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ORIGINAL ARTICLE

Methylation of *GATA-4* and *GATA-5* and development of sporadic gastric carcinomas

Xian-Zi Wen, Yoshimitsu Akiyama, Kai-Feng Pan, Zhao-Jun Liu, Zhe-Ming Lu, Jing Zhou, Lian-Kun Gu, Cai-Xuan Dong, Bu-Dong Zhu, Jia-Fu Ji, Wei-Cheng You, Da-Jun Deng

Xian-Zi Wen, Zhao-Jun Liu, Zhe-Ming Lu, Jing Zhou, Lian-Kun Gu, Da-Jun Deng, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Etiology, Peking University School of Oncology and Beijing Cancer Hospital & Institute, Fucheng Road No. 52, Haidian District, Beijing 100142, China
Yoshimitsu Akiyama, Department of Molecular Oncology, Graduate School of Medicine and Dentistry, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
Kai-Feng Pan, Cai-Xuan Dong, Wei-Cheng You, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Epidemiology, Peking University School of Oncology and Beijing Cancer Hospital, Fucheng Road No. 52, Haidian District, Beijing 100142, China
Bu-Dong Zhu, Department of Internal Medicine, Peking University School of Oncology and Beijing Cancer Hospital, Fucheng Road No. 52, Haidian District, Beijing 100142, China
Jia-Fu Ji, Department of Surgery, Peking University School of Oncology and Beijing Cancer Hospital, Fucheng Road No. 52, Haidian District, Beijing 100142, China
Author contributions: Wen XZ participated in the study design and conducted methylation specific polymerase chain reaction, clone sequencing, immunohistochemical staining assays and wrote the manuscript; Akiyama Y participated in the study design; Pan KF, Dong CX and You WC conducted the ^{13}C-breath test and collected gastric dysplasia samples from subjects in Linqu County; Liu ZJ quantified GATA-4 methylation by denatured high performance liquid chromatography; Lu ZM analyzed *Helicobacter pylori* by polymerase chain reaction; Zhou J extracted genetic DNA from all tissue samples; Gu LK prepared gastric cancer tissue sections; Zhu BD collected the gastric biopsies from the outpatients; Ji JF collected the paired sporadic gastric carcinomas at Beijing Cancer Hospital; Deng DJ designed and coordinated the study, analyzed the data, and revised the manuscript.
Correspondence to: Da-Jun Deng, Professor, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Etiology, Peking University School of Oncology and Beijing Cancer Hospital & Institute, Fucheng Road No. 52, Haidian District, Beijing 100142, China. dengdajun@bjmu.edu.cn
Telephone: +86-10-88196752   Fax: +86-10-88122437

Abstract

AIM: To understand the implication of *GATA-4* and *GATA-5* methylation in gastric carcinogenesis.

METHODS: Methylation status of *GATA-4* and *GATA-5* CpG islands in human gastric mucosa samples, including normal gastric biopsies from 45 outpatients, gastric dysplasia [low-grade gastric intraepithelial neoplasia (GIN), $n = 30$; indefinite, $n = 77$], and 80 paired sporadic gastric carcinomas (SGC) as well as the adjacent non-neoplastic gastric tissues was analyzed by methylation specific polymerase chain reaction (MSP) and confirmed by denatured high performance liquid chromatography (DHPLC). Immunohistochemical staining was used to detect protein expression. The correlation between *GATA-4* and *GATA-5* methylation and clinicopathological characteristics of patients including *Helicobacter pylori* (*H. pylori*) infection was analyzed.

RESULTS: *GATA-4* and *GATA-5* methylation was frequently observed in SGCs (53.8% and 61.3%, respectively) and their corresponding normal tissues (41.3% and 46.3%) by MSP. The result of MSP was consistent with that of DHPLC. Loss of both GATA-4 and GATA-5 proteins was associated with their methylation in SGCs ($P = 0.01$). Moreover, a high frequency of *GATA-4* and *GATA-5* methylation was found in both gastric low-grade GIN (57.1% and 69.0%) and indefinite for dysplasia (42.9% and 46.7%), respectively. However, *GATA-4* and *GATA-5* methylation was detected only in 4/32 (12.5%) and 3/39 (7.7%) of normal gastric biopsies. *GATA-4* methylation in both normal gastric mucosa and low-grade GIN was also significantly associated with *H. pylori* infection ($P = 0.023$ and 0.027, two-sides).

CONCLUSION: Epigenetic inactivation of *GATA-4* (and *GATA-5*) by methylation of CpG islands is an early frequent event during gastric carcinogenesis and is significantly correlated with *H. pylori* infection.

Key words: Dysplasia; Gastric carcinoma; *GATA-4*; *GATA-5*; *Helicobacter pylori*; Methylation

INTRODUCTION

GATA proteins comprise a small family of transcriptional factors defined by a highly conserved DNA-binding domain that interacts specifically with DNA *cis*-elements. Six distinct vertebrate GATA proteins have been characterized and classified into two subfamilies based on their structural and expression patterns. GATA-1, -2 and -3 are important in the development and differentiation of the hematopoietic cell lineage, while GATA-4, -5 and -6 guide development and differentiation in the endoderm-derived organs, and specification of proper gut embryogenesis[1-3]. GATA-4 deficient cells exhibit an intrinsic defect in gastric epithelial cell differentiation to parietal cells in mice[4]. Overexpression of GATA-5 induced TFF1 expression[5]. Thus, GATA-4, -5 and -6 may play a critical role in the regulation of stomach-specific gene expression and cancer development. GATA-6 may function as an oncogene since it is often upregulated in proliferating progenitor cells[6-8]. In contrast, GATA-4 and GATA-5 may function as tumor suppressors to drive endodermal-specific differentiation because they are potential upregulators of the differentiation-related genes in the endoderm-derived organs[9].

The endoderm gives rise to the lining epithelium of the gastrointestinal tract. Loss of GATA-4 and GATA-5 might affect gastrointestinal epithelial cell differentiation, leading to tumorigenesis of these cells. In fact, there is growing evidence linking loss of *GATA-4* and *GATA-5* expression to many malignancies, such as primary esophageal, colorectal, gastric and pulmonary cancers[5,10-12]. Moreover, in addition to genetic inactivation, epigenetic changes such as methylation of CpG islands may play a major role in the loss of *GATA-4* and *GATA-5* function[5,10,12]. Since methylation of *GATA-4* and *GATA-5* is a frequent event in gastric cancer cell lines[5], it is interesting to know that methylation silences of *GATA-4* and *GATA-5* during gastric carcinogenesis.

MATERIALS AND METHODS

Gastric tissue samples
Primary sporadic gastric carcinoma (SGC) and the corresponding normal gastric samples: Fresh-frozen surgical SGC and their corresponding adjacent non-neoplastic "normal" samples were from the Tissue Bank at Beijing Cancer Hospital ($n = 80$; 62 males and 18 females, aged 35-81 years, average age 58.5 years). The clinical and histological information for each case was also collected according to approved institutional guidelines. The 1997 UICC-TNM criteria were used for classification of gastric cancers.

Gastric dysplasia samples: Biopsies of gastric dysplasia lesions [low-grade noninvasive gastric intraepithelial neoplasia (GIN), $n = 30$; indefinite for dysplasia, $n = 77$] (51 males and 56 female, aged 41-65 years, average age 51.7 years) were collected from patients without malignant disease enrolled in a gastro-endoscopic survey in Linqu County, a rural area in Shandong Province, China which has one of the world's highest rates of gastric cancer[13]. Histopathological diagnosis of each case was made by three senior pathologists at the Department of Pathology in Beijing Cancer Hospital, according to the Padova international classification[14]. Information on *Helicobacter pylori* (*H. pylori*) infection by the ^{13}C-urea breath test was also collected[15].

Gastric biopsies from patients with and without chronic gastritis: Gastric mucosa biopsies were collected from outpatients undergoing gastro-endoscopic examination at Beijing Cancer Hospital. Of 45 gastric biopsies used in the present study, 18 patients were diagnosed with superficial chronic gastritis and 27 without obvious pathological changes (43 males and 2 female, aged 19-47 years, average age 30.7 years).

Informed consent was obtained from all subjects and the institutional review committee approved this study.

Detection of H. pylori
H. pylori in the normal or corresponding normal gastric samples was analyzed with a *H. pylori*-specific 23S *rRNA*-polymerase chain reaction (PCR) assay as described[16].

Methylation-specific PCR (MSP)
DNA extraction, bisulfite treatment, and MSP were performed as described previously[17,18]. The MSP primer sequences for *GATA-4* and *GATA-5* were as follows: GATA-4M sense, 5'-GTATAGTTTCGT AGTTTGCGTTTAGC-3'; GATA-4M antisense, 5'-AACTCGCGACTCGAATCCCCG-3'; GATA-4U sense, 5'-TTTGTATAGTTTTGTAGTTTGTGTTTAGT-3'; GATA-4U antisense, 5'-CCCAACTCACAACTCAAATC CCCA-3'; GATA-5M sense, 5'-AGTTCGTTTTTAGGT TAGTTTTCGGC-3'; GATA-5M antisense, 5'-CCAATA CAACTAAACGAACGAACCG-3'; and GATA-5U sense, 5'-TGGAGTTTGTTTTTAGGTTAGTTTTTGGT-3'; GATA-5U antisense, 5'-CAAACCAATACAACTAAACA AACAAACCA-3' as described by Gou *et al*[12].
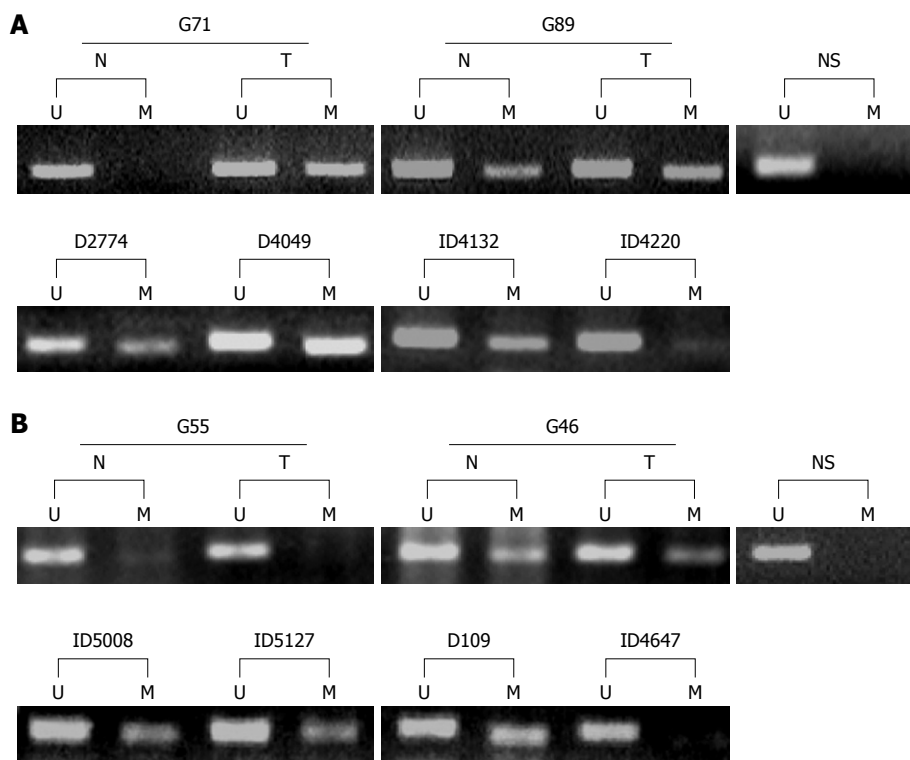
Figure 1 Methylation specific polymerase chain reaction (MSP) analyses of methylation status of the *GATA-4* (A) and *GATA-5* (B) CpG islands in human gastric mucosa samples. The upper panel shows MSP results of gastric cancer tissues (T) and their corresponding non-neoplastic tissues (N). The lower panel shows results of gastric dysplasia. The unmethylated (lanes U) and methylated (lanes M) MSP products were displayed on 2.5% agarose gels. NS: Normal stomach mucosa; D: Definite dysplasia; ID: Indefinite dysplasia.

Sequencing of the MSP products

The MSP products for *GATA-4* and *GATA-5* were subcloned with the pEASY-T1 simple clone system (Trans-Gen Biotech Company, Beijing, China), and sequenced on an ABI PRISM 3730 DNA Analyzer.

Separation and quantification of the methylated GATA-4 by denatured high performance liquid chromatography (DHPLC)[19,20]

Both methylated and unmethylated *GATA-4* CpG islands were amplified by a universal primer set without CpG (GATA-4uni sense 5'-GGAGATTTTAGAGTTT GGAT-3' and antisense 5'-CTCCCACTAACTACCTCT CT-3') under thermal cycle conditions [95℃ for 15 min → (95℃ for 30 s → 52.8℃ for 30 s → 72℃ for 50 s) × 40 cycles → 72℃ for 10 min]. The 385-bp PCR product of the methylated and unmethylated *GATA-4* were separated by DHPLC at a partial denaturing temperature of 57.1℃ and detected by a fluorescence (FL)-detector. The proportion of the methylated *GATA-4* in the testing samples was calculated according to the ratio of the peak area for the methylated *GATA-4* to the total peak area for both the methylated and unmethylated *GATA-4*. Genomic DNA of the HCT116 cell line was used as a *GATA-4* methylation positive control.

Immunohistochemical staining (IHC)

Paraffin sections (4 μm) were dewaxed and rehydrated in xylene and ethanol. For antigen retrieval, the sections were autoclaved in 1.0 mmol/L EDTA for 5 min, followed by immersion in 3% H_2O_2 methanol for 15 min to block endogenous peroxidase. The sections were then blocked in 15% normal goat serum in phosphate-buffered saline, followed by incubation with polyclonal GATA-4 or GATA-5 antibodies (Sigma-Aldrich, Inc., Missouri, USA) (1:100) overnight at 4℃. Then the sections were incubated with biotinylated goat anti-rabbit IgG working solution for 15 min at room temperature. The standard SP (Streptavidin/peroxidase) process was then performed according to the instructions for the Histostain™-Plus Kits (Beijng Zhongshan Goldenbridge Biotechnology Company, Beijng, China). Diaminobenzidine was used as a chromogen, followed by counterstaining with hematoxylin. Normal gastric mucosa samples were used as a positive control. PBS was used as a negative control to replace GATA-4/-5 antibody. We regarded GATA-4 and GATA-5 expression as positive when 10% or more cancer cells exhibited GATA-4 and GATA-5 expression.

RESULTS

Aberrant CpG island methylation of the GATA-4 and GATA-5 promoter in primary gastric cancer and their corresponding normal tissues

The promoter methylation status of *GATA-4* and *GATA-5* in eighty SGCs and their corresponding normal samples were analyzed by MSP. *GATA-4* and *GATA-5* methylation was observed in 43 and 49 SGCs (53.8% and 61.3%), respectively (Figure 1, Figure 2A and Table 1). To our surprise, these genes were also fre-
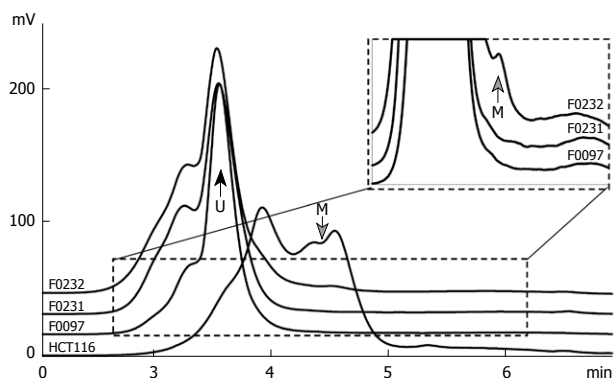
Figure 2 Summary of MSP results of *GATA-4* and *GATA-5* methylation in SGCs (A), gastric dysplasia (B), and normal gastric mucosa samples (C). Each row represents a single sample. G4: *GATA-4*; G5: *GATA-5*; M in black, detection of methylated alleles; u in white, detection of unmethylated alleles only; ND in white, methylation information not available. T: Sporadic gastric carcinomas (SGC) tissue; N: SGC-paired corresponding normal tissue; S: Clinical stage (1-4) of SGCs; *Hp*: *H. pylori* infection positive (P in gray) or negative (N in light gray), detected with the 23S *rRNA*-PCR for the normal and corresponding normal gastric mucosa samples and with the ^{13}C-urea breath test for subjects with gastric dysplasia. The *GATA-4* methylation positive rate in low-grade GIN from patients with *H. pylori* infection was significantly higher than that from patients without infection (15/21 *vs* 1/7, *P* = 0.023). The *GATA-5* methylation positive rate in low-grade GIN was significantly higher than that in indefinite for dysplasia (20/29 *vs* 35/75, *P* = 0.042). The *GATA-4* and *GATA-5* methylation rates in normal gastric biopsies were significantly lower than those in gastric dysplasia and SGC and the adjacent normal tissues (*P* < 0.001).

quently methylated in the corresponding non-neoplastic samples, 33 for *GATA-4* (41.3%) and 37 for *GATA-5* (46.3%), which were only slightly lower than those in SGCs (Figure 2A and Table 1).

To confirm the results of the MSP assay, we established a DHPLC assay to detect the frequency and proportion of the methylated *GATA-4* in 16 paired-SGC representative samples, in which *GATA-4* methylation was observed in both SGCs and their corresponding normal tissues by MSP. The methylated and unmethylated *GATA-4* was completely separated by DHPLC at a partial denaturing temperature of 57.1℃ (Figure 3). *GATA-4* methylation was detected in 15 SGCs (93.8%) and 12 adjacent normal samples (75.0%). The proportion of the methylated *GATA-4* CpG islands in the DHPLC-positive SGC samples was slightly higher than that in the DHPLC-

positive corresponding normal tissues (1.33% ± 0.45% *vs* 1.10% ± 0.56%, paired *t*-test, *P* = 0.054). The DHPLC results corresponded well with the above MSP results.

Aberrant CpG island methylation of the GATA-4 and GATA-5 promoter in gastric dysplasia lesions

We analyzed the methylation status of these genes in gastric biopsies with low-grade GIN or indefinite for dysplasia in the subjects from Linqu County, a high risk area for stomach cancer in China. Results showed that *GATA-4* and *GATA-5* were also frequently methylated in these dysplasia lesions, with strong methylated signals in 46 of 98 (46.9%) and 55 of 104 (52.9%), respectively (Figure 2B and Table 2). More *GATA-4* and *GATA-5* methylation was observed in tissues with GIN (57.1% and 69.0%, respectively) than in indefinite for dysplasia

Figure 3 Chromatogram of the methylated and unmethylated *GATA-4* CpG islands by denatured high performance liquid chromatography (DHPLC). The 385-bp PCR product of the methylated and unmethylated *GATA-4* were separated by DHPLC at a partial denaturing temperature 57.1℃ and detected by a fluorescence (FL)-detector. The proportion of methylated *GATA-4* in the tested samples was calculated according to the ratio of the peak area for the methylated *GATA-4* to the total peak area for both the methylated and unmethylated *GATA-4*. The gray arrow points to the peak for the methylated *GATA-4* (M) at the retention time 4.5 min; The black arrow points to the peak for the unmethylated *GATA-4* (U) at the retention time 3.6 min. Genomic DNA of HCT116 was used as the *GATA-4* methylation positive control. The inserted chart represents the dashed line surrounding the area. *GATA-4* methylation was detected in the tested sample F0232.

Table 1 Comparison of *GATA-4* and *GATA-5* methylation status with clinicopathological characteristics of SGC

Clinicopathological characteristics	Methylation positive rate (%)			
	GATA-4		*GATA-5*	
	SGC	N	SGC	N
SGC invasion				
T1&T2 (n = 20)	45.0[a]	35.0[b]	45.0	45.0
T3 (n = 50)	54.0	42.0	68.0	48.0
T4 (n = 10)	70.0	50.0	60.0	40.0
Metastasis				
Yes (n = 44)	52.3	31.8	61.4	36.4
No (n = 36)	55.6	52.8	61.1	38.9
H. pylori infection				
Positive (n = 42)	61.9	45.2	64.3	50.0
Negative (n = 34)	41.2	38.2	55.9	44.1
Age (yr)				
⩽ 59 (n = 42)	45.2	33.3	57.1	28.6
⩾ 60 (n = 38)	63.2	50.0	65.8	65.8[c]
Sex				
Male (n = 62)	51.6	41.9	61.3	51.6
Female (n = 18)	61.1	38.9	61.1	27.6[d]
Total				
n = 80	53.8	41.3	61.3	46.3[e]

SGC: Sporadic gastric carcinomas; N: Adjacent corresponding non-neoplastic gastric tissue; *H. pylori*: *Helicobacter pylori* detected by the 23S rRNA-PCR assay[16]; [a,b]Analysis for linear trend by EpiInfo 6.0 software, positive rates *vs* extent of penetration of the stomach wall, P = 0.198 and 0.434, respectively; [c]χ^2 test by EpiInfo 6.0 software, samples from young patients (⩽ 59 years old) *vs* old patients (⩾ 60 years old), P < 0.001; [d]Male *vs* female, P = 0.074; [e]SGCs *vs* the normal, P = 0.057.

(42.9% and 46.7%), especially for *GATA-5* (P < 0.05) (Table 2). *GATA-4* and *GATA-5* methylation in two representative dysplasia samples were confirmed by bisulfite clone sequencing (Figure 4). In contrast, these genes were rarely methylated in the relatively normal gastric biopsies.

Table 2 *GATA-4* and *GATA-5* methylation frequency (%) in gastric dysplasia lesions in subjects from Linqu County, a high risk area for stomach cancer

Classification of gastric dysplasia	*GATA-4*	*GATA-5*
Low-grade noninvasive neoplasia	57.1 (16/28)	69.0 (20/29)
Hp-infection		
With	71.4 (15/21)	63.6 (14/22)
Without	14.3 (1/7)[a]	85.7 (6/7)
Indefinite for dysplasia[b]	42.9 (30/70)	46.7 (35/75)[c]
Total	46.9 (46/98)	52.9 (55/104)

[a]χ^2 test by EpiInfo 6.0 software, with *vs* without Hp-infection, P = 0.023; [b]Only two cases without *Hp*-infection; [c]χ^2 test by EpiInfo 6.0 software, Low-grade noninvasive neoplasia (GIN) *vs* indefinite for dysplasia, P = 0.042.

Table 3 Correlation of the *GATA-4* and *GATA-5* methylation status with their protein expression in sporadic gastric carcinomas with IHC

Protein expression, by IHC	*GATA-4* methylation status			*GATA-5* methylation status		
	M	U	P-value[a]	M	U	P-value
Positive (+ ~ +++)	7	11	0.012	5	9	0.011
Negative (- ~ ±)	11	3		16	3	

IHC: Immunohistochemical staining; +~+++: 10% or more cancer cells exhibited a similar positive staining pattern; - or weak: Positive expression in less than 10% cancer cells or a weak staining pattern compared with a non-cancerous area of the same section; M: Methylated; U: Unmethylated; [a]Fisher's exact test by EpiInfo 6.0 software.

Among the 45 gastric biopsies (27 normal and 18 superficial chronic gastritis), *GATA-4* and *GATA-5* methylation was only 4/32 (12.5%) and 3/39 (7.7%), respectively (Figure 2C), significantly lower than the methylation in gastric dysplasia and SGCs as well as the adjacent normal samples (P < 0.001).

GATA-4 and GATA-5 expression was correlated with their methylation status

We investigated the expression of GATA-4 and GATA-5 in normal and gastric carcinoma tissues using IHC. These genes were expressed in the gland region of normal gastric mucosa (Figure 5A and D). In contrast, GATA-4 and GATA-5 expression was obviously decreased in many gastric carcinomas (Figure 5B and E, Table 3). A significant inverse relationship was observed between *GATA-4* (or *GATA-5*) methylation and GATA-4 (or GATA-5) protein expression in the gastric samples tested using IHC (Figure 3 and Table 3).

The relationship between methylation frequencies of GATA-4 and GATA-5 and clinicopathological parameters including H. pylori infection

As shown in Table 1, an increased trend of *GATA-4* methylation was observed in SGCs and the corresponding normal samples with depth of tumor invasion (T1-2, T3, T4), but was not statistically significant. The *GATA-5* methylation positive rate in the corresponding normal tis-
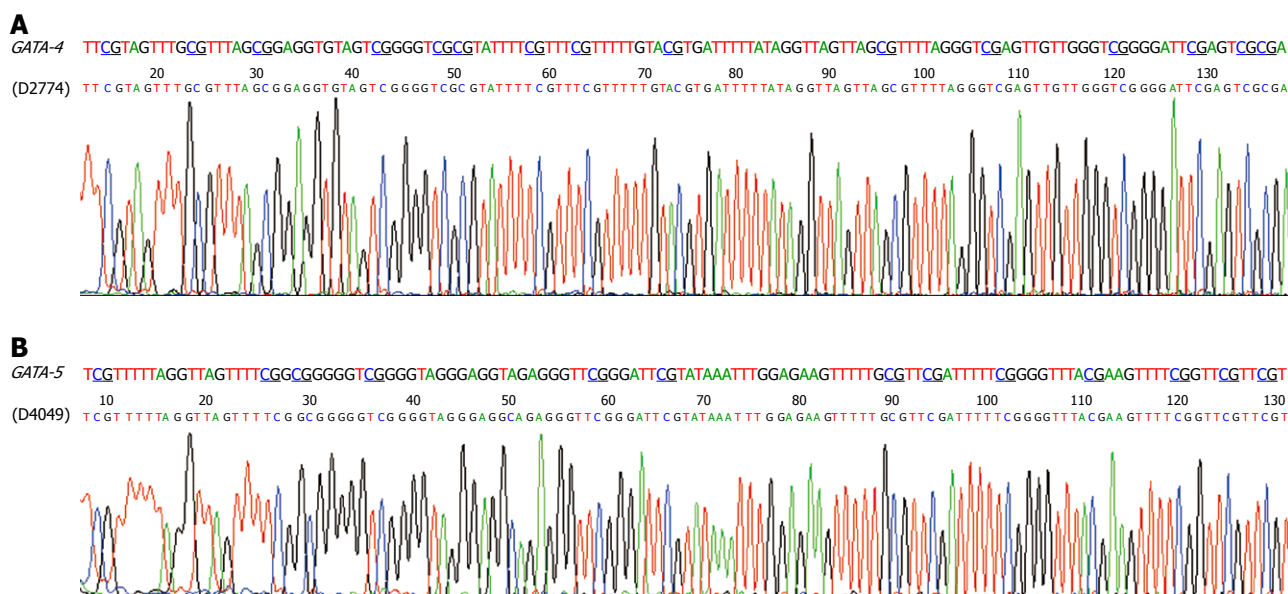
A

GATA-4 TTCGTAGTTTGCGTTTAGCGGAGGTGTAGTCGGGGTCGCGTATTTTCGTTTCGTTTTTGTACGTGATTTTTATAGGTTAGTTAGCGTTTTAGGGTCGAGTTGTTGGGTCGGGGATTCGAGTCGCGA

(D2774)

B

GATA-5 TCGTTTTTAGGTTAGTTTTCGGCGGGGGTCGGGGTAGGGAGGTAGAGGGTTCGGGATTCGTATAAATTTGGAGAAGTTTTTGCGTTCGATTTTTCGGGGTTTACGAAGTTTTCGGTTCGTTCGT

(D4049)

Figure 4 Sequencing of the methylated MSP products for *GATA-4* (A) and *GATA-5* (B) from two representative gastric dysplasia samples. The predicted sequence of bisulfite-modified *GATA-4* and *GATA-5* CpG islands are listed in each panel at the top. The methylated CpG sites are underlined.
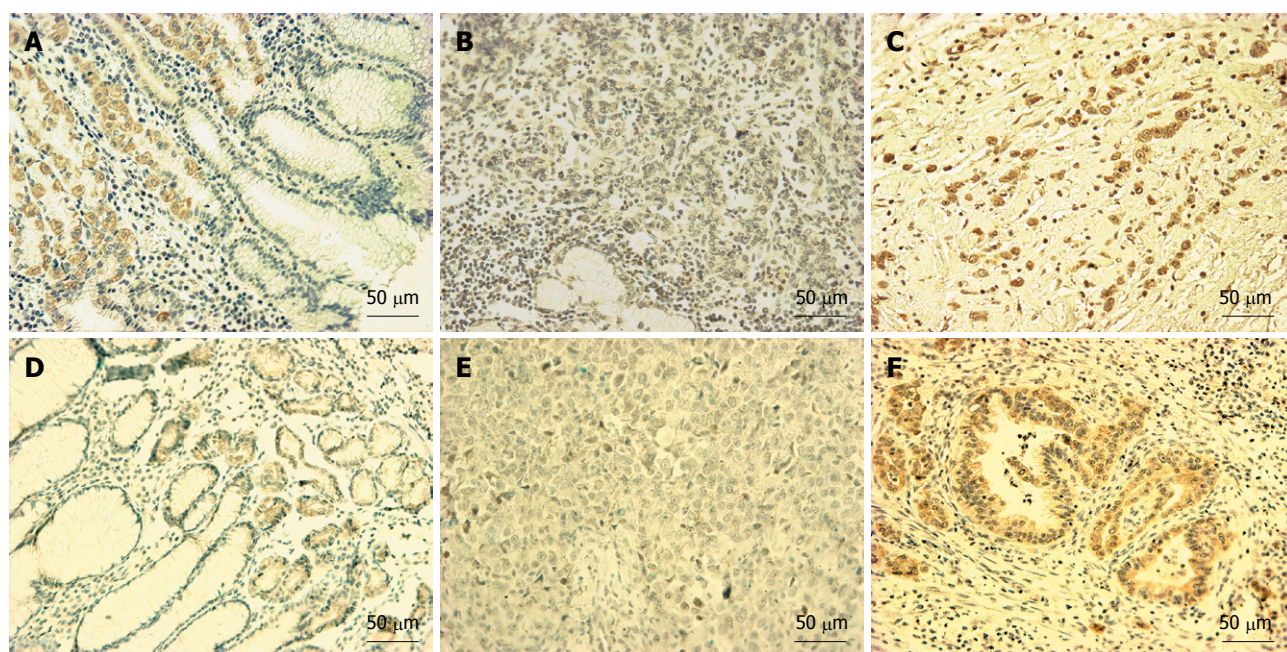
Figure 5 GATA-4 (A-C) or GATA-5 (D-F) protein in various gastric mucosa samples by immunohistochemical staining (IHC). A and D: GATA-4 and GATA-5 expression were confined to the gland region in the normal gastric mucosa; B and E: Carcinoma cells containing methylated alleles of *GATA-4* (G89) or *GATA-5* (G48) exhibited negative staining; C and F: Unmethylated cancer cells exhibited positive staining in case G27 and G45, respectively. Diaminobenzidine was used as a chromogen, followed by counterstaining with hematoxylin. Black bar, 50 μm in length.

sues from elderly and male patients was significantly higher than that from young and female patients, respectively (Table 1).

Moreover, a correlation was observed between *GATA-4* (or *GATA-5*) methylation and *H. pylori* infection. More *GATA-4* and *GATA-5* methylation was detected in SGCs and their corresponding normal samples with *H. pylori* infection than in those without infection, but was not statistically significant ($P = 0.071$) (Figure 2B). In the gastric dysplasia tissues, particularly in those with low-grade GIN, the *GATA-4* methylation positive rate for the patients with *H. pylori* infection was significantly higher than that in patients without *H. pylori* infection (15/21 *vs* 1/7, $P = 0.023$, Table 2).

In addition, all 4 biopsies with *GATA-4* methylation were from *H. pylori* infected patients ($n = 14$). No *GATA-4* methylation was observed in biopsies from subjects without *H. pylori* infection ($n = 18$, $P = 0.027$, two-sides). Of 3 biopsies with *GATA-5* methylation, 2 were from *H. pylori* infected patients (Figure 2C).

DISCUSSION

Epigenetic silencing of *GATA-4* and *GATA-5* by DNA methylation has been reported in gastrointestinal cancer cell lines and primary carcinomas[5,10,12]. However, the implication of *GATA-4* and *GATA-5* methylation in the development of gastric cancer is unclear. Hence, we analyzed the methylation status of *GATA-4* and *GATA-5* CpG islands in gastric tissues from different kinds of lesions. We found that *GATA-4* and *GATA-5* methylation was detected in 53.8% and 61.3% of SGCs by MSP, respectively. These results were confirmed by the quantitative DHPLC assay. Furthermore, a significant inverse relationship was observed between methylation status and their protein expression in the gastric samples tested using IHC. These results indicate that *GATA-4* and *GATA-5* may be frequently inactivated in SGCs by DNA methylation. Moreover, the high prevalence of *GATA-4* and *GATA-5* methylation in the corresponding normal tissues suggests that these aberrant methylations may be a gastric field-effect, a phenomenon which happens within the whole target regions during carcinogenesis[21].

GATA-4 and *GATA-5* were also methylated in 46.9% and 52.9%, respectively, of gastric dysplasia, a precancerous lesion of gastric carcinomas. However, both of these genes were seldom methylated in gastric tissues with or without chronic gastritis [4/32 (12.5%) and 3/39 (7.7%), respectively]. These results indicate that *GATA-4* and *GATA-5* methylation is an early frequent event during the development of gastric carcinomas.

In addition, we found that the *GATA-5* methylation positive rate in the corresponding normal tissues from elderly and male patients was significantly higher than that from young and female patients, respectively. These results are consistent with the higher incidence of gastric carcinoma in males than in females, and the increasing prevalence of gastric carcinomas among elderly subjects.

H. pylori infection is the main cause of chronic atrophic gastritis, which may play an important role in gastric carcinogenesis. A number of tumor-related genes such as *p16* could be inactivated by DNA methylation in gastric mucosa lesions with *H. pylori* infection[22,23]. In the present study, we found that more *GATA-4* and *GATA-5* methylation in gastric samples from patients with *H. pylori* infection were detected than in those without *H. pylori* infection, especially for *GATA-4* methylation. The *GATA-4* methylation positive rate in the low-grade GIN patients with *H. pylori* infection was significantly higher than that in patients without *H. pylori* infection (15/21 *vs* 1/7, *P* = 0.023). These results suggest that *GATA-4* and *GATA-5* methylation could be initiated in the precancerous stage by *H. pylori* infection.

In addition, among gastric tissues with or without chronic gastritis, all 4 biopsies with *GATA-4* methylation were from *H. pylori* infected patients (*n* = 14), no *GATA-4* methylation was observed in biopsies from subjects without *H. pylori* infection (*n* =18; *P* = 0.027, two-sides), and of 3 biopsies with *GATA-5* methylation, 2 were from *H. pylori* infected patients. These results again support the hypothesis that *H. pylori* infection may contribute to epigenetic inactivation of these genes in gastric mucosa.

In conclusion, *GATA-4* and *GATA-5* methylation was an early frequent field-effect and significantly correlated with the severity of pathological changes during gastric carcinogenesis. *H. pylori* infection may contribute to *GATA-4* and *GATA-5* methylation in the human stomach.

COMMENTS

Background
Tumor suppressor genes *GATA-4* and *GATA-5* are important for development of the stomach during embryogenesis. Epigenetic inactivation of these genes by DNA hypermethylation was previously reported in esophageal and lung cancer.

Innovations and breakthroughs
In the present study, Dr. Wen *et al* found that aberrant *GATA-4* and *GATA-5* methylation was also a frequent event in gastric carcinomas and their adjacent tissues. Interestingly, their work demonstrated that epigenetic inactivation of *GATA-4* and *GATA-5* was observed in about 50% of gastric mucosa samples with epithelial dysplasia, a precancerous lesion in the stomach. However, such a phenomenon was very rare in gastric mucosa biopsies from healthy subjects or patients with chronic gastritis. They also observed that *Helicobacter pylori* (*H. pylori*) infection correlated well with *GATA-4* and *GATA-5* methylation. These results indicate that epigenetic inactivation of *GATA-4* and *GATA-5* is an early frequent event during gastric carcinogenesis by *H. pylori* and might be used to screen patients with a high risk of stomach cancer.

Peer review
The authors have tried to show that methylation of *GATA-4* and *GATA-5* could be important in the oncogenesis of gastric mucosa in patients coming from a Chinese area where gastric cancer is common. They showed that in cancer and adjacent mucosa methylation of these two antioncogenes is common and apparently related mostly with chronic *H. pylori* infection.

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