Aberrant methylation status of known methylation-sensitive CpG islands in gastrointestinal stromal tumors without any correlation to the state of *c-kit* and *PDGFRA* gene mutations and their malignancy

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(Received July 6, 2007/Revised September 29, 2007/Accepted October 1, 2007/Online publication February 4, 2008)

To identify additional alterations to *c-kit* or platelet-derived growth factor receptor α (*PDGFRA*) genes in gastrointestinal stromal tumors (GIST), we investigated the methylation status of nine known methylation-sensitive CpG islands (*p15*, *p16*, *p73*, 0-6-methylguanine-DNA methyltransferase, *E-cadherin*, mutL homolog 1, colon cancer nonpolyposis type 2 (escherichia), methylated in tumors [*MINT*] *1*, *MINT2*, and *MINT31*), and compared the results with the malignant potential and gain-of-function mutation types of GIST. Thirty-five GIST (*c-kit* mutations in 25 cases, *PDGFRA* mutations in seven cases, and lacking either mutation in three cases) were subjected to methylation-specific polymerase chain reaction to detect the methylation status of the nine methylation-sensitive CpG islands. Aberrant DNA methylation of these loci was found in 94% of all GIST. The rates of DNA methylation at each locus were as follows: *hMLH1*, 60%; *MINT2*, 51%; *MGMT*, 49%; *p73*, 49%; *p16*, 20%; *E-cadherin*, 14%; *MINT1*, 9%; *p15*, 6%; and *MINT31*, 0%. CpG islands methylator phenotype, which was defined as methylation involving more than three gene promoters, was found in 57% of GIST with *c-kit* or *PDGFRA* gene mutations. According to the risk categories, CpG islands methylator phenotype was present in 55% of low-risk GIST, and in 58% of high-risk GIST. Our results suggested that in addition to *c-kit* or *PDGFRA* mutations, the aberrant methylation of CpG islands, especially of mismatch-repair genes, may have a role in the tumorigenesis of GIST. (*Cancer Sci* 2008; 99: 253–259)

Gastrointestinal stromal tumors (GIST) are thought to be the most common nonepithelial tumors in the gastrointestinal tract with an annual incidence estimated at 10–20 cases per million.[1] During tumorigenesis of GIST, the most frequent changes are reported to be gain-of-function mutations in *c-kit* protooncogene. These mutations can result in autophosphorylation, namely KIT ligand-independent kinase activity.[2] *C-kit* mutations occur in up to 90% of GIST. Also, approximately 5% of GIST are characterized by a mutation in the related receptor tyrosine kinase platelet-derived growth factor receptor α (*PDGFRA*) exons 12 and 18.[3,4] Very recently, Lasota *et al*. reported *PDGFRA* exon 14 mutations in 11 of 200 GIST negative for mutations in *c-kit* exons 9, 11, 13, and 17, and *PDGFRA* exons 12 and 18.[5] However, approximately 5% of all GIST do not have a detectable mutation in either *c-kit* or *PDGFRA* genes.

Although most GIST are characterized by a gene mutation in either *c-kit* or *PDGFRA*, the clinical behavior of each GIST differs widely from benign to malignant with widespread metastasis. Therefore, we speculated that additional genetic alterations, other than the *c-kit* and *PDGFRA* genes, were required for the progression of GIST.

We previously reported allelic losses of 1p, 14q, and 22q in both low- and high-risk GIST.[6] Moreover, additional chromosomal losses and microsatellite instability (MSI) were observed in high-risk GIST. Fluorescence *in situ* hybridization (FISH) or comparative genomic hybridization (CGH) methods have also demonstrated the same chromosomal changes in high-risk GIST.[7–10]

Recent advances in molecular biology have elucidated relevant epigenetic modifications in gene regulation, and have shown that epigenetic modifications play important roles in tumorigenesis and tumor progression as well as previously known genetic alterations in oncogenes and tumor-suppressor genes. In particular, aberrant epigenetic hypermethylation of the gene-promoter region and the subsequent loss of gene expression are closely related to the development and progression of several human cancers.[11] For example, the hypermethylation of a mismatch-repair gene closely correlates with the development of MSI-positive colon cancer.[12,13] The distinct methylation status of various genes has revealed characteristic differences among tumor phenotypes and clinical behaviors in several human cancers such as colon, breast,[14] and liver cancers,[15] and mucosa-associated lymphoid tissue (MALT) lymphoma.[16] The methylation status of various genes greatly influences the diagnosis and prognosis of several tumors.

However, only one report has analyzed the methylation status of GIST.[17] Therefore, the present study aimed to identify the role of hypermethylation in the development and progression of GIST. We selected nine well-known methylation-sensitive CpG islands, evaluated the aberrant methylations in these loci, and compared the results of the mutational status of *c-kit* and *PDGFRA*, and the malignant potential of GIST.

Materials and Methods

Patients and tumor tissues. We included 35 GIST patients who underwent potentially curative surgery without preoperative therapy at Gunma University Hospital and Jichi Medical School Hospital. The study was approved by the Institutional Review Board of the Gunma University Graduate School of Medicine and the Jichi Medical School.

The risk grade of the GIST was evaluated according to the methods of Fletcher *et al*.[18] Tumors that were <2 cm in diameter with 0–4 mitoses/50 high-power fields (HPF) were considered to be of very low risk, and tumors that were 2–5 cm in diameter with 0–4 mitoses/50 HPF were considered to be of low risk.

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doi: 10.1111/j.1349-7006.2007.00682.x

Table 1. Sequences of primers used in the mutational analysis of *c-kit* and *PDGFRA* genes

Exon	Sequence
c-kit	
Exon 9F	5′-ATGCTCTGCTTCTGTACTGCC-3′
Exon 9R	5′-CAGAGCCTAAACATCCCCTTA-3′
Exon 11F	5′-CCAGAGTGCTCTAATGACTG-3′
Exon 11R	5′-ACCCAAAAAGGTGACATGGA-3′
Exon 13F	5′-CATCAGTTTGCCAGTTGTGC-3′
Exon 13R	5′-ACACGGCTTTACCTCCAAATG-3′
Exon 17F	5′-TGTATTCACAGAGACTTGGC-3′
Exon 17R	5′-GGATTTACATTATGAAAGTCACAGG-3′
PDGFRA	
Exon 12F	5′-TCCAGTCACTGTGCTGCTTG-3′
Exon 12R	5′-GCAAGGGAAAAGGGAGTCTT-3′
Exon 14F	5′-GTAGCTCAGCTGGACTGATA-3′
Exon 14R	5′-AATCCTCACTCCAGGTCAGT-3′
Exon 18F	5′-ACCATGGATCAGCCAGTCTT-3′
Exon 18R	5′-AAGTGTGGGAGGATGAGCCTG-3′

PDGFRA, platelet-derived growth factor receptor α.

Tumors <5 cm in diameter with 6–10 mitoses/50 HPF or tumors 5–10 cm in diameter with 0–4 mitoses/50 HPF were considered to be of intermediate risk. Tumors >5 cm in diameter with a mitotic count higher than 5/50 HPF, or tumors >10 cm in diameter with any mitotic rate, or tumors with >10 mitoses/50 HPF were classified as high-risk tumors. Tumors with necrosis were also classified as high-risk tumors.

Mutational analysis of *c-kit* and *PDGFRA*. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tumor tissues using standard proteinase K digestion and phenol/chloroform extraction methods, and was used for the following molecular analysis. To avoid contamination from normal tissues, only tumor tissues were scraped and obtained from each paraffin section, guided by serial hematoxylin–eosin stained sections.

Exons 9, 11, 13, and 17 of *c-kit*, and exons 12, 14, and 18 of *PDGFRA* were amplified by polymerase chain reaction (PCR) using previously published primer sets (Table 1).[5,19] Each of the amplified fragments was purified from a polyacrylamide gel, and direct sequencing was carried out using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3100 DNA Sequencer (Applied Biosystems). All sequencing reactions were carried out in forward and reverse directions.

Methylation-specific polymerase chain reaction. We selected nine methylation-sensitive CpG islands as follows: tumor-suppressor genes and related genes (*p15*, *p16*, *p73*, *E-cadherin*), DNA repair genes (0-6-methylguanine-DNA methyltransferase, mutL homolog 1, colon cancer nonpolyposis type 2 (escherichia)), and the three methylated clones methylated in tumors (*MINT*) *1*, *MINT2*, and *MINT31*, originally recovered from a colorectal carcinoma cell line.[20] The methylation status of the CpG islands in the promoter region of each gene was analyzed by the bisulfite modification technique using a CpGenome DNA modification kit (Chemicon, Temecula, CA, USA) according to the manufacturer's protocol. The primer sequences used in this methylation-specific polymerase chain reaction (MSP) study are listed in Table 2, and the PCR conditions were as described previously.[16,17] Each of the PCR products (10-μL volume) was directly loaded onto 8% polyacrylamide gels, stained with ethidium bromide, and visualized directly under ultraviolet illumination (Fig. 1).

Statistical analysis. Fisher's exact probability test, χ^2-test, or Student's *t*-test were used to analyze correlations between the methylation status of CpG islands and the clinicopathological characteristics of GIST, including the mutation statuses. *P*-values less than 0.05 were considered statistically significant.

Results

Clinicopathological characteristics. The clinicopathological characteristics of the patients are shown in Table 3. Tumor localizations were as follows: 26 GIST were in the stomach (74%), six were in the small intestine (17%), two were in the rectum (6%), and one was in the omentum (3%). The mean tumor size (maximum diameter) was 5.1 cm (range, 1.6–22 cm). Histopathologically, 28 GIST consisted of spindle tumor cells and the remaining seven were myxoid epithelioid GIST, a subtype of GIST closely correlated with *PDGFRA* gene

Table 2. Sequences of primers used in methylation-specific polymerase chain reaction

Gene	5′ primer	3′ primer
Methylated		
p15	GCGTTCGTATTTTGCGGTT	CGTACAATAACCGAACGACCGA
p16	TTATTAGAGGGTGGGGCGGATCGC	GACCCCGAACCGCGACCGTAA
p73	GGACGTAGCGAAATCGGGGTTC	CGTCGCAACCCCGAACATCG
MGMT	TTTCGACGTTCGTAGGTTTTCGC	GCACTCTTCCGAAAACGAAACG
E-cadherin	TGTAGTTACGTATTTATTTTTAGTGGCGTC	CGAATACGATCGAATCGAACCG
hMLH1	TTAATAGGAAGAGCGGATAGC	TCTATAAATTACTAAATCTCTTCG
MINT1	AATTTTTTTATATATATTTTCGAAGC	AAAAACCTCAACCCCGCG
MINT2	TTGTTAAAGTGTTGAGTTCGTC	AATAACGACGATTCCGTACG
MINT31	TGTTGGGGAAGTGTTTTTCGGC	CGAAAACGAAACGCCGCG
Unmethylated		
p15	TGTGATGTGTTTGTATTTTGTGGTT	CCATACAATAACCAAACAACCAA
p16	TTATTAGAGGGTGGGGTGGATTGT	CAACCCCAAACCACAACCATAA
p73	AGGGGATGTAGTGAAATTGGGGTTT	ATCACAACCCCAAACATCAACATCCA
MGMT	TTTGTGTTTTGATGTTTGTAGGTTTTTGT	AACTCCACACTCTTCCAAAAACAAAACA
E-cadherin	TGGTTGTAGTTATGTATTTATTTTTAGTGGTGTT	ACACCAAATACAATCAAATCAAACCAAA
hMLH1	TTAATAGGAAGAGTGGATAGTG	TCTATAAATTACTAAATCTCTTCA
MINT1	AATTTTTTTATATATATTTTTGAAGTGT	AACAAAAAACCTCAACCCCACA
MINT2	GATTTTGTTAAAGTGTTGAGTTTGTT	CAAAATAATAACAACAATTCCATACA
MINT31	TAGATGTTGGGGAAGTGTTTTTTGGT	TAAATACCCAAAAACAAAACACCACA

hMLH, mutL homolog 1, colon cancer nonpolyposis type 2 (escherichia); MGMT, 0-6-methylguanine-DNA methyltransferase; MINT, methylated in tumors.

doi: 10.1111/j.1349-7006.2007.00682.x

Table 3. Clinicopathological characteristics of 35 gastrointestinal stromal tumors

Variable	Value
Age (years, mean ± SD)	61.9 ± 12.0
Sex	
Male	17 (49%)
Female	18 (51%)
Tumor location	
Stomach	26 (74%)
Small intestine	6 (17%)
Rectum	2 (6%)
Omentum	1 (3%)
Tumor size (cm, average ± SD)	5.09 ± 3.90
Grade	
High	12 (34%)
Intermediate	1 (3%)
Low	22 (63%)
Histological type	
Spindle	28 (80%)
Myxoid epithelioid	7 (20%)
Recurrence	
Absent	28 (80%)
Present	7 (20%)
Mutation status	
c-kit	25 (71%)
PDGFRA	7 (20%)
No mutation	3 (9%)

PDGFRA, platelet-derived growth factor receptor α.

Fig. 1. Representative methylation-specific polymerase chain reaction (PCR) experiments for methylation analysis. PCR products amplified with unmethylated (U) and methylated (M) sequence-specific primers.

mutations, as we reported previously.[18] According to the risk categories, 22 cases were classified into the low-risk group, one into the intermediate-risk group and 12 into the high-risk group. The median follow-up time was 62 months (range, 5–125 months). Among the 35 patients, seven (20%) experienced tumor relapse, and two died of a recurrent tumor. The sites of recurrence were local (pelvic cavity and peritoneal dissemination, each in two cases), and distant metastasis (one each in liver, lung, and bone). The median tumor-free time was 26 months (range, 5–63 months).

Mutation analysis of the *c-kit* and *PDGFRA* genes. As shown in Table 3 and Figure 2a, 25 of 35 GIST showed mutations in *c-kit*: one (case 22) in exon 9 (duplication) and 24 in exon 11 (10 point mutations, nine in-frame deletions, and five internal tandem duplications). No mutations in exons 13 or 17 were found in any of the tumors. As reported previously,[19] all seven myxoid epithelioid GIST had mutations in *PDGFRA* exon 18 (four in-frame deletions and three point mutations).[19] The three remaining GIST showed no mutations in either the *c-kit* or *PDGFRA* genes.

Methylation analysis. The results of MSP analysis are summarized in Figure 2a. Unmethylated alleles of each locus were amplified successfully in all samples examined in the present study, confirming the accuracy of bisulfate modification in the MSP protocol.

The frequency rates for hypermethylation of the nine CpG islands are listed in Table 4. High frequency rates were observed

Table 4. Frequency rates of aberrant methylation in each CpG island

CpG island	Frequency (%)
hMLH1	60
MINT2	51
MGMT	49
p73	49
p16	20
E-cadherin	14
MINT1	9
p15	6
MINT31	0

in *hMLH1* (60%), *MGMT* (49%), *p73* (49%), and *MINT2* (51%), whereas low frequency rates were observed in *p15* (5.7%), *MINT1* (8.6%), and *MINT31* (0%). The average number of methylated genes per tumor was 2.6. The majority of GIST (33 of 35, 94.3%) showed aberrant DNA methylation of at least one CpG island. Two GIST (cases 9 and 23) showed no methylation of the CpG islands examined. On average, 2.6 (0–5) loci were methylated in GIST with *c-kit* gene mutations and 2.3 (1–4) loci were methylated in those with *PDGFRA* mutations (Fig. 2b).

No statistical difference was found in the frequency rate of hypermethylation between GIST with *c-kit* and *PDGFRA* gene mutations. Comparing GIST with *c-kit* or *PDGFRA* gene mutations, the three GIST without any mutations showed higher frequency rates of methylation (3–4, average 3.3), although there was no statistical difference (Fig. 2b). The results of MSP in each risk grade are summarized in Figure 3a. On average, 2.5 (0–5) loci were methylated in low-risk GIST, whereas 2.7 (1–4) loci were methylated in high-risk GIST (Fig. 3b). No statistical difference was also found among the risk categories.

The hypermethylated phenotype (CpG islands methylator phenotype; CIMP), which was methylated at three or more of the nine loci, as described previously,[21] was also analyzed. CIMP-positive tumors were found in 20 of 35 (57%) GIST. We analyzed the relationship between CIMP status and clinicopathological data (age, sex, tumor location, tumor size, histological type, mitotic score, mindbomb homolog 1 labeling index, risk grade, presence of recurrence, and mutation status) (Table 5). For risk grade, CIMP was present in 12 (55%) of the low-risk GIST, and in seven (58%) of the high-risk GIST (Fig. 3b). For the mutation status, 14 (56%) of 25 GIST with *c-kit* mutations, three (43%) of seven with *PDGFRA* mutations, and all three (100%) of three GIST with no mutation were CIMP positive. However, there was no significant correlation between CIMP status and any clinicopathological characteristic.

Discussion

In an earlier report, we demonstrated the presence of loss of heterozygosity (LOH) and MSI preferentially in high-risk GIST at a rate of 11–40%.[6] Similar results are reported in other studies using FISH or CGH.[7–10] Moreover, MSI was observed in 50% of GIST. In low-risk GIST, MSI was detected at a single locus in two of nine tumors. In contrast, it was observed in 69% of high-risk GIST; two at a single locus, five at two loci, one at three loci, and one at five loci.[6] It is known that the silencing of tumor-related genes by promoter hypermethylation at CpG islands results in gene alterations.[6] Therefore, these results indicated that a higher frequency of LOH and the accumulation of MSI might relate to the progression of GIST from low to high risk.

In the present study, to elucidate additional molecular mechanisms that participate in the development or malignant conversion of GIST from low to high risk, we characterized the

(a)

Case No.	Mutation status	MGMT	hMLH1	MINT1	MINT2	MINT31	p15	p16	p73	E-cadherin
1	c-kit	■	■		■				■	
2		■	■							
3			■	■	■				■	
4		■							■	
5									■	
6			■		■		■			
7		■			■					
8		■	■						■	
9										
10		■								
11			■		■				■	
12		■	■	■				■		
13			■					■		
14		■	■		■				■	■
15			■		■			■		
16					■					
17			■		■				■	■
18		■	■		■			■		
19		■	■		■					■
20			■							
21			■		■				■	
22				■	■				■	■
23										
24		■								
25					■				■	
26	PDGFRA	■	■					■		
27									■	
28					■					
29		■			■				■	■
30		■			■				■	
31			■						■	
32			■							
33	No mutation	■	■						■	
34		■	■					■	■	
35		■					■	■		

(b)

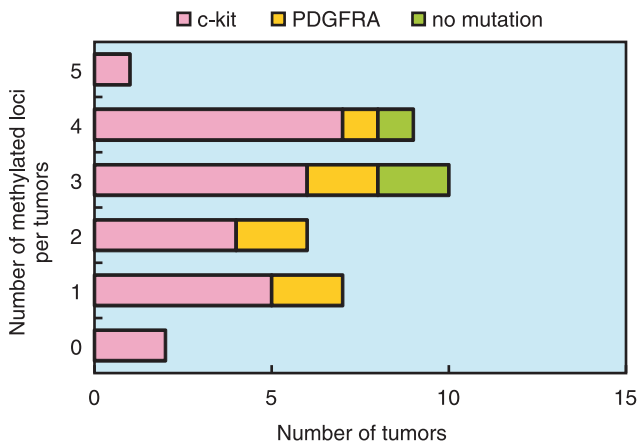

Fig. 2. (a) Methylation profiles of nine CpG islands and mutation statuses of *c-kit* and platelet-derived growth factor receptor α (*PDGFRA*) genes. The black grid squares represent positive methylation. The open squares denote no detectable methylation. (b) Correlation between the number of methylated loci and the mutational status of gastrointestinal stromal tumors (GIST). Although GIST lacking either mutation showed relatively higher rates of multigene methylation, there were no statistical differences between GIST with *c-kit* or *PDGFRA* mutations or those without those mutations.

methylation status of GIST using a panel of nine independent CpG islands. In many previous studies analyzing hypermethylation of human malignancies,[11] the target genes inactivated by DNA methylation were diverse and the list of such genes continues to grow. Therefore, a standard list of CpG islands that should be analyzed when profiling hypermethylation is not settled. Here, we selected mismatch repair genes, tumor suppressor genes, and related genes that, when hypermethylated, have been reported to occur in many kinds of tumors. As well as these, we analyzed the hypermethylation of *MINT1*, *MINT2*, and *MINT31*. These three regions of *MINT* are CpG islands that were observed in colorectal carcinoma by Toyota *et al*. in 1999.[20,21] In those studies, Toyota *et al*. subclassified colorectal carcinoma into CIMP-positive and -negative tumors and found a close correlation between CIMP and MSI. Subsequently, CIMP has been reported in various tumors, such as hepatocellular carcinoma,[15] pancreatic adenocarcinoma,[22] and gastric adenocarcinoma.[23] Moreover, we reported a distinct methylation pattern in *Helicobacter pylori*-dependent and -independent gastric MALT lymphoma.[16] In these studies,[15,16,20–23] *MINT1*, *MINT2*, and *MINT31* were methylated in various tumors, and were thought to be useful markers of CIMP. However, no study has analyzed the methylation status

doi: 10.1111/j.1349-7006.2007.00682.x

(a)

Case No.	grade	MGMT	hMLH1	MINT1	MINT2	MINT31	p15	p16	p73	E-cadherin
3	low risk		■	■	■				■	
5									■	
6			■		■		■			
8		■							■	
9										
10		■								
11			■		■				■	
12		■	■	■				■		
13			■							
14		■	■		■				■	■
15			■		■			■		
17			■		■				■	■
18		■	■		■			■		
23										
24		■								
27			■						■	
28					■					
30		■			■				■	
31			■						■	
32			■							
34		■	■					■	■	
35		■					■	■		
22	intermediate risk			■	■				■	■
1	high risk	■	■		■				■	
2		■	■							
4		■			■				■	
7		■			■					
16					■					
19		■	■		■					■
20			■							
21			■		■				■	
25					■					
26		■	■					■		
29		■			■				■	■
33		■	■						■	

(b)

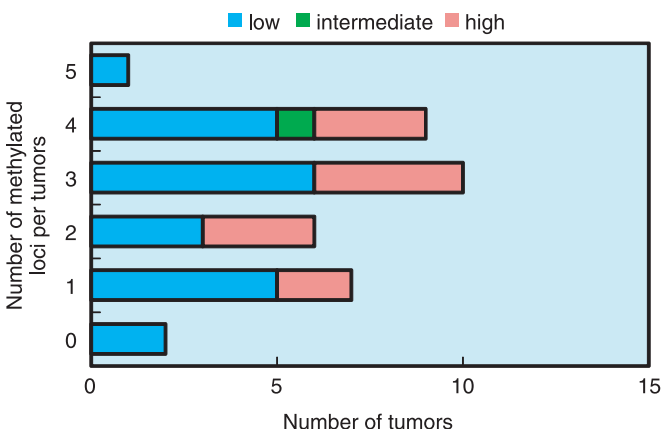

Fig. 3. (a) Methylation profiles for the risk categories of gastrointestinal stromal tumors (GIST) by Fletcher *et al*. The black grid squares represent positive methylation. The open squares denote no detectable methylation. (b) Correlation between the number of methylated loci and risk categories of GIST. There were no statistical differences among the risk categories.

of MINT and CIMP in mesenchymal tumors, including GIST. Thus we selected *MINT1*, *MINT2*, and *MINT31* for the present study.

We demonstrated that *hMLH1* and *MGMT* had the highest rates of hypermethylation of the mismatch-repair genes. As the silencing of hMLH1 by promoter hypermethylation at a CpG island resulted in MSI, these results seem to be consistent with our previous report,[6] in which MSI was observed frequently in high-risk GIST.

Interestingly, there were no correlations between methylation status and any clinicopathological characteristics, including the mutational status of *c-kit* and *PDGFRA*. In a previous study using an oligonucleotide microarray,[24] GIST with either *c-kit* or *PDGFRA* gene mutation showed different gene expression profiles, which might indicate that GIST with different gene mutations progress by different additional mechanisms. However, FISH analysis revealed a similar cytogenetic profile for GIST with *PDGFRA* gene mutations and GIST with *c-kit* gene mutations, and losses of 1p, 14q, and 22q were seen in both types of GIST.[25] The similar methylation status of GIST with different mutations in the present study supports the latter result of FISH analysis, which might indicate that GIST, irrespective of mutation status, have common pathways for tumor progression.

Variable	CIMP		
	Positive (n = 20)	Negative (n = 15)	P-value
Age (years, mean ± SD)	60.9 ± 10.4	63.3 ± 14.0	0.551
Sex (male:female)	9:11	8:7	0.738
Tumor location (St/SI/R/Ome)	15/3/1/1	11/3/1/0	0.922
Tumor size (cm, average ± SD)	4.98 ± 2.78	4.18 ± 2.27	0.370
Histological type (spindle/epithelioid)	17/3	11/4	0.430
Mitotic score	4.75 ± 4.99	7.00 ± 10.32	0.399
MIB-1 labeling index	5.14 ± 4.13	6.65 ± 5.37	0.353
Grade (high/intermediate/low)	7/1/12	5/0/10	0.655
Recurrence (absent/present)	18/2	10/5	0.088
Mutation (*c-kit*/*PDGFRA*/negative)	14/3/3	11/4/0	0.241

Ome, omentum; PDGFRA, platelet-derived growth factor receptor α; R, rectum; SI, small intestine; St, stomach.

Table 5. Comparison of clinicopathological variables between CpG islands methylator phenotype (CIMP)-positive and -negative gastrointestinal stromal tumors

However, GIST without any mutation, although a minority, were all CIMP positive. This suggests that hypermethylation may play a more important role in tumorigenesis of GIST without mutation than those with *c-kit* or *PDGFRA* mutations.

To date, only one study has reported the methylation status of GIST. House *et al*. revealed hypermethylation of at least 1 of 11 candidate gene regions in 84% of the 38 KIT-positive GIST.[17] In that study, they indicated that the presence of methylated *E-cadherin* alleles or the absence of methylated *hMLH1* alleles correlated with increased early tumor recurrence for GIST. However, in our study, hypermethylation of *hMLH1* and *E-cadherin* was found at different rates to those of House *et al*. (hMLH1, 60 vs 34%; E-cadherin, 37 vs 14%), and no correlation was found between the hypermethylation of *hMLH1* or *E-cadherin* and the risk categories of GIST. Although we could not explain the reason for these differences, the early tumor recurrence of GIST as shown by House *et al*. might not necessarily be consistent with the high-risk GIST investigated in the present study. Longer follow-up periods will be required to clarify the exact correlation between the aberrant methylation status of GIST and the prognosis of patients.

The standard therapy for primary GIST is complete surgical resection, which is carried out in approximately 85% of patients with this as a primary disease.[26] Nevertheless, tumor recurrence is frequent, and 5-year survival after removal of the primary localized GIST is approximately 50%.[26,27] Imatinib mesylate (STI571, Gleevec; Novartis Pharmaceuticals, Basel, Switzerland) is an oral agent that selectively inhibits specific tyrosine kinases including KIT, ABL, BCR-ABL, and PDGFR,[28] and can induce a partial response or stable disease in up to 80% of patients with metastatic or unresectable GIST.[29,30] However, GIST without mutations in *c-kit* and *PDGFRA* do not respond to imatinib.[31] Moreover, acquired resistance to imatinib in GIST with mutations has been reported by several groups and is often caused by secondary *c-kit* mutations.[32–34] It is now clear that many patients subsequently develop resistance to this agent. The frequent aberrant methylation found in GIST in our study might indicate that some therapeutic strategies using demethylating drugs could be effective against GIST, especially those without *c-kit* or *PDGFRA* gene mutations.

In conclusion, we reported aberrant methylation status using nine methylation-sensitive CpG islands in GIST with and without mutations. The frequencies of methylation for each locus were similar in GIST with *c-kit* or with *PDGFRA* mutations. However, CIMP was present in 100% of GIST without mutation, although there was no statistical difference between GIST with or without gene mutations. From these results, we hypothesized that aberrant methylation of multiple gene promoters, as well as the *c-kit* and *PDGFRA* genes, plays a role in the tumorigenesis of GIST.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (C), Scientific Research on Priority Areas and Initiatives for Attractive Education in Graduate Schools from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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