significant differences of each score between serum CagA antibody positive and negative group (Table 2). However, the correlation between serum CagA antibody titer and histological score was examined; the inflammation in the corpus was significantly correlated with serum CagA antibody titer ($r = 0.26$, $P = 0.01$) (Fig. 2). Mucosal activity in the corpus was tended to be correlated with serum CagA antibody titer; however, there was no statistical significance ($P = 0.07$). These correlations was not found in the antrum ($P = 0.47$ for the inflammation, $P = 0.60$ for the activity). On the other hand, there was no association between serum CagA antibody titer and bacterial density both in the antrum and corpus ($P = 0.87$ and 0.79, respectively; Fig. 2). This suggests that low bacterial density cannot be a reason for low serum CagA antibody titer. Neither atrophy nor intestinal metaplasia both in the antrum and corpus was correlated with serum CagA antibody titer.

Table 2 The comparison of histological scores according to the status of serum CagA antibody

	Serum CagA antibody positive	Serum CagA antibody negative	*P*
n	66	22	
Antrum			
Inflammation	2.38 ± 0.51	2.41 ± 0.59	0.73
Activity	1.29 ± 0.73	1.55 ± 0.91	0.22
Atrophy	1.41 ± 0.65	1.36 ± 0.79	0.86
Intestinal metaplasia	0.26 ± 0.68	0.55 ± 0.96	0.09
Bacterial density	1.20 ± 0.76	1.20 ± 0.76	0.75
Corpus			
Inflammation	2.30 ± 0.60	2.14 ± 0.56	0.23
Activity	1.48 ± 0.70	1.36 ± 0.65	0.51
Atrophy	0.65 ± 0.85	0.55 ± 0.85	0.48
Intestinal metaplasia	0.09 ± 0.33	0.18 ± 0.50	0.38
Bacterial density	1.65 ± 0.64	1.64 ± 0.79	0.89

PG II was significantly correlated with inflammation and activity in the corpus ($P < 0.001$, $P < 0.001$, respectively). These correlations was not found in the antrum ($P = 0.20$ for the inflammation, $P = 0.15$ for the activity). Bacterial density in the antrum was significantly correlated with activity and inflammation in the antrum ($P = 0.001$ and $P < 0.001$, respectively), whereas bacterial density in the corpus was not correlated with any histological score. Even when only serum CagA antibody positive group was selected, serum CagA antibody titer was significantly correlated with inflammation and activity in the corpus ($r = 0.26$, $P = 0.04$ for inflammation, $r = 0.24$, $P = 0.04$ for activity, respectively).

Association between serum CagA antibody and bacterial CagA expression. The presence of *cagA* in the strain was examined by PCR using randomly selected 28 patients including 19 serum CagA antibody positive and 9 negative cases. PCR showed that all 28 samples were *cagA*-positive. To examine whether the difference of serum CagA antibody titer is attribute to