

Abnormal MGMT Promoter Methylation in Gastrointestinal Stromal Tumors: Genetic Susceptibility and Association with Clinical Outcome

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Purpose: KIT/PDGFR α wild-type (WT) gastrointestinal stromal tumors (GISTs) represent a heterogeneous subgroup of GISTs that lack KIT or PDGFR α mutations and possess distinct genetic alterations and primary resistance to imatinib. Succinate dehydrogenase (SDH)-deficient GISTs comprise the largest subpopulation of WT GISTs that are characterized by loss-of-function of SDH. O6-methylguanine-DNA methyltransferase (MGMT) is a specific DNA repair enzyme that has been identified as a predictor of positive treatment response to alkylating agents in a variety of cancers. The aim of this study was to evaluate the expression of MGMT and the prevalence of MGMT promoter methylation in GISTs and to determine the association between MGMT promoter methylation and clinicopathological characteristics and clinical outcomes.

Patients and Methods: A heterogeneous cohort of 137 primary GISTs that confirmed by immunohistochemistry and KIT/PDGFR α mutation analysis were retrospectively selected and analyzed for MGMT expression and MGMT promoter methylation using immunohistochemical staining and methylation-specific PCR (MSP). A concordance analysis between MGMT promoter methylation and clinicopathological characteristics and prognosis was also performed.

Results: A total of 44.5% (65/137) of GIST patients displayed loss of MGMT protein expression, and 10.9% (15/137) of these patients exhibited MGMT promoter methylation. However, no significant correlation was observed between the loss of MGMT protein expression and MGMT promoter methylation. WT GISTs possessing an epithelioid or mixed phenotype, particularly those that were SDH-deficient, displayed a markedly higher prevalence of MGMT promoter methylation compared to that in KIT/PDGFR α mutated GISTs. Moreover, MGMT promoter methylation was identified as a potential independent prognostic factor for OS and DFS in patients with GIST.

Conclusion: MGMT promoter methylation is particularly frequent in SDH-deficient GISTs and in WT GISTs possessing an epithelioid/mixed phenotype, and knowledge of this methylation status may offer a novel potential therapeutic option for WT GISTs.

Keywords: gastrointestinal stromal tumor, O6-methylguanine-DNA methyltransferase, wild-type, succinate dehydrogenase deficiency

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract, and they harbor mutually exclusive mutations in

c-kit receptor tyrosine kinase (KIT) or platelet-derived growth factor receptor alpha (PDGFRA) in approximately 85% of cases.^{1,2} However, the remaining 10–15% of GISTs are categorized as KIT/PDGFRA wild-type (WT), as they do not harbor any detectable mutations in the *KIT* and *PDGFRA* genes.^{3,4} Recently, several studies have suggested that WT GISTs comprise different subtypes with distinct genetic alterations and can be subdivided into succinate dehydrogenase (SDH)-deficient and non-SDH-deficient groups.⁵ Among these, SDH-deficient GISTs represent the largest subpopulation of WT GISTs that are characterized by a loss of SDH function,⁶ while non-SDH-deficient GISTs are comprised of neurofibromatosis type 1 (NF1) -associated, serine/threonine protein kinase B-RAF (BRAF) mutation, RAS gene mutation, and quadruple wild-type GISTs.^{7–9} Therefore, WT GISTs are currently regarded as a heterogeneous subgroup of tumors that are accompanied by different clinicopathological, genetic, and biological characteristics.^{10,11} Moreover, WT GISTs often display a primary resistance to targeted tyrosine kinase inhibitor (TKI) treatment using imatinib.^{12,13} Therefore, further identification of the genetic alterations associated with the pathogenesis of WT GISTs may contribute to the development of new therapeutic strategies and may aid in ameliorating the clinical management of GISTs.

O6-methylguanine-DNA methyltransferase (MGMT) is a specific DNA repair enzyme that has been demonstrated to repair DNA alkylation damage by removing the methyl group from the O⁶-guanine residues and then transferring them to its cysteine residues.^{14,15} MGMT promoter methylation-related gene silencing and loss of function have been detected in various cancers, including gliomas,¹⁶ lung cancer,¹⁷ breast cancer,¹⁸ colorectal cancer,¹⁹ and gastric cancer.²⁰ Moreover, aberrant MGMT promoter methylation was identified as a predictor of positive treatment response to alkylating agents.^{21–23} Recently, MGMT promoter methylation has been reported in a few GISTs.^{24–27} However, the preponderance of MGMT promoter methylation in GISTs possessing different genetic subtypes remains unclear. Therefore, we conducted immunohistochemical staining and methylation-specific PCR (MSP) analyses to identify the expression of MGMT and the prevalence of MGMT promoter methylation in a heterogeneous cohort of 137 GISTs. Additionally, the distinct MGMT promoter methylation status and its association with clinicopathological characteristics and prognosis of patients with GIST were evaluated.

Materials and Methods

Patients and Samples

A total of 137 formalin-fixed paraffin-embedded samples of primary GIST with available clinical and pathological characteristics were retrospectively selected from more than 1000 patients who underwent surgery at Tongji Hospital between January 2013 and July 2019. None of the patients received imatinib treatment prior to surgery. Supportive immunohistochemical and mutational analyses were performed for each case. This study was approved by the Ethics Committee of Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, P.R. China. Written informed consent was obtained from the participants themselves or from their parents. All experiments were conducted in accordance with the World Medical Association Declaration of Helsinki.

Histological and Immunohistochemical Analyses

Histological diagnosis was established by the Department of Pathology at Tongji Hospital according to the current World Health Organization (WHO) classification of tumors. Hematoxylin and eosin-stained (H&E) slides were reviewed independently by 2 experienced gastrointestinal pathologists for the presence of histomorphological features. The tumor risk grade was assessed based on the modified National Institute of Health (NIH) stratification criteria. Immunohistochemistry was performed on 4 μ m paraffin sections using an UltraVision Quanto Detection System HRP DAB (Thermo Scientific, CA, USA) for the detection of CD117 (rabbit polyclonal, 1:400 dilution, DAKO, Glostrup, Denmark), DOG-1 (rabbit polyclonal, ready to use, Zhongshan Golden Bridge Biotechnology Co., Ltd., ZGBBT, Beijing), CD34 (rabbit polyclonal, 1:400 dilution, DAKO, Glostrup, Denmark), SMA (mouse polyclonal, 1:400 dilution, DAKO, Glostrup, Denmark), Caldesmon (mouse polyclonal, ZGBBT, Beijing), S-100 (rabbit polyclonal, ready to use, ZGBBT, Beijing), Desmin (mouse polyclonal, ready to use, ZGBBT, Beijing), and Ki67 (rabbit polyclonal, 1:400 dilution, ZGBBT, Beijing) according to the manufacturer's protocols. Immunostaining for SDHB (succinate dehydrogenase complex subunit B) and MGMT were performed on all samples using the mouse monoclonal antibody anti-SDHB antibody (1:32 dilution, ZGBBT, Beijing) and the mouse monoclonal antibody anti-MGMT antibody (ready

to use, ZGBBT, Beijing), regardless of the anatomic site and morphologic subtype.

The expression of the MGMT protein was evaluated in tumor cells using a semi-quantitative scoring method that was based on the percentage of positive cells and the staining intensity. The percentage of positive tumor cells was scored as follows: 0 (<10% nuclear staining), 1 (10–25% nuclear staining), 2 (26–50% nuclear staining), and 3 (>50% nuclear staining). The staining intensity of tumor cells was ranked as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). An immunoreactive score (IRS) was generated by multiplying the percentage of positive cells by the staining intensity. Finally, samples with IRS >2 were classified as positive, while samples with IRS ≤2 were classified as negative.

DNA Extraction

For paraffin-embedded specimens, the MagCore Genomic DNA Tissue Kit C401 (RBC Bioscience Corp, Xiamen, China) was used to isolate genomic DNA from 8–10 sections of 10 μm thickness according to the recommendations of the manufacturer. DNA concentrations were quantified using a SMA4000 spectrophotometer (Merinton Instrument, Inc., Beijing, China). Subsequently, 1000 ng of genomic DNA was modified by exposure to a sodium bisulfite solution using the EpiTect Bisulfite kit (Qiagen, Hilden, Germany).

KIT and *PDGFRA* Gene Mutation Analysis

Mutation analysis of *KIT* exons 9, 11, 12, 13, 14, and 17 and *PDGFRA* exons 12, 14, and 18 were performed using a commercial kit based on fluorescence PCR and capillary electrophoresis sequencing according to the manufacturer's protocols (SinoMDgene Technology Co., Ltd., Beijing, China). The amplification was carried out on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster city, CA, USA) with an initial denaturation at 95°C for 3 min, then 40 cycles of 94°C for 15 s and 60°C for 45 s. The PCR amplification products were identified by direct sequencing using a commercially available kit (SinoMDgene Technology Co., Ltd., Beijing, China) on an ABI 3500DX Genetic Analyzer (Applied Biosystems, USA). Gene sequencing results were compared to the National Center for Biotechnology Information (NCBI) database sequences for *KIT* (NM_001093772.1) and *PDGFRA* (NM_006206.4).

MGMT Methylation Assessment by Methylation-Specific PCR (MSP)

After sodium bisulfite conversion, genomic DNA was amplified for the MGMT promoter using a commercially available kit obtained from SinoMDgene Technology Co., Ltd., Beijing, China, according to the manufacturer's instruments. Each reaction mixture contained 2 μL of bisulfite-treated DNA, 12.5 μL of SYBR Green PCR Master Mix, and 5.5 μL of forward and reverse primers to provide a final volume of 20 μL. The amplification was performed on a Bio-Rad CFX96 Real-Time PCR Detection System instrument (Bio-Rad Laboratories) with an initial denaturation at 95°C for 3 min, then 45 cycles of 95°C for 15 s, 60°C for 45 s, and 72°C for 15 s, and a final extension for 10 min at 72°C. Analysis was performed using the Bio-Rad CFX Manager 3.1 software (Bio-Rad Laboratories).

Statistical Analysis

Contingency tables and associations were estimated using the chi-square (χ^2) test and Fisher's exact test. The Kaplan-Meier method was applied to evaluate the overall survival (OS) and disease-free survival (DFS), and the comparisons of survival curves between groups were assessed using the Log rank test. The prognostic value of MGMT promoter methylation in GIST patients was determined by multivariate analyses using the Cox proportional hazard model. A two-tailed P value < 0.05 was considered as statistically significant.

Results

Clinicopathological Characteristics

A total of 137 patients with GIST were recruited for this study, and these patients included 70 men (51.1%) and 67 women (48.9%) ([Supplementary Table 1](#)). The ages of the patients ranged from 5 to 81 years with a mean age of 54.6 years. Tumor size ranged from 4 to 350 mm with a mean size of 66 mm. Metastasis was identified in 6 patients, and recurrence was identified in 9 patients. The primary tumors predominantly occurred in the stomach (50.4%) and small intestine (32.8%), and the liver was the most frequent site of metastases. The most common pathomorphological appearance was spindle cell type (73.0%), and this was followed by epithelioid (20.4%) and mixed (6.6%) cell types ([Table 1](#)). A high mitotic rate (>5/50 HPF) was observed in 24 cases (17.5%), and 26 cases (19.0%) exhibited necrosis ([Table 1](#)). Based on the NIH

Table 1 Clinicopathological Features of 137 Patients with GIST in This Study

Clinicopathological Features	KIT/PDGFR Wild-Type	KIT/PDGFR Mutant	P value
Age (years)			
<55	29(56.9%)	33(38.4%)	0.036
≥55	22(43.1%)	53(61.6%)	
Gender			
Male	24(47.1%)	46(53.5%)	0.467
Female	27(52.9%)	40(46.5%)	
Primer sites			
Stomach	27(52.9%)	42(48.8%)	0.074
Small intestine	15(29.4%)	30(34.9%)	
Colon	5(9.8%)	1(1.2%)	
EGIST	4(7.9%)	13(15.1%)	
Tumor size (cm)			
≤5	33(64.7%)	43(50.0%)	0.094
>5	18(35.3%)	43(50.0%)	
Histological type			
Spindle phenotype	30(58.8%)	70(81.4%)	0.012
Epithelioid phenotype	15(29.4%)	13(15.1%)	
Mixed phenotype	6(11.8%)	3(3.5%)	
Mitotic rate (per 50 HPFs)			
≤5	45(88.2%)	69(80.2%)	0.226
>5	6(11.8%)	17(19.8%)	
NIH risk score			
Very low risk	18(35.3%)	7(8.1%)	0.001
Low risk	15(29.4%)	30(34.9%)	
Intermediate risk	6(11.8%)	12(14.0%)	
High risk	12(23.5%)	37(43.0%)	
Tumor necrosis			
Yes	6(11.8%)	20(23.3%)	0.097
No	45(88.2%)	66(76.7%)	
CD117			
Positive	50(98.0%)	85(98.8%)	1.000
Negative	1(2.0%)	1(1.2%)	
DOG1			
Positive	50(98.0%)	81(94.2%)	0.526
Negative	1(2.0%)	5(5.8%)	
CD34			
Positive	43(84.3%)	67(77.9%)	0.362
Negative	8(15.7%)	19(22.1%)	
Recurrence or metastasis			
Yes	1(2.0%)	14(16.3%)	0.021
No	50(98.0%)	72(83.7%)	

risk categories, tumors were categorized as very low-risk (25/137, 18.2%), low-risk (45/137, 32.8%), intermediate risk (18/137, 13.1%), and high risk (49/137, 35.9%). The mean follow-up time was 29.1 months (range, 9–75 months).

Based on the supporting immunohistochemical analyses, CD117, DOG1, and CD34 antibodies exhibited diffuse positive expression in 135 (98.5%), 131 (95.6%), and 110 (80.3%) cases of GIST (Table 1, Figure 1). Focal positive expressions of SMA, Caldesmon, and desmin were observed in 12.4%, 27.7%, and 2.9% of the tumors. None of the cases were positive for S-100. The Ki-67 index ranged from 1% to 70%, with a mean of 2.2%, 2.9%, 4.9%, and 14.8% in GISTs with very low, low, intermediate, and high risk, respectively. The absence of SDHB expression was noted in 7/139 (5.1%) cases.

Mutations in KIT and PDGFRA Genes

Among the 137 cases of GISTs included in this study, 75 (54.7%) harbored KIT gene mutations in exons 11 (n=41), 9 (n=12), 13 (n=6), and 17 (n=6), respectively. Of the remaining 62 patients, 11 (8.0%) cases possessed PDGFRA gene mutations, while the remaining 51 (37.3%) cases showed no detectable mutations and were regarded as KIT/PDGFR WT GISTs. KIT exon 11 mutations appeared as deletions, insertions, point mutations, and complex mutations were detected in 41 (29.9%) tumors. The 12 cases with KIT exon 9 mutations all displayed a 6 bp insertion that resulted in duplication of codons A502 and T503. More than 90% of PDGFRA mutations affecting exons 12, 14, and 18 were associated with epithelioid morphology. Univariate analyses revealed that KIT/PDGFR WT GISTs exhibited a younger age, a tendency towards epithelioid or mixed morphology, and a lower recurrence or metastasis rate compared to that of KIT/PDGFR mutated GISTs (Table 1). Furthermore, seven of the WT GISTs were found to possess negative staining for SDHB according to IHC (Figure 1H). Each of these seven cases exhibited positive staining for KIT and DOG1 and possessed typical morphological characteristics that have been recognized as indicative of SDH-deficient GISTs (Figure 1B, D and F). All seven patients with SDH-deficient GIST exhibited tumors that occurred in the stomach, and these patients possessed a mean age of 21 years (range 10–36 years). Their tumors demonstrated a predominantly multinodular growth pattern (5/7) and possessed an epithelioid (5/7) or mixed (2/7) histologic subtype (Table 2). Secondary

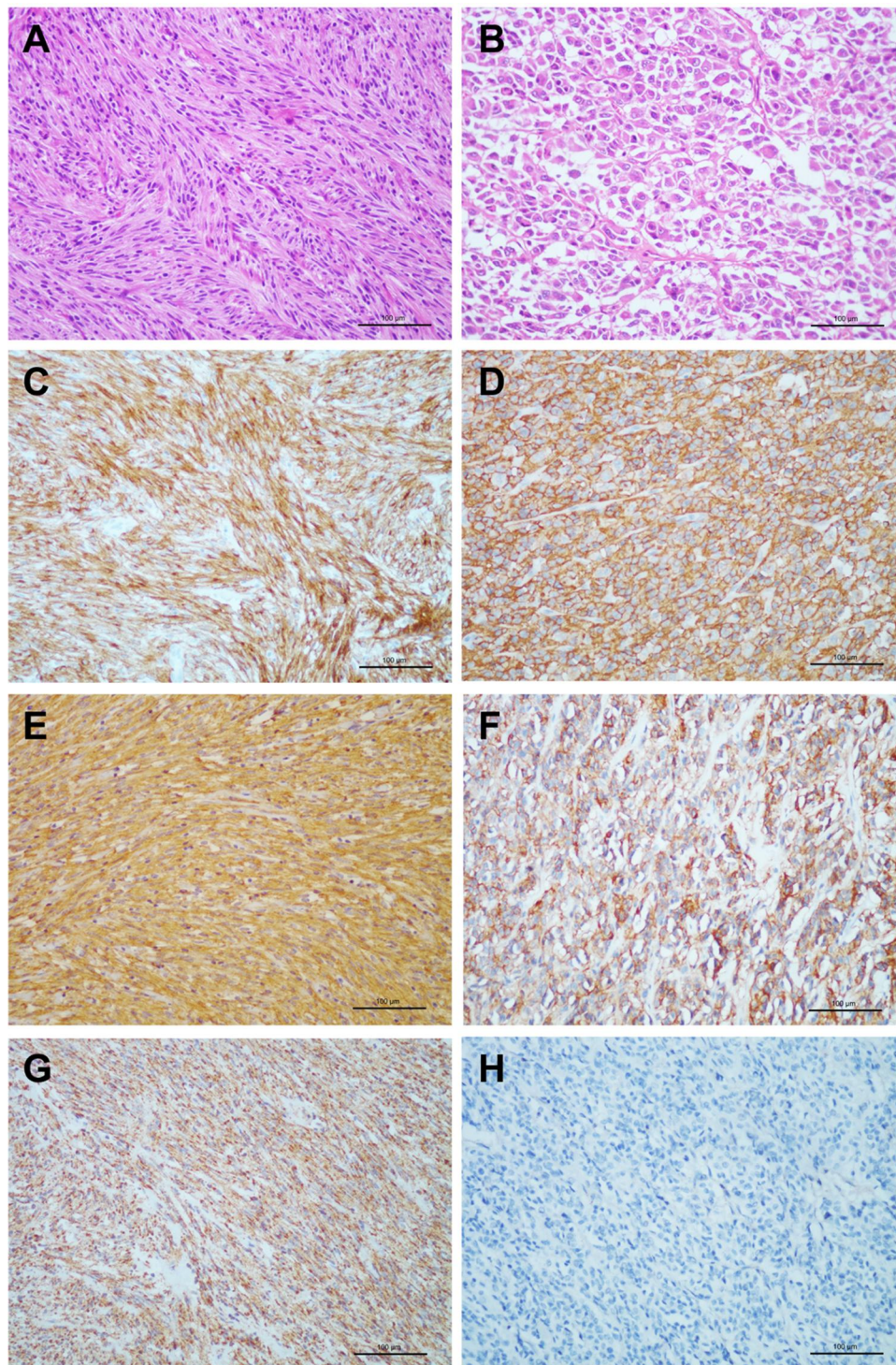


Figure 1 H&E staining and immunohistochemistry analysis of GIST specimens. **(A)** KIT-mutant GISTs showing spindle cell morphology. KIT mutated GISTs with spindle cell morphology showing diffuse positive expression of CD117 **(C)**, DOG1 **(E)** and SDHB **(G)** by Immunohistochemistry. **(B)** SDH-deficient GISTs showing epithelioid morphology. Immunohistochemical staining showing diffuse positive expression of CD117 **(D)** and DOG1 **(F)** in SDH-deficient GISTs. **(H)** SDH-deficient GISTs showing loss expression of SDHB by Immunohistochemistry. Scale bar 100 μ m.

mutations in KIT that predominantly presented in exons 13, 14, and 17 were observed in 10 out of 15 patients with recurrence or metastasis. All of the mutations are presented in [Supplementary Table 1](#).

MGMT Promoter Methylation Analysis

According to MSP, methylation of the MGMT promoter was detected in 10.9% (n=15) of tumors. The mean age of GIST patients with MGMT promoter methylation was

Table 2 Clinicopathological Features of 7 Patients with SDH-Deficient GIST

Clinicopathological Features	N (%)
Age (years)	
<40	7(100.0%)
≥40	0(0.0%)
Gender	
Male	2(28.6%)
Female	5(71.4%)
Primer sites (gastric)	
Corpus	3(42.8%)
Antrum	2(28.6%)
Fundus	2(28.6%)
Tumor size (mean; range), cm	6.7(5–8)
Histological type	
Spindle phenotype	0(0.0%)
Epithelioid phenotype	5(71.4%)
Mixed phenotype	2(28.6%)
Mitotic rate per 50 HPFs (mean, range)	3.7(2–5)
NIH risk score	
Low risk	2(28.6%)
Intermediate risk	5(71.4%)
High risk	0(0.0%)
Multinodular pattern	5(71.4%)
Tumor necrosis	3(42.9%)
Distance metastasis at presentation	0(0.0%)
IHC	
CD117 Positive	7(100.0%)
DOG1 Positive	7(100.0%)
CD34 Positive	6(85.7%)
SMA Positive	1(14.3%)
Caldesmon Positive	2(28.6%)
S-100 Positive	0(0.0%)
MGMT Negative	5(33.3%)
Ki-67(mean, range) (%)	4.3(1–8)

44.1±15.8 (95% CI 35.4–52.9) years, and this was markedly younger than that of patients without MGMT promoter methylation (55.8±12.1 years, 95% CI 53.6–57.9; $p=0.001$). Further subgroup analysis indicated that the MGMT promoter was methylated in only 3 out of 86 (3.5%) KIT/PDGFR α mutated GISTs and in 12 of 51 (23.5%) WT GISTs. Among the GISTs lacking KIT/PDGFR α mutations, eight out of 44 (18.2%) and four out of 7 (57.1%) cases demonstrated MGMT promoter methylation in the non-SDHB-deficient and SDHB-proficient subgroups. Comparative analyses revealed that WT GISTs, particularly those with SDH-deficiency, demonstrated a markedly higher prevalence of MGMT

promoter methylation compared to that of KIT- or PDGFR α -mutant GISTs ($P<0.001$, Table 3). Moreover, GISTs possessing epithelioid or mixed morphology displayed a higher frequency of MGMT promoter methylation than spindle cell GISTs ($P=0.004$, Table 3). These findings indicated that MGMT promoter methylation may exhibit a trend toward a higher frequency in epithelioid/mixed-type WT GISTs and in SDH-deficient GISTs. However, no statistically significant relationships were observed between MGMT promoter methylation and patient sex, primary tumor localization, primary tumor size, mitotic rate, risk grade, recurrence, or metastasis in this study.

MGMT Immunohistochemical Expression and Its Correlation with MGMT Promoter Methylation

The expression of the MGMT protein was identified in 15 cases with MGMT promoter methylation and in 122 cases without MGMT promoter methylation according to IHC staining. Positive staining for the MGMT protein was localized primarily within the nucleus of tumor cells (Figure 2E–H). The loss of MGMT protein expression was detected in 61 (44.5%) cases of 137 GISTs (Figure 2A–D, Table 3). Among the 15 cases with methylated MGMT promoter, 5 (33.3%) cases exhibited a loss of MGMT protein expression, and 10 (66.7%) cases demonstrated moderate to high expression of MGMT protein. Meanwhile, 51 (41.8%) cases displayed a loss of MGMT protein expression, and 71 (58.2%) cases exhibited moderate to high expression of MGMT protein among the 122 cases with non-methylated MGMT promoters. However, no significant correlation was observed between the loss of MGMT protein expression and MGMT promoter methylation status ($P=0.067$, Table 3).

Association Between MGMT Promoter Methylation and Prognosis of Patients with GIST

The Kaplan–Meier method with Log rank test indicated that GIST patients carrying MGMT promoter methylation exhibited longer overall survival (OS) and disease-free survival (DFS) compared to that in patients without MGMT promoter methylation (Figure 3). Moreover, multivariate Cox proportional hazards regression analysis demonstrated that MGMT promoter methylation could be considered as a potential independent prognostic factor for

Table 3 Correlation of MGMT Promoter Methylation with Clinicopathological Parameters

Clinical and Pathological Parameters	N	MGMT Methylation		P value
		Yes (%)	No (%)	
Age (years)				
<55	62	11(17.7%)	51(82.3%)	0.021
≥55	75	4(5.3%)	71(94.7%)	
Gender				
Male	70	6(8.6%)	64(91.4%)	0.362
Female	67	9(13.4%)	58(86.6%)	
Primer sites				
Stomach	69	10(14.5%)	59(85.5%)	0.075
Small intestine	45	3(6.7%)	42(93.3%)	
Colon	6	2(33.3%)	4(66.7%)	
EGIST	17	0(0.0%)	17(100.0%)	
Tumor size (cm)				
≤5	76	6(7.9%)	70(92.1%)	0.201
>5	61	9(14.8%)	52(85.2%)	
Histological type				
Spindle phenotype	100	6(6.0%)	95(94.0%)	0.004
Epithelioid phenotype	28	6(21.4%)	22(78.6%)	
Mixed phenotype	9	3(33.3%)	6(66.7%)	
Mitotic rate (per 50 HPFs)				
≤5	113	13(11.5%)	100(88.5%)	0.927
>5	24	2(8.3%)	22(91.7%)	
NIH risk score				
Very low risk	25	3(12.0%)	22(88.0%)	0.113
Low risk	45	3(6.7%)	42(93.3%)	
Intermediate risk	18	5(27.8%)	13(75.0%)	
High risk	49	4(8.2%)	45(91.8%)	
Mutation status				
KIT mutant	75	3(4.00%)	72(96.00%)	<0.001
PDGFRA mutant	11	0(0.00%)	11(100.00%)	
Wild-type (SDH deficient)	7	4(57.14%)	3(42.86%)	
Wild-type (non-SDH deficient)	44	8(18.18%)	36(81.82%)	
Tumor necrosis				
Yes	26	4(15.4%)	22(84.6%)	0.649
No	111	11(9.7%)	100(90.3%)	
CD117				
Positive	135	15(11.1%)	120(88.9%)	1.000
Negative	2	0(0.0%)	2(100.0%)	
DOG1				
Positive	131	14(10.7%)	117(89.3%)	1.000
Negative	6	1(16.7%)	5(83.3%)	
CD34				
Positive	110	13(11.8%)	97(88.2%)	0.754
Negative	27	2(7.4%)	25(92.6%)	

(Continued)

Table 3 (Continued).

Clinical and Pathological Parameters	N	MGMT Methylation		P value
		Yes (%)	No (%)	
MGMT IHC expression				
Positive	76	5(6.6%)	71(93.4%)	0.067
Negative	61	10(16.4%)	51(83.6%)	
Recurrence or metastasis				
Yes	15	0(0.0%)	15(100%)	0.372
No	122	15(12.3%)	107(87.7%)	

OS (HR=3.711; 95% CI 1.645–8.372; P=0.002) and DFS (HR=3.287; 95% CI 1.169–9.245; P=0.024) in GIST patients. These results suggest that MGMT promoter methylation may predict improved clinical outcomes in patients with GISTs.

Discussion

KIT/PDGFR α WT GISTs represent a genetically heterogeneous subgroup of GISTs that lack *KIT* or *PDGFR α* mutations.^{3,10} These WT GISTs exhibit identical clinicopathological, genetic, and biological characteristics that are indistinguishable from those of KIT/PDGFR α mutated GISTs.²⁸ SDH-deficient GISTs are the largest subpopulation of WT GISTs that are characterized by loss-of-function mutations in any of the SDH-subunits A, B, C, or.^{6,11} Although most WT GISTs exhibit relatively benign clinical behaviors, there are still a few WT GISTs that exhibit malignant potential.^{28,29} In the present study, we selected a cohort of KIT/PDGFR α -mutated GISTs and WT GISTs, and we found that WT GISTs displayed a tendency to exhibit epithelioid or mixed morphology, and a lower recurrence or metastasis rate compared to these values in KIT/PDGFR α mutated GISTs. Meanwhile, of the WT GISTs examined, 7/51 (13.7%) cases were observed to be negative for SDHB and were identified as SDH-deficient GISTs in our study. Based on the present findings, SDH-deficient GISTs typically share unique clinicopathological features that include gastric primary tumor localization, multinodular growth patterns, and an epithelioid or mixed cell morphological phenotype.³⁰ Lymph node metastasis and local recurrence are also present more frequently in SDH-deficient GISTs.³⁰ However, even with local recurrence or lymph node metastasis, the majority of SDH-deficient GISTs still tend to follow a relatively indolent clinical course in contrast to other GISTs.^{11,30} Similarly, in this study, we observed that all seven cases occurred exclusively in the stomach and exhibited a predominantly

multinodular growth pattern with epithelioid or mixed histologic subtype and a male/female ratio of 1:2.5 with a mean age of 21 years. Additionally, none of the patients developed local recurrence or distant metastasis after surgery, and no patients died of this disease at the longest follow-up of 67 months.

MGMT is a specific DNA repair enzyme that protects against mutagenic and alkylating agent-induced carcinogenesis.³¹ Aberrant MGMT promoter methylation has been identified as an inheritable epigenetic alteration that occurs in a broad spectrum of human cancers.^{16–20} To date, MGMT promoter methylation has been reported in only a few GISTs. In two previous studies that were conducted in 2003 and 2008, MGMT promoter methylation was observed to exhibit a comparatively high frequency rate in the study cohort of gastric GISTs, and this contradicts the notion that MGMT methylation is a relatively rare event in GISTs.^{26,27} Recently, MGMT methylation was detected for the first time in nine SDH-deficient GISTs when these tumors were compared to other pathogenetic GIST subsets, including 15 WT GISTs and 24 KIT/PDGFR α mutated GISTs. These findings indicated that MGMT methylation was clearly enriched in SDH-deficient GISTs and in WT GISTs in comparison to levels in KIT/PDGFR α mutated GISTs.²⁴ However, these results warrant further validation studies using larger GIST series with distinct genetic status and in different populations. Thus, we detected the expression of MGMT and MGMT promoter methylation status in a heterogeneous cohort of GISTs that included 86 KIT/PDGFR α mutant GISTs and 51 WT GISTs through the use of IHC staining and MSP. Our results indicated that 44.5% (65/137) of the GISTs displayed a loss of MGMT protein expression and that 10.9% (15/137) of the GISTs exhibited MGMT promoter methylation. However, no significant correlation was observed between the loss of

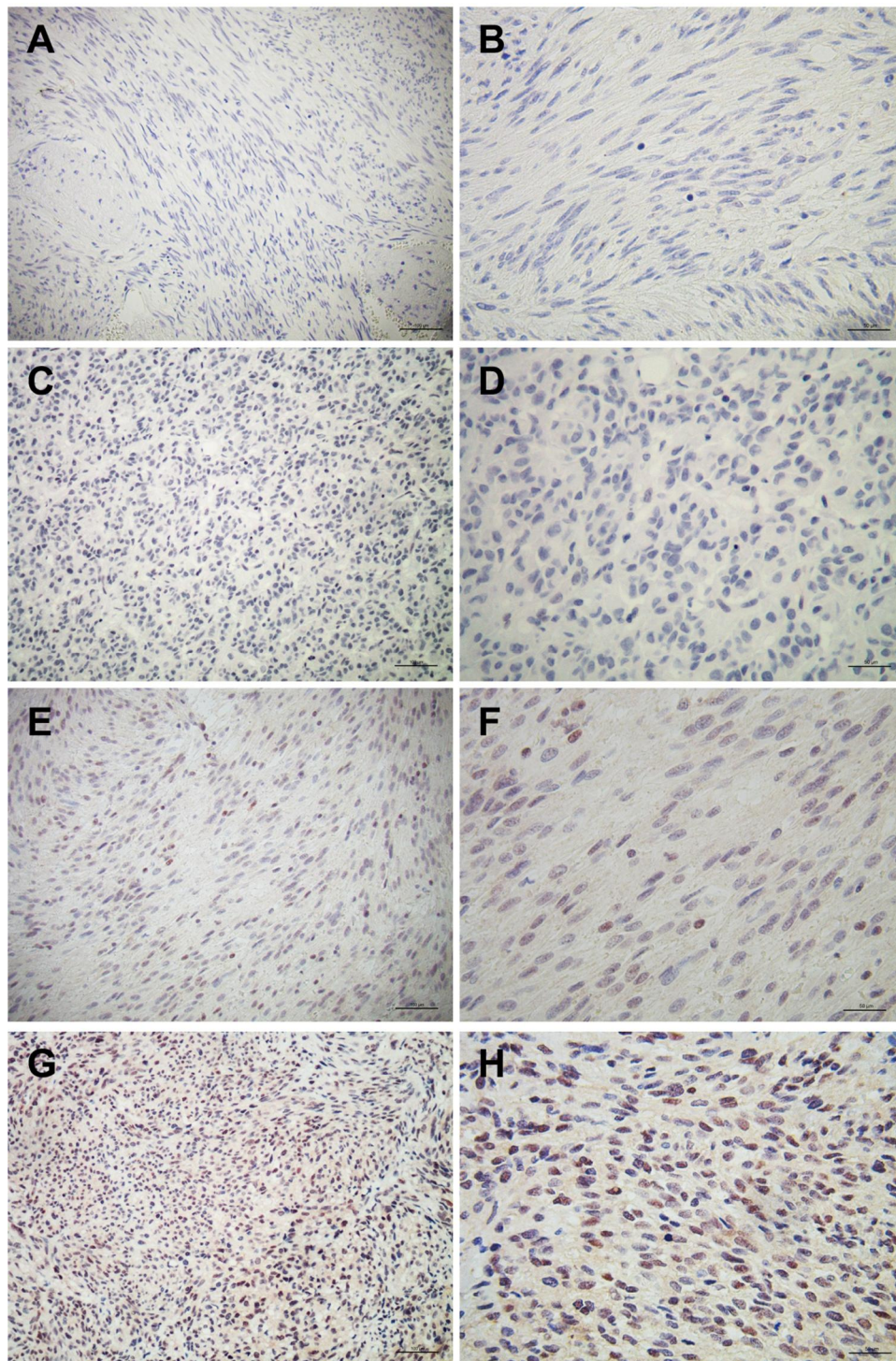


Figure 2 Immunohistochemistry staining of MGMT in GIST specimens. Negative nuclear staining of MGMT in KIT mutated GISTs with spindle cell morphology (A-B, case 57) and SDH-deficient GISTs with epithelioid morphology (C, D, case 87). (E, F) Moderate nuclear staining of MGMT in GISTs (case 7). (G, H) Strong nuclei staining of MGMT in GISTs (case 75). Scale bar (A), (C), (E) and (G) 100 μ m; (B), (D), (F) and (H) 50 μ m.

MGMT protein expression and MGMT promoter methylation status. Interestingly, we found that WT GISTs possessing an epithelioid or mixed phenotype, particularly SDH-deficient ones, exhibited a markedly higher

prevalence of MGMT promoter methylation in comparison to that in KIT/PDGFR α mutated GISTs. These findings indicated that MGMT promoter methylation is a less frequent epigenetic alteration in GISTs and that MGMT

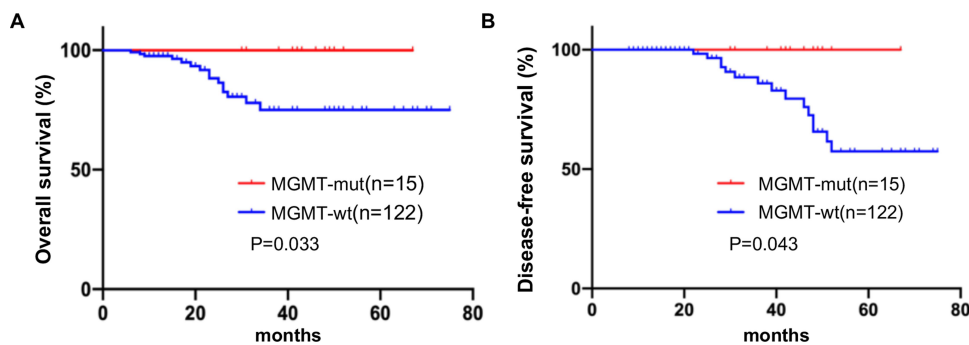


Figure 3 Association between MGMT promoter methylation and prognosis of patients with GIST. Kaplan-Meier analysis of OS (A) and DFS (B) in GIST patients according to the MGMT promoter methylation status.

promoter methylation tends to be more frequent in SDH-deficient GISTs and in WT GISTs with an epithelioid/mixed phenotype. Therefore, the detection of MGMT promoter methylation would only be recommended for WT GISTs. Furthermore, multivariate Cox regression analysis indicated that MGMT promoter methylation could be considered as a potential independent prognostic factor for OS and DFS in GIST patients, suggesting that MGMT promoter methylation may predict better clinical outcomes in patients with GISTs.

Multiple studies have suggested that WT GISTs, including SDH-deficient ones, often exhibit primary resistance to imatinib and lack efficacious therapy options.^{12,13} This indicates that other molecular genetic alterations may also be involved in the tumorigenesis of this distinct subgroup of GISTs. Recently, methylation of the MGMT promoter has emerged as a predictive factor of positive therapeutic response to alkylating agents.^{21–23} Based on these premises, it is reasonable to hypothesize that WT GISTs and SDH-deficient GISTs harboring MGMT promoter methylation may benefit from treatment with alkylating agents. Although the current trials studying the effects of the alkylating agents temozolomide and carmustine on GISTs were published with negative results, we still cannot completely deny the possible efficacy of these drugs in the treatment of a subset of WT GISTs, particularly on SDH-deficient GISTs.^{32–34} Additionally, these negative results are based on studies limited to only a few GIST patients who enrolled in past trials examining sarcomas without selecting the genotype and detecting the SDH status of the tumors. Subsequently, MGMT promoter methylation status was not detected in all cases of these GISTs, with the exception of two cases in which MGMT protein expression was evaluated by IHC.³⁴ Additionally, none of these trials applied Choi criteria to evaluate the

GIST response to drug treatment.³⁵ Therefore, further clinical studies are required to validate this hypothesis. Currently, a Phase II trial of temozolomide in metastatic SDH-deficient GIST is ongoing (ClinicalTrials.gov/NCT03556384). In the event of acquiring positive results, this clinical trial may offer a novel potential therapeutic option for WT GISTs.

Conclusion

Our study provides additional evidence that MGMT promoter methylation is particularly frequent in SDH-deficient GISTs and in KIT/PDGFR α WT GISTs with an epithelioid/mixed phenotype. Moreover, GIST patients harboring MGMT promoter methylation exhibited improved clinical outcomes with longer OS and DFS. These findings support the use of MGMT promoter methylation as a prognostic predictor in GISTs, and our findings offer a novel potential therapeutic option for WT GISTs. However, the predictive value of MGMT promoter methylation for the application of alkylating agents as a new treatment strategy in KIT/PDGFR α WT GISTs required further exploration in future clinical studies.

Abbreviations

GISTs, gastrointestinal stromal tumors; WT, wild-type; SDH, succinate dehydrogenase; MGMT, O⁶-methylguanine-DNA methyltransferase; MSP, methylation-specific PCR; KIT, C-kit receptor tyrosine kinase; PDGFR α , platelet-derived growth factor receptor alpha; NF1, neurofibromatosis type 1; BRAF, serine/threonine protein kinase B-RAF; TKI, tyrosine kinase inhibitor; DFS, disease-free survival; H&E, hematoxylin and eosin-stained; NCBI, National Center for Biotechnology Information; NIH, National Institute of Health; OS, overall survival; WHO, World Health Organization; IRS,

immunoreactive score; IHC, immunohistochemistry; HPF, high-power field.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed on the journal to which the article will be submitted; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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