



## Aberrant DNA methylation of *MGMT* and *hMLH1* genes in prediction of gastric cancer

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**ABSTRACT.** We aimed to explore the association between aberrant DNA methylation of the O(6)-methylguanine-DNA methyltransferase (*MGMT*) and human mutL homolog 1 (*hMLH1*) genes with gastric cancer. A total of 283 gastric cancer patients who were confirmed by pathological diagnosis were included in our study. Aberrant DNA methylation of *MGMT* and *hMLH1* were detected. The proportions of DNA hypermethylation in *MGMT* and *hMLH1* in cancer tissues were significantly higher than those in remote normal-appearing tissues. The DNA hypermethylation of *MGMT* was correlated with the tumor-necrosis-metastasis stage in gastric cancer tissues. Results showed that individuals with gastric cancer in the N1 and M1 stages had a significantly higher risk of DNA hypermethylation of *MGMT* in cancer tissues [odds ratio (OR) = 1.97, 95% confidence interval (CI) = 1.15-3.37 for the N1 stage; OR (95%CI) = 5.39 (2.08-14.98) for the M1 stage]. In conclusion, we found that aberrant hypermethylation of *MGMT* could be a predictive biomarker for detecting gastric cancer.

**Key words:** Aberrant DNA methylation; *MGMT*; *hMLH1*;  
Gastric cancer

## INTRODUCTION

Gastric cancer is one of the most common cancers in the world (IARC, 2008). Almost two-thirds of gastric cancer cases occur in less developed regions (IARC, 2008). Infection with *Helicobacter pylori* is a well-established cause of gastric cancer. However, polymorphisms in various genes may also influence the susceptibility to gastric cancer (IARC, 1994). DNA methylation is an important epigenetic feature of DNA, which plays important roles in eukaryotes including gene regulation, cellular differentiation mechanisms, X chromosome inactivation, aging, and carcinogenesis (Sharp et al., 2011). Alterations of DNA methylation patterns in the genome are found in various cancers and induce the over-expression of oncogenes and the silencing of tumor suppressor genes in the process of carcinogenesis. However, few studies have been conducted on the aberrant hypermethylation of cancer-related genes, such as the O(6)-methylguanine-DNA methyltransferase (*MGMT*) and human mutL homolog 1 (*hMLH1*) genes. Therefore, we aimed to explore the association between the aberrant DNA methylation of *MGMT* and *hMLH1* and gastric cancer.

## MATERIAL AND METHODS

### Subjects

This study recruited gastric cancer patients who were confirmed by pathological diagnosis at the Central South Hospital (Wuhan, China) between March 2009 and December 2011. Patients with cardiac adenocarcinoma, secondary or recurrent tumors, a history of other malignant neoplasms, and previous eradication therapy for *H. pylori* were excluded from our study.

All patients received surgery, and cancer lesions and remote normal-appearing tissues were excised and stored at  $-70^{\circ}\text{C}$ . Twenty normal gastric tissue samples were also obtained from surgery and stored.

### DNA extraction and quantification

Five milliliters venous blood were drawn from each case and control subject. The blood was kept at  $-20^{\circ}\text{C}$ , and 1.5-2.2 mg/mL ethylenediaminetetraacetic acid was used as the anticoagulant. Total DNA was extracted from the buffy coat layer using a TIANamp blood DNA kit (Tiangen Biotech; Beijing, China) and centrifuged for 3 min at 13,400 g (12,000 rpm). The methylation status of *MGMT* and *hMLH1* was determined by the methylation-specific polymerase chain reaction (PCR) method after sodium bisulfate modification of DNA (Wang et al., 2008). The pairs of primers used were designed with the Assay Design 3.1 software (Sequenom; San Diego, CA, USA; Table 1). Samples of 1.5 to 2.0  $\mu\text{g}$  genomic DNA were dissolved in  $\text{H}_2\text{O}$  and incubated in 5.5  $\mu\text{L}$  NaOH for 10 min at  $37^{\circ}\text{C}$ , and then treated with hydroquinone and  $\text{NaHSO}_3$ . After these procedures, the unmethylated cytosine would be converted to uracil and determined as thymine by *Taq* polymerase during the PCR process according to manufacturer instructions. The PCR amplification consisted of 40 cycles of  $95^{\circ}\text{C}$  for 5 s and  $60^{\circ}\text{C}$  for 30 s after an initial denaturation step of  $95^{\circ}\text{C}$  for 10 s.

**Table 1.** Primers in the PCR process.

Gene	Primer	Sequence (5'→3')	Product size (bp)	Annealing temperature (°C)	
<i>MGMT</i>	M	F	GCGTTTCGACGTTTCGTAGGT	83	65
		B	CACTCTCCGAAAACGAAACG		
	U	F	TTTGTGTTTTGATGTTTGTAGGTTTT	93	59
<i>hMLH1</i>	M	F	AACTCCACACTCTCCAAAAACAAA	87	53
		B	CGTTATATATCGTTCGTAGTATTTCGTGTTT		
	U	F	CTATCGCCGCCATCATCGT	124	60
		F	TTTTGATGATAGTTTTATTAGGGTTGT		
		B	ACCACCTCATCATAACTACCCACA		

M = methylated; U = unmethylated; F = forward; B = backward.

## Statistical analysis

All statistical analyses were performed using the SPSS version 11.0 software (SPSS Inc.; Chicago, IL, USA) for Windows. Continuous variables are reported as means  $\pm$  SD and were analyzed using the independent sample Student *t*-test. Categorical variables are presented as percentage of subjects and were analyzed using the  $\chi^2$  test. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to assess the influence of *MTHFR* and *hMLH1* hypermethylation on the risk of gastric cancer. All comparisons were two-sided, and  $P < 0.05$  was regarded as statistically significant.

## RESULTS

A total of 311 gastric cancer patients were included in our study, and 283 patients were involved in the final analysis (participation rate: 91%; 152 males and 131 females). The average age of the 283 patients was  $53.7 \pm 10.3$  years. The DNA hypermethylation status of *MGMT* and *hMLH1* in cancer tissues and in paracancerous normal tissues is shown in Table 2. The proportions of DNA hypermethylation in *MGMT* and *hMLH1* in gastric cancer tissues were 31.8% (90/283) and 5.7% (16/283), respectively. Of the remote normal-appearing tissues, 17.3% (49/283) showed hypermethylation in the *MGMT* gene, whereas no hypermethylation was found in the *hMLH1* gene. The proportions of DNA hypermethylation in *MGMT* and *hMLH1* in cancer tissues were significantly higher than those in remote normal-appearing tissues. We did not find any significant association of DNA hypermethylation with gender or tumor sites either in gastric cancer tissues or in remote normal-appearing tissues.

The hypermethylation of *MGMT* was significantly correlated with the different clinical characteristics (Table 2). We found a significantly higher proportion of DNA hypermethylation of *MGMT* in the N1 and M1 tumor-necrosis-metastasis (TNM) stages ( $P < 0.05$ ).

The association of the DNA hypermethylation of *MGMT* and *hMLH1* with clinical characteristics of gastric cancer is shown in Table 3. Individuals with gastric cancer at the N1 and M1 stages had a significantly higher risk of DNA hypermethylation of *MGMT* in cancer tissues: OR (95%CI) = 1.97 (1.15-3.37) for the N1 stage; OR (95%CI) = 5.39 (2.08-14.98) for the M1 stage.

**Table 2.** Association of DNA hypermethylation with demographic and clinical characteristics of gastric cancer.

Variables	N = 283	Cancer tissue			%	Remote normal-appearing tissues			%
		MGMT	%	hMLH1		MGMT	%	hMLH1	
Mean age (years)	53.7 ± 10.3								
Gender									
Male	152	49	32.24	8	5.26	23	15.13	0	0
Female	131	41	31.29	8	6.11	26	19.84	0	0
P value		0.87		0.8		0.35		1	
Site									
Upper	53	17	32.30	4	7.60	9	17.10	0	0
Middle	134	39	29.01	7	5.21	22	16.37	0	0
Low	96	34	35.44	5	5.21	18	18.76	0	0
P value		0.82		0.81		0.9		1	
TNM stage									
T									
T1	39	11	28.17	2	5.12	7	17.92	0	0
T2	83	23	27.64	4	4.81	14	16.83	0	0
T3	87	24	27.62	4	4.60	15	17.26	0	0
T4	74	32	43.32	6	8.12	13	17.60	0	0
P value		0.11		0.77		0.98		1	
N									
N0	158	40	25.28	6	3.79	22	13.91	0	0
N1	125	50	40.06	10	8.01	27	21.63	0	0
P value		0.01*		0.19		0.14		1	
M									
M0	258	73	28.25	13	5.03	42	16.26	0	0
M1	25	17	69.05	3	12.18	7	28.43	0	0
P value		0.001*		0.16		0.16		1	

\*P value was <0.05.

**Table 3.** Association of DNA hypermethylation with clinical characteristics of gastric cancer.

Variables	OR (95%CI)			
	Cancer tissue		Remote normal-appearing tissues	
	MGMT	hMLH1	MGMT	hMLH1
Gender				
Male	1.0 (Ref.)	1.0 (Ref.)	-1.0 (Ref.)	-
Female	0.86 (0.56-1.63)	1.17 (0.37-3.69)	1.42 (0.73-2.76)	-
TNM stage				
T				
T1	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	-
T2	0.98 (0.39-2.54)	0.94 (0.13-10.8)	0.93 (0.31-2.99)	-
T3	0.97 (0.39-2.51)	0.89 (0.12-10.3)	0.95 (0.33-3.04)	-
T4	1.94 (0.78-4.97)	1.63 (0.27-17.25)	0.97 (0.32-3.19)	-
N				
N0	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	-
N1	1.97 (1.15-3.37)*	2.20 (0.67-7.58)	1.70 (0.88-3.33)	-
M				
M0	1.0 (Ref.)	1.0 (Ref.)	1.0 (Ref.)	-
M1	5.39 (2.08-14.98)*	2.57 (0.43-10.35)	2.0 (0.66-5.40)	-

\*P value was <0.05.

## DISCUSSION

Changes in the activity of folate metabolic enzymes induced by genetic polymorphisms may affect the methylation status of these genes and induce carcinogenesis (Chen et al., 2012; Gao et al., 2013). A relationship between the DNA methylation of the *MGMT* and

*hMLH1* genes and cancer risk has been demonstrated in several studies (Chen et al., 2012; Gomes et al., 2012; Cheng et al., 2012; de Cássia Carvalho Barbosa et al., 2012). However, few studies have evaluated their association with gastric cancer risk. The findings from the present study indicated increased hypermethylation of *MGMT* and *hMLH1* in gastric cancer tissues than in remote-normal-appearing gastric tissues. Hypermethylation of *MGMT* was related to the TNM stage of gastric cancer tissues.

Aberrant methylation, global hypomethylation in genomic DNA, and hypermethylation in specific gene promoters commonly occurs in cancers (Mompalmer and Bovenzi, 2000). The lack of global DNA methylation may cause instability of the gene, and thus promote cancer development (Sato and Meltzer, 2006), whereas promoter hypermethylation might induce inactivity of gene transcription (Sato and Meltzer, 2006). Previous studies indicated that DNA methylation primarily influences the cytosine of symmetrical dinucleotide CpG islands in humans (Issa et al., 2004), and that the subsequent pattern of DNA methylation is transmitted through mitosis and maintained after DNA replication (Gius et al., 2005); thereby, aberrant CpG island methylation could promote carcinogenesis. It was previously reported that the *COX2*, *MGMT*, *hMSH2*, and *hMLH1* genes are more frequently found in cancer tissues than in remote normal-appearing tissues, and hypermethylation of these genes is not found in normal tissues (Wang et al., 2008; Chen et al., 2012; Gao et al., 2013). The present study indicated that individuals with methylation of *MGMT* might have an increased risk of gastric cancer, which suggests that DNA methylation might play a role in the development of gastric cancer.

Consistent with other studies, no methylation of *MGMT* and *hMLH1* was detected in normal tissues in the present study. By contrast, methylation of *MGMT* and *hMLH1* was detected in approximately 35.7% of gastric cancer patients (101 patients). The overall findings suggest that methylation of *MGMT* and *hMLH1* occurs frequently and may be associated with gastric cancer. Furthermore, the highest prevalence of methylation of *MGMT* and *hMLH1* was observed in cancer tissues. This indicates that methylation of particular genes may preferentially occur in particular types of cells, and that promoter hypermethylation might also be a cell type-specific event. Therefore, great precaution should be taken when selecting methylated DNA markers for cancer detection.

In conclusion, we found that the aberrant hypermethylation of cancer-related genes, such as *MGMT* and *hMLH1*, could be predictive biomarkers for detecting gastric cancer. The aberrant hypermethylation of *MGMT* was associated with the N1 and M1 stages. Further large-scale studies are required to confirm the association between *MGMT* and *hMLH1* and the risk of gastric cancer.

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