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DNA Methylation of Multiple Genes in Gastric Cancer: Association with CpG Island Methylator Phenotype and *Helicobacter pylori* Infection

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Purpose: Methylation of gene regulatory elements plays an important role in gene inactivation without genetic alteration. Gastric cancer is one of the tumors that exhibit a high frequency of CpG island hypermethylation. The purpose of this study was to investigate the occurrence of CpG island hypermethylation in gastric carcinoma in relation to *H. pylori* infection, CIMP and clinicopathologic variables.
Materials and Methods: We investigated the promoter methylation status of six genes (hMLH1, p16, p14, COX-2, MGMT, E-cadherin) and CIMP in 36 gastric carcinoma tissues as well as in nontumor tissues. CIMP status was investigated by examining the methylation status of MINT 1, 2, 12, 25 and 31. The methylation status of the promoter was examined by methylation-specific PCR (MSP) and H. pylori infection was examined by histological diagnosis after staining with Warthin-Starry silver.
Results: Among the 36 gastric carcinoma tissues, DNA hypermethylation was detected in the following frequencies: 14 (38.9%) for p14, 13 (36.1%) for p16, 8 (22.2%) for MGMT, 10 (27.8%) for COX-2, 21 (58.3%) for E-cadherin, and 6 (16.7%) for hMLH1. The frequencies for MINT1 and MINT25 hypermethylation were significantly higher in tumor tissues than in nontumor tissues. 16 (44.4%) of the 36 gastric carcinoma tissues were positive for the CIMP. CIMP-H tumors were associated with older patients and larger tumor size than CIMP-L tumors. We found a significant association between the presence of the CIMP and hypermethylation of p16. Hypermethylation of p16

and MINT2 were significantly different when compared by age. MINT1 gene methylation was significantly associated with H. pylori infection (P=0.004).
Conclusion: Our results suggest that aberrant hypermethylation of multiple tumor related genes (hMLH1, p16, p14, COX-2, MGMT, E-cadherin, MINT1, 2, 12, 25, 31) occurs frequently in gastric carcinoma tissues. The hypermethylation of MINT1 was significantly higher in the tumor tissues and was associated with H. pylori infection.

INTRODUCTION

Epigenetic change is an inherited change in the pattern of gene expression without a change in DNA sequences. The promoters of about half of all human genes have CpG islands containing a G+C content of greater than 60% which is usually unmethylated in normal tissues.(1) Aberrant hypermethylation of CpG islands in 5'-promoter regions acts as an alternative to genetic changes for the inactivation of tumor suppressor genes.(2,3) CpG island methylation is associated with changes in chromatin organization and consequent repression of gene transcription.(4,5) Gastric cancer has been shown to have a high frequency of DNA hypermethylation.(6) Genes that are inactivated by CpG island hypermethylation have been reported in gastric cancer, involving tumor suppressor, cell-cycle regulator, tissue-invasion-related and DNA mismatch repair genes.(7,8) Recently, the CpG island methylator phenotype (CIMP), which is characterized by simultaneous methylation of the CpG islands of multiple genes, has been recognized as one of the important mechanisms in the deve-

lopment of colon, stomach and pancreas cancer.

H. pylori is considered a major risk factor for gastric cancer. Chronic infection with *H. pylori* causes the inflammation of the gastric mucosa with mononuclear/neutrophil infiltration and results in the destruction of parietal cells followed by a decrease of gastric acid secretion. Superoxide molecules in the gastric mucosa play an important role in the initiation of the carcinogenesis by mediating carcinogenic nitrosamine formation, DNA damage and tissue injury. H. pylori infection is associated with the enhanced expression of iNOS by tissue neutrophils and mononuclear cells and the iNOS producing gastritis is associated with gastric cancer. Chan et al(9) suggested an another mechanism of gastric cancer induction by *H. pylori* infection, that is, *H. pylori* might augment DNA hypermethylation in normal gastric tissue. There are few reports that have shown an association between *H. pylori* infection and hypermethylation of CpG islands in gastric carcinoma.

The present study investigated whether *H. pylori* infection and CIMP affect DNA methylation in gastric carcinoma tissues as well as corresponding non-tumor mucosa. The aim of the present study was to evaluate the occurrence of CpG island hypermethylation in gastric carcinoma in relation to *H. pylori* infection, CIMP, and clinicopathologic variables. We examined 11 genes, whose expression is frequently silenced by aberrant CpG island methylation in gastric cancer. The tested genes were COX-2, p14, p16, hMLH1, MGMT, E-cadherin and 5 methylated in tumors (MINT) loci. The MSP method was used for the analysis of methylation frequency, and the findings were correlated with the *H. pylori* infection and clinicopathological parameters.

MATERIALS AND METHODS

1) Samples and DNA extractions

A total 36 samples were obtained at St. Vincent's Hospital, the Catholic University of Korea, between January 2003 and December 2003. All of the patients had provided informed consent before collection of the samples. For all of these tumors, paired normal tissues were also obtained. Tumor tissues were removed surgically, frozen immediately in liquid nitrogen, and stored at $-80°C$ until use. We confirmed microscopically that the 36 tumor specimens consisted mainly ($>80\%$) of carcinoma tissue. Genomic DNAs were extracted with a genomic DNA purification kit (Promega, Madison, WI).

2) Bisulfite treatment and methylation-specific PCR

Both methylation-specific PCR (MSP) and bisulfite-PCR take

advantage of the fact that unmethylated cystosine is efficiently converted to uracil after 16 hours of Na-bisulfite treatment, whereas methylated cytosines remain unchanged. To examine the DNA methylation patterns, we treated genomic DNA with sodium bisulfite, as described by Herman et al(10) In brief, 2 μg of genomic DNA was denatured by treatment with NaOH and modified with 3M sodium bisulfite for 16 hours. DNA samples were purified with Wizard DNA purification resin (Promega, Madison, WI), treated with NaOH, precipitated with ethanol and resuspended in 25 μl of water. MSP was used to examine the methylation status of 6 genes (MGMT, p16, hMLH1, COX-2, p14, E-cadherin) and 5 cancer-specific MINT clones (MINT 1, MINT 2, MINT 12, MINT 25, and MINT 31). The primer sequences of all genes for the methylated and unmethylated reactions, and the annealing temperature, are described in Table 1. PCR was performed in 10-μl reaction volumes, containing 10× PCR buffer, deoxynucleotide triphosphates (each at 2 nmol/L), primers (5 pmol each) and 0.2 unit of *Taq* polymerase. PCR products underwent electrophoresis on 2.5% agarose gel and were then visualized under UV illumination using an ethidium bromide stain. Samples were scored as methylated when there was a clearly visible band on the gel with the methylated primers. Cases with methylation at more than three of five loci (MINT1, 2, 12, 25, and 31) were defined as CIMP-H (high), those methylated at less than two loci were defined as CIMP-L (low) and the cases where no loci were methylated were defined as CIMP-N (negative).

3) Analysis of *Helicobactor pylori* infection

All patients underwent gastroduodenoscopic examinations, and routine biopsy specimens were taken from the gastric antrum for histological diagnosis. *H. pylori* was considered to be present when histological diagnosis after staining with Warthin-Starry silver, was positive. When the staining was negative, the patient was considered to be *H. pylori* negative.

4) Statistical analysis

Statistical significance of differences was assessed with the chi-square test, Fisher's exact test and unpaired *t*-test using SPSS software (version 8.01 SPSS, Chicago, IL). Two-sided tests were used to determine significance and P values less than 0.05 were regarded as statistically significant.

RESULTS

1) DNA methylation pattern according to age

The mean age of the patients was 58.9 years; the ages ranged

Table 1. Primer sequences used in MSP

Genes		Sequences	Product size (bp)	AT* (°C)
p14	Unmethylated	5'-TTTTTGGTGTTAAAGGGTGGTGTAGT-3' 5'-CACAAAAACCCTCACTCACAACAA-3'	132	57
	Methylated	5'-GTGTTAAAGGGCGGCGTAGC-3' 5'-AAAACCCTCACTCGCGACGA-3'	122	60
p16	Unmethylated	5'-TTATTAGAGGGTGGGGTGGATTGT-3' 5'-CAACCCCAAACCACAACCATAA-3'	151	60
	Methylated	5'-TTATTAGAGGGTGGGGCGCATCGC-3' 5'-GACCCCGAACCGCGACCGTAA-3'	150	65
MGMT	Unmethylated	5'-GTAGGTTGTTTGTATGTTTGT-3' 5'-AACCAATACAAACCAAACA-3'	121	43
	Methylated	5'-TTTCGACGTTCGTAGGTTTTCGC-3' 5'-GCACTCTTCCGAAAACGAAACG-3'	118	59
COX-2	Unmethylated	5'-ATAGATTAGATATGGTGGTGGTGGT-3' 5'-CACAATCTTTACCCAAACACTTCCA-3'	171	61
	Methylated	5'-TTAGATACGGCGGCGGCGGC-3' 5'-TCTTTACCCGAACGCTTCCG-3'	161	61
E-cadherin	Unmethylated	5'-TAATTTTAGGTTAGAGGGGTTATTGT-3' 5'-CACAACCAATCAACAACACA-3'	97	53
	Methylated	5'-TTAGGTTAGAGGGTTATCGCGT-3' 5'-TAACTAAAAATTCACCTACCGAC-3'	116	57
hMLH1	Unmethylated	5'-TTTTGATGTAGATGTTTTATTAGGGTTGT-3' 5'-ACCACCTCATCATAACTACCCACA-3'	100	60
	Methylated	5'-TATATCGTTCGTAGTATTCGTGT-3' 5'-TCCGACCCGAATAAACCCAA-3'	92	60
MINT1	Unmethylated	5'-AATTTTTTTATATATATTTTTGAAGTGT-3' 5'-AACAAAAAACCTCAACCCCACA-3'	100	48
	Methylated	5'-GGGTTGAGGTTTTTTGTTAGC-3' 5'-CTACTTCGCCTAACCTAACG-3'	96	64
MINT2	Unmethylated	5'-GATTTTGTTAAAGTGTTGAGTTTGTT-3' 5'-CAAAATAATAACAACAATTCCATACA-3'	100	51
	Methylated	5'-AATCGAATTTGTCGTCGTTTC-3' 5'-AAATAAATAAATAAAAAAAAACGCG-3'	90	60
MINT12	Unmethylated	5'-TGGGAGTTTATTTAGGTTGG-3' 5'-AAACACAACAATCTTCCAAAT-3'	155	55
	Methylated	5'-TTGGGAGTTTATTTAGGTCG-3' 5'-ACAACGATCTTCCGAATTTA-3'	152	55
MINT25	Unmethylated	5'-GTAATTTTGTGGAAGGTGTT-3' 5'-ACAAAACACCACACCCAACAC-3'	109	45
	Methylated	5'-GTTCGTTAGAGTAATTTTGCG-3' 5'-TTATAACTAACGAAACACCGC-3'	101	55
MINT31	Unmethylated	5'-TAGATGTTGGGGAAGTGTTTTTTGGT-3' 5'-TAAATACCCAAAAACAAAACACCACA-3'	96	57
	Methylated	5'-TTGAGACGATTTTAATTTTTTGC-3' 5'-AAAACCATCACCCCTAAACG-3'	84	62

*Annealing temperature.

Table 2. MSP results of multiple genes according to age

		Age group (%)				P value
		~39	40~49	50~59	60~	
p14	Methylated	1 (7.1)	3 (21.4)	1 (7.1)	9 (64.3)	0.2960
	Unmethylated	1 (4.6)	5 (22.7)	7 (31.8)	9 (40.9)	
p16	Methylated	−	1 (7.7)	1 (7.7)	11 (84.6)	0.0137
	Unmethylated	2 (8.7)	7 (30.4)	7 (30.4)	7 (30.4)	
MGMT	Methylated	1 (12.5)	4 (50.0)	1 (12.5)	2 (25.0)	0.0748
	Unmethylated	1 (3.6)	4 (14.3)	7 (25.0)	16 (57.1)	
COX-2	Methylated	−	2 (20.0)	1 (10.0)	7 (70.0)	0.4796
	Unmethylated	2 (7.7)	6 (23.1)	7 (26.9)	11 (42.3)	
E-cadherin	Methylated	2 (9.5)	6 (28.6)	3 (14.3)	10 (47.6)	0.3771
	Unmethylated	−	2 (13.3)	5 (33.3)	8 (53.3)	
hMLH1	Methylated	−	1 (16.7)	2 (33.3)	3 (50.0)	0.8995
	Unmethylated	2 (6.7)	7 (23.3)	6 (20.0)	15 (50.0)	
MINT1	Methylated	1 (4.6)	7 (31.8)	3 (35.7)	11 (50.0)	0.2004
	Unmethylated	1 (7.1)	1 (7.1)	5 (35.7)	7 (50.0)	
MINT2	Methylated	1 (7.7)	2 (15.4)	−	10 (76.9)	0.0241
	Unmethylated	1 (4.4)	6 (26.1)	8 (34.8)	8 (34.8)	
MINT12	Methylated	−	2 (25.0)	1 (12.5)	5 (62.5)	0.9207
	Unmethylated	2 (7.1)	6 (21.4)	7 (25.0)	13 (46.4)	
MINT25	Methylated	2 (6.9)	6 (20.7)	6 (20.7)	15 (51.7)	0.8358
	Unmethylated	−	2 (28.6)	2 (28.6)	3 (42.9)	
MINT31	Methylated	1 (14.3)	−	2 (28.6)	4 (57.1)	0.3277
	Unmethylated	1 (3.5)	8 (27.6)	6 (20.7)	14 (48.3)	

from 35 to 82 years. Two genes (p16, MINT2) showed a general increase in the methylation frequency as a function of aging, whereas the other genes (p14, MGMT, COX-2, E-cadherin, hMLH1, MINT1, MINT12, MINT25, MINT31) were only rarely methylated (Table 2).

2) DNA methylation pattern of gastric carcinoma

Thirty-six gastric carcinoma tissues and adjacent nontumor tissues were examined for methylation status of CpG islands of 6 cancer-related genes and 5 cancer-specific MINT loci. Representative data for MSP of thep14, p16, MGMT, COX-2, E-cadherin and hMLH1 genes are shown in Fig. 1. Aberrant methylations was more frequent in gastric carcinoma tissues than in nontumor tissues but was not statistically significant. Among the 36 gastric carcinoma tissues, DNA hypermethlyation was detected at the following frequencies: 14 (38.9%) for p14, 13 (36.1%) for p16, 8 (22.2%) for MGMT, 10 (27.8%) for COX-2, 21 (58.3%) for E-cadherin and 6 (16.7%) for hMLH1. There was no significant association between methylation status of other genes in. the tumor tissues.

Of the 36 gastric carcinoma tissues, CpG island hypermethylation of the MINT loci was detected at the following frequencies: 22 (61.1%) for MINT1, 13 (36.1%) for MINT2,
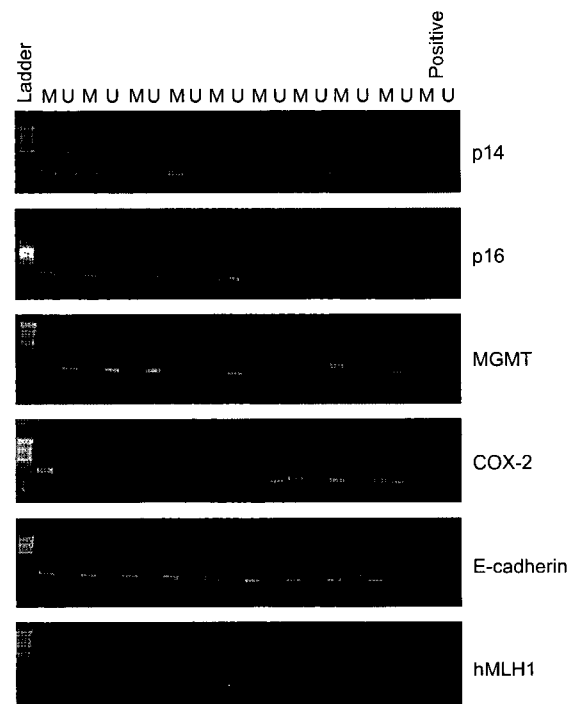

Fig. 1. Representative gel electrophoresis pictures demonstrating aberrant methylation in p14, p16, MGMT, COX-2, E-cadherin and hMLH1 for gastric cancer. C = cancer; N = normal; UM = unmethylated; M = methylated positive control was RKO cell line.
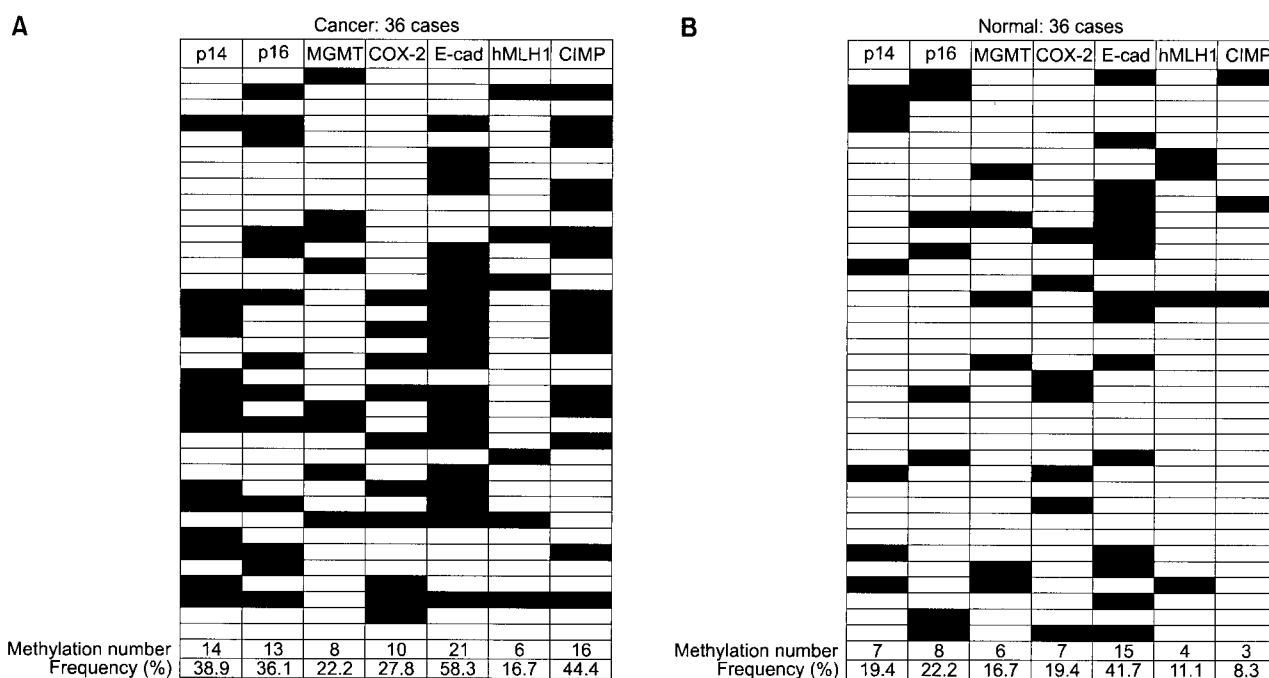
Fig. 2. Summary of methylation of p14, p16, MGMT, COX-2, E-cadherin and hMLH1 in primary gastric cancer (A) and nontumor tissue (B). White boxes represent samples that are not methylated or CIMP-negative and black boxes represent samples that are methylated or CIMP-positive.

8 (22.2%) for MINT12, 29 (80.6%) for MINT25 and 7 (19.4%) for MINT31. The frequencies for MINT1 and MINT25 hypermethylation were significantly higher in tumor tissues than in nontumor tissues. The overall results are shown in Fig. 2.

3) Association between CIMP and DNA hypermethylation

The frequency of CIMP-H was 16 (44.4%) in the gastric carcinoma tissues but 3 (8.4%) in the nontumor tissues. The CIMP-H rates were significantly higher in tumor tissues than in nontumor tissues (Table 3). There were significant differences in comparisons between CIMP-H tumors and CIMP-L tumors with regard to age and tumor size. CIMP-H tumors were generally from older patients and were larger in size than CIMP-L tumors (Table 4). When we compared the DNA methylation status of the six genes with the CIMP status, we found a significant association between the presence of the CIMP and p16 gene hypermethylation (Table 5).

4) Association between the clinicopathological features including *H. pylori* infection and DNA methylation

Table 6 summarizes the clinicopathological features of patients with gastric carcinoma and aberrant methylation. Aberrant methylation in gastric tumors had no correlation with

Table 3. Methylation status of MINT

	Cancer (%)	Normal (%)	Total (%)	P value
MINT				
All negative	2 (5.6)	6 (16.7)	8 (11.1)	0.0115
1 positive	10 (27.8)	15 (41.7)	25 (34.7)	
2 positive	8 (22.2)	12 (33.3)	20 (27.8)	
3 positive	11 (30.6)	2 (5.6)	13 (18.1)	
4 positive	5 (13.9)	1 (2.8)	6 (8.3)	
positive (more than 3)	16 (44.4)	3 (8.3)	19 (26.3)	0.0005
Total	36	36		

demographic data except in p16 and hMLH1. The frequencies of hypermethylation for p16 and hMLH1 were significantly lower in poorly differentiated histology. The hypermthylation for p16 was lower in grossly nonprotruded masses and higher in the intestinal types.

A significant association was found between the methylation of the MINT1 gene and *H. pylori* infection (P=0.004). In tumor tissues, 75% of cases with hypermethylated MINT1 were infected with *H. pylori*. By contrast, 75% of cases with unmethylated MINT1 were not infected with *H. pylori*.

Table 4. Association between CIMP and clinicopathologic features

| | CIMP | | P value |
	High (%)	Low (%)	
Sex			0.5023
Male	7 (43.7)	11 (55.0)	
Female	9 (56.3)	9 (45.0)	
Age			0.0442
≤60 years	5 (31.3)	13 (65.0)	
>60 years	11 (68.7)	7 (35.0)	
Size			0.0126
Small (≤3 cm)	3 (18.8)	12 (60.0)	
Large (>3 cm)	13 (81.2)	8 (40.0)	
Site			0.5317
Upper	3 (18.7)	6 (30.0)	
Middle	1 (6.3)	3 (15.0)	
Lower	12 (75.0)	11 (55.0)	
Lauren			0.2797
Intestinal	10 (62.5)	7 (35.0)	
Diffuse	5 (31.2)	6 (30.0)	
Mixed	1 (6.3)	7 (35.0)	
H. pylori			1.0000
(+)	3 (18.8)	9 (45.0)	
(−)	13 (81.2)	11 (55.0)	
Differentiation			1.0000
Well	1 (5.0)	1 (6.3)	
Moderately	7 (40.0)	8 (43.7)	
Poorly	8 (55.0)	11 (50.0)	
Stage			0.0995
I	4 (25.0)	12 (60.0)	
II	5 (31.2)	4 (20.0)	
III	4 (25.0)	3 (15.0)	
IV	3 (18.8)	1 (5.0)	

Table 5. Correlation of promoter methylation status and CIMP

| | | CIMP | | P value |
		High (%)	Low (%)	
p14	Methylated	8 (50.0)	6 (30.0)	0.2213
	Unmethylated	8 (50.0)	14 (70.0)	
p16	Methylated	9 (56.2)	4 (20.0)	0.0244
	Unmethylated	7 (43.8)	16 (80.0)	
MGMT	Methylated	2 (12.5)	6 (30.0)	0.2571
	Unmethylated	14 (87.5)	14 (70.0)	
COX-2	Methylated	5 (31.3)	5 (25.0)	0.7225
	Unmethylated	11 (68.7)	15 (75.0)	
E-cadherin	Methylated	11 (68.7)	10 (50.0)	0.2568
	Unmethylated	5 (31.3)	10 (50.0)	
hMLH1	Methylated	3 (15.0)	3 (18.8)	1.0000
	Unmethylated	13 (85.0)	17 (81.2)	

and CIMP, clinicopathological data and *H. pylori* infection.

Cyclin-dependent kinase inhibitor, p16, inhibits the cell cycle and inactivation of p16 accelerates uncontrolled cell growth.(11) In gastric cancer, genetic mutation or homozygous deletion of p16 is infrequent, this suggests that transcriptional silencing by hypermethylation may be a major pathway of p16 inactivation.(12) p14 acts like a tumor suppressor by inhibiting the MDM2, which is a p53 antagonist.(13) Lida et al(14) showed that diffuse type gastric carcinomas had higher frequencies of p14 hypermethylation, however, in this study hypermethylation of p14 was not significantly different in comparisons between the intestinal type and the diffuse type. MGMT is a DNA damage repair enzyme that removes alkyl groups from O^6-methylguanine.(15) The loss of MGMT expression is infrequently associated with genetic mutation. Oue et al.(16) showed that 31% of gastric cancers were hypermethylated in the MGMT gene and that hypermethylation of MGMT was correlated with the loss of protein expression. COX-2 is a rate-limiting enzyme in the synthesis of prostaglandins and its overexpression has been reported in multiple types of gastrointestinal tumors including gastric cancer. Recently reported data indicate that COX-2 plays an important role in angiogenesis, apoptosis and cell adhesion.(17,18) Baylin et al(1) suggested that DNA hypermethylation plays a critical role in the transcriptional silencing of COX-2. E-cadherin is a calcium-dependent adhesion molecule and loss of E-cadherin expression is associated with tumor progression and metastasis. hMLH1 is a DNA mismatch repair gene, and its hypermethylation is restricted to sporadic tumors with microsatellite

DISCUSSION

CpG island methylation plays an important role in transcriptional inactivation of multiple genes.(2,3) CIMP, which means simultaneous hypermethylation of multiple CpG islands, has been shown to be responsible for gastric cancer formation and progression in early stage.(8) In this study, we demonstrated aberrant methylation of multiple gastric cancer-associated genes involved in tumorigenesis, including cell cycle regulation (p16, p14, and COX2), DNA repair (hMLH1 and MGMT), metastasis or invasion (E-cadherin) and 5 cancer-specific MINT loci (MINT1, 2, 12, 25, and 31). We also investigated the association between DNA hypermethylation

Table 6. Clinicopathological characteristics of gastric cancer patients with aberrant methylation

	p14			p16			MGMT			COX-2			E-cadherin			hMLH1		
	M*	U†	P†	M	U	P	M	U	P	M	U	P	M	U	P	M	U	P
Sex			NS§			NS			NS			NS			NS			NS
Male	7	11		5	13		2	16		5	13		8	10		2	16	
Female	7	11		8	10		6	12		5	13		13	5		4	14	
Lauren			NS			0.0296			NS			NS			NS			NS
Intestinal	6	11		10	7		4	13		5	12		9	8		5	12	
Diffuse	5	6		2	9		2	9		2	9		8	3		1	10	
Mixed	3	5		1	7		2	6		3	5		4	4		–	8	
Differentiation			NS			0.0188			NS			NS			NS			0.0295
Well	1	1		1	1		–	2		1	1		1	1		2	–	
Moderately	6	9		9	6		5	10		4	11		8	7		2	13	
Poorly	7	12		3	16		3	16		5	14		12	7		2	17	
Gross			NS			0.0151			NS			NS			NS			NS
Protruded	6	12		3	15		4	14		6	12		10	8		2	16	
Nonprotruded	8	10		10	8		4	14		4	14		11	7		4	14	
Site			NS			NS			NS			NS			NS			NS
Upper	1	8		1	8		4	5		1	8		6	3		2	7	
Middle	3	1		1	3		–	4		2	2		2	2		–	4	
Lower	10	13		11	12		4	19		7	16		13	10		4	19	
H. pylori			NS			NS			NS			NS			NS			NS
Negative	11	13		11	13		6	18		7	17		16	8		3	21	
Positive	3	9		2	10		2	10		3	9		5	7		3	9	
Depth of invasion			NS			NS			NS			NS			NS			NS
Early	5	6		6	5		1	18		3	8		5	6		2	9	
Advanced	9	16		7	18		7	10		7	18		16	9		4	21	
LN metastasis			NS			NS			NS			NS			NS			NS
Negative	9	8		7	10		2	15		6	11		9	8		2	15	
Positive	5	14		6	13		6	13		4	15		12	7		4	15	
Stage			NS			NS			NS			NS			NS			NS
I	7	9		7	9		3	13		4	12		7	9		2	14	
II	4	5		3	6		2	7		3	6		7	2		3	6	
III	2	5		1	6		1	6		2	5		4	3		–	7	
IV	1	3		2	2		2	2		1	3		3	1		1	3	

*Methylated; †Unmethylated; †P value; §Not significant

instability (MSI) such as endometrial, colon and stomach cancers. In the present study, hypermethylation of six cancer related genes was more frequent in the tumor tissues evaluated than in the nontumor tissues however, the difference were not statistically significant. This may be due to the small sample size, and further study for confirmation is required.

A previous report showed that methylation of 5 MINT loci was exclusively detected in gastric cancers but was absent in most normal gastric mucosa tissues.(19) In the present study, there was a hypermethylation pattern at MINT loci in nontumor tissues and on average the number of methylated MINT loci was significantly greater in tumor tissues than in nontumor tissues. We compared the clinicopathologic parameters of CIMP-H and CIMP-L. Our results showed that CIMP-H tumors were significantly found in older patients with larger tumor size compared to CIMP-L. Toyota et al(19) suggested that concordant methylation of MINT loci and CIMP is an early event in gastric cancer that leads to cancer formation and progression through the transcriptional silencing of tumor suppressor genes, and that CIMP postitive gastric cancers have a relatively earlier

stage when compared with CIMP negative tumors. However, our results showed that there is no association between the status of CIMP and stage.

Our findings showed a significant association between CIMP-H and p16 gene hypermethylation in tumor tissues. Previous reports have shown that CIMP-positive gastric carcinomas are frequently associated with p16 gene hypermetylation, and that CIMP and hypermethylation of the p16 gene are more frequently found in the intestinal and diffuse-adherent types compared to the diffuse-scattered type.(20) We evaluated the association between p16 hypermethylation and relevant parameters. We found that aberrant hypermethylation of p16 was detected more frequently in the intestinal type and less frequently with poorly differentiated histology and grossly nonprotruded masses. These data suggest that the p16 gene had an association with a better prognosis, but further studies are necessary to determine the association.

Although, there has been controversy, Maekita et al(21) suggested that H. pylori infection potently induces aberrant methylation in multiple CpG islands. In the present study, the hypermethylation of MINT1 was significantly higher in tumor tissues than in nontumor tissues and the proportion of aberrant methylation of MINT1 increased significantly in the H. pylori infected patients. These findings suggest that methylation of MINT1 influences the development of gastric cancer induced by H. pylori infection.

CpG islands are normally protected from DNA methylation, but they are aberrantly methylated with aging. Previous reports have demonstrated that some genes are not methylated in pediatric gastric mucosa or at a frequency significantly lower than that in gastric mucosa from adults, and that p16, E-cadherin and hMLH1 showed a progressive increase of methylation frequency with ageing.(22-24) In this study, hypermethylation of p16 and MINT2 increased with age. These data suggest that some genes show an age-specific methylation pattern, but the reason for this is unknown.

CONCLUSION

The results of our study demonstrated that CIMP-H was significantly more frequent in tumor tissues than in nontumor tissues and that CIMP-H tumors were found more significantly in older patients and were larger in size than CIMP-L tumors. In addition, there was a significant association between CIMP-H and p16 gene hypermethylation in tumor tissues, and hypermethylation of p16 gene was lower in poorly differentiated histology, with gross nonprotruded masses and in

the diffuse type. Moreover, the hypermethylation of MINT1 was significantly higher in tumor tissues and was associated with H. pylori infection. The explanation for the association between hypermethylation of MINT1 and H. pylori infection, in gastric cancer, requires further study.

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위암에서 유전자 메틸화와 CpG Island Methylator Phenotype 및 *Helicobacter pylori*균 감염과의 연관성

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목적: 유전자 메틸화는 유전자의 서열에 영향을 주지 않으면서 유전자의 발현을 억제하고 세포분열 후 그대로 보존되는 후성적 변화이다. 위암조직과 정상위조직에서 hMLH1, p16, p14, COX-2, MGMT, E-cadherin 유전자와 MINT (MINT1, 2, 12, 25, 31)의 메틸화 상태를 검사하여 위암의 발생 과정에서의 작용과 CIMP 및 *Helicobacter pylori*균 감염을 포함한 임상병리학적인자와의 연관성을 알아보고자 하였다.

대상 및 방법: 위암과 정상위 신선 동결 조직 각각 36예를 대상으로 MSP (methylation-specific PCR) 방법을 이용하여 메틸화 상태를 분석하였고 CIMP의 분석은 MINT1, MINT2, MINT12, MINT25, MINT31의 5개 marker를 대상으로 시행하였다. Helicobacter pylori균 감염여부는 Warthin-Starry silver 염색을 통하여 분류하였다.

결과: 위암 관련 유전자인 p14, p16, MGMT, COX-2, E-cadherin, hMLH1의 메틸화는 각각 14예(38.9%), 13예(36.1%), 8예 (22.2%), 10예(27.8%), 21예(58.3%), 6예(16.7%)였다. MINT1과 MINT25의 메틸화는 위암조직에서 정상위조직에서보다 통계학적으로 유의하게 높게 관찰되었다. CIMP 양성률은 위암조직에서 44.4%로 높게 나타났으며 CIMP-H 위암은 환자의 연령과 종양크기와 연관이 있었다. CIMP 양성 위암은 p16 유전자의 메틸화와 연관이 있었고 p16 유전자의 메틸화는 조직학적으로 저분화, 미만형, 궤양형성하는 위암에서 낮게 나타났다. MINT1의 메틸화는 *Helicobacter pylori*균과 연관성이 있었다.

결론: 위암에서 hMLH1, p16, p14, COX-2, MGMT, E-cadherin, MINT (MINT1, 2, 12, 25, 31)의 불활성화에 DNA 메틸화가 작용함을 알 수 있었고, *Helicobacter pylori*균에 의한 위암발생에 MINT1의 메틸화가 연관이 있음을 알 수 있었다.
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중심 단어: 위암, MSP, CIMP, *Helicobacter pylori*