Methylation of the *MGMT* Gene is Frequently Detected in Advanced Gastric Carcinoma

KENJI HIBI, MAKIKO SAKATA, KAZUAKI YOKOMIZO, YO-HEI KITAMURA, KAZUMA SAKURABA, ATSUSHI SHIRAHATA, TETSUHIRO GOTO, HIROKI MIZUKAMI, MITSUO SAITO, KAZUYOSHI ISHIBASHI, GAKU KIGAWA, HIROSHI NEMOTO and YUTAKA SANADA

Department of Surgery, Showa University Fujigaoka Hospital, Yokohama, Japan

Abstract. *Background: Recently, Herfarth et al. reported that a subset of colorectal tumors was characterized by a specific methylation pattern in the MGMT promoter associated with reduced MGMT expression. Materials and Methods: Methylation status of the MGMT gene was examined in primary carcinomas and the corresponding normal tissues derived from 38 patients with gastric cancer using quantitative methylation-specific PCR (qMSP) and the correlation between the methylation status and the clinicopathological findings was evaluated. Results: Aberrant methylation of the MGMT gene was detected in 4 out of the 38 (11%) primary gastric carcinomas, suggesting that the methylation of MGMT is observed in gastric carcinomas as well as colorectal ones. The clinicopathological data were correlated with the methylation results. A significant difference was observed in the extent of tumor (p=0.0470), lymph node metastasis (p=0.0470), and TNM stage (p=0.0377) (Table I). Moreover, a trend was shown toward large maximal tumor size in methylated tumors (p=0.134). Conclusion: MGMT was more frequently methylated in advanced gastric carcinomas.*

Accumulating evidence indicates that gastric cancer is the result of various genetic and epigenetic alterations of oncogenes, tumor suppressor genes, DNA repair genes, cell-cycle regulators, and cell adhesion molecules (1). Aberrant methylation of CpG-rich sequences (CpG islands) is an epigenetic change that is common in human cancer (2). In gastric cancer, the inactivation of human mutL homolog 1 (hMLH1), O-6-methylguanine-DNA methyltransferase *(MGMT)*, tissue inhibitor of metalloproteinase 3 *(TIMP-3)* and *p16* by promoter hypermethylation has been demonstrated (3-6). There has been substantial interest in attempting to adapt such cancer-associated aberrant gene methylation for clinical use.

The human enzyme MGMT protects the cell from guanine methylation by irreversibly transferring the alkyl group of O^6-methylguanine to a specific cysteine residue within the molecule. The expression of MGMT varies widely among different tissues (7). Previously, Herfarth *et al*. reported that a subset of colorectal tumors was characterized by a specific methylation pattern in the *MGMT* promoter associated with reduced MGMT expression (8). However, the relationship between the *MGMT* methylation and the characteristics of these tumors remains unknown.

In the present study, the methylation status of the *MGMT* gene was examined in primary carcinomas and the corresponding normal tissues derived from 38 patients with gastric carcinomas, and the correlation between the methylation status and the clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Thirty-eight primary tumor and corresponding normal tissue specimens were collected consecutively at Showa University Fujigaoka Hospital from gastric cancer patients during gastric surgery. All the tissue specimens were confirmed histologically. Written informed consent, as required by the Institutional Review Board, was obtained from all the patients. The samples were stored immediately at –80°C until analysis. The DNA was prepared as described elsewhere (9). Briefly, tumor samples were digested overnight by proteinase K and treated by phenol/chloroform. DNA was precipitated by ethanol and ammonium acetate. The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

Sodium bisulfite modification. One μg of the genomic DNA extracted from the tumor and the corresponding normal gastric tissue specimens was subjected to bisulfite treatment using an Epitect Bisulfite Kit (Qiagen, Hilden, Germany).

Correspondence to: Kenji Hibi, Department of Surgery, Showa University Fujigaoka Hospital, 1-30 Fujigaoka, Aoba-ku, Yokohama 227-8501, Japan. Tel: +81 459711151, Fax: +81 459717125, e-mail: kenjih-ngy@umin.ac.jp

Key Words: MGMT, quantitative methylation-specific PCR, gastric cancer.

Quantitative methylation-specific PCR (qMSP). The bisulfite-treated DNA was amplified with qMSP conducted in a Thermal Cycler Dice® Real-Time System TP800 (Takara Bio Inc., Otsu, Japan). Thermocycling was performed in a final volume of 25 μl containing 1.0 μl of the DNA sample, 100 nM each of the *MGMT* or *β-actin* primers (forward and reverse), and 12.5 μl of SYBR Premix Ex Taq II (Takara Bio Inc.), which consists of Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for *MGMT* have been described elsewhere and were: *MGMT* MS (sense) 5'-CGAATATACTAAAACAACCCGCG-3' and *MGMT* MAS (antisense) 5'-GTATTTTTTCGGGAGCGAGGC-3'. The PCR amplification consisted of 40 cycles (95˚C for 5 s and 60˚C for 30 s) after an initial denaturation step (95˚C for 10 s). The bisulfite-treated DNA obtained from L132 cells, which are fully methylated by *SssI* methylase, was used as a positive control. To correct for differences in both quality and quantity between samples, *β-actin* was used as an internal control. The targets were obtained from the same bisulfite-treated DNA.

MGMT methylation scores. The relative amounts of *MGMT* methylated DNA in the gastric carcinomas and the corresponding normal tissues normalized to the internal control *β-actin* were calculated. The *MGMT* methylation score in each tissue was defined as follows: relative amount of *MGMT* in tumor/average relative amount of *MGMT* in all corresponding normal tissues. *MGMT* methylation was positive when the methylation score was more than 3.0.

Statistical analysis. The associations between *MGMT* methylation and clinicopathological parameters were analyzed using Chi-square tests or Student's *t*-tests. A p-value <0.05 indicated statistical significance.

Results

Methylation of the *MGMT* gene was detected in 4 out of the 38 (11%) primary gastric carcinomas, suggesting that the methylation of *MGMT* was observed in gastric carcinoma as well as colorectal ones.

The clinicopathological data were tested for correlation with the methylation results. No significant correlations were observed between the presentation of methylation in the gastric carcinomas and patient gender or age, maximal tumor size, or histology (Table I). A significant difference was observed in the extent of tumor (p=0.0470), lymph node metastasis (p=0.0470), and TNM stage (p=0.0377) (Table I). Moreover, a trend was shown toward large maximal tumor size in methylated tumors (p=0.134), thus indicating that *MGMT* was more frequently methylated in advanced gastric carcinomas.

Discussion

Gastric cancer is one of the most common malignancies worldwide (10). In order to remove this fatal cancer from patients, we perform surgical operations and subsequent chemotherapy and radiotherapy. For this purpose, it is important to identify the occurrence of genetic alterations as a new parameter to estimate the malignancy of the cancer.

Previously, we examined the methylation status of the *MGMT* gene in primary carcinomas and the corresponding normal tissues derived from 48 patients with colorectal cancer using qMSP and the correlation between the methylation status and the clinicopathological findings was evaluated (11). Aberrant methylation of the *MGMT* gene was detected in 10 out of the 48 (21%) primary colorectal tumors. All stages of colorectal cancer presented *MGMT* methylation, even in early stages. In the present study, we compared *MGMT* gene methylation in gastric carcinoma patients with their clinicopathological features and demonstrated that while *MGMT* gene methylation was less frequently observed in gastric carcinoma patients than in these with colorectal cancer, it was strongly associated with advanced stage cancer. Therefore, it might be possible that *MGMT* gene methylation might play different roles depending on the tissue types along the digestive tract. *MGMT* methylation could be used as a tumor marker in clinical samples such as serum for the detection of gastric carcinomas (12-14).

This study provides a solid basis for additional studies on the molecular mechanism of MGMT in gastric carcinoma and our findings also suggest that MGMT may play a role in the carcinogenic pathway in some patients with gastric carcinomas. Since gastric carcinoma is one of the most aggressive of all cancer types, we may not be able to improve the overall survival rate using information about *MGMT* methylation alone. However, *MGMT* methylation could prove useful as a marker for advanced gastric carcinoma. *MGMT* methylation should therefore be the target of future therapies for gastric carcinomas.

References

1 Yasui W, Yokozaki H, Fujimoto J, Naka K, Kuniyasu H and Tahara E: Genetic and epigenetic alterations in multistep carcinogenesis of the stomach. J Gastroenterol *35*: 111-115, 2000.

2 Chen W-D, Han ZJ, Skoletsky J, Olson J, Sah J, Myeroff L, Platzer P, Lu S, Dawson D, Willis J, Pretlow TP, Lutterbaugh J, Kasturi L, Willson JKV, Rao JS, Shuber A and Markowitz SD: Detection in fecal DNA of colon cancer-specific methylation of the non-expressed *vimentin* gene. J Natl Cancer Inst *97*: 1124-1132, 2005.

3 Leung SY, Yuen ST, Chung LP, Chu KM, Chan AS and Ho JC: *hMLH1* promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. Cancer Res *59*: 159-164, 1999.

4 Oue N, Shigeishi H, Kuniyasu H, Yokozaki H, Kuraoka K, Ito R and Yasui W: Promoter hypermethylation of *MGMT* is associated with protein loss in gastric carcinoma. Int J Cancer *93*: 805-809, 2001.

5 Kang SH, Choi HH, Kim SG, Jong HS, Kim NK, Kim SJ and Bang YJ: Transcriptional inactivation of the tissue inhibitor of metalloproteinase-3 gene by DNA hypermethylation of the 5'-CpG island in human gastric cancer cell lines. Int J Cancer *86*: 632-635, 2000.

Table I. *Clinicopathological features and MGMT methylation in gastric cancer.*

Clinicopathological feature	Variable	No. of cases	*MGMT* methylation		*p*-Value
			+	–	
Gender	Male	31	4	27	0.188†
	Female	7	0	7	
Age (years)	50-82	38	72.3±6.9*	68.9±9.8*	0.520‡
Maximal tumor size (mm)	10-130	38	78.8±23.2*	58.7±24.9*	0.134‡
Extent of tumor	≤ss	14	0	14	0.0470†
	>ss	24	4	20	
Histology	Well	15	1	14	0.520†
	Poor	23	3	20	
Lymph node metastasis	+	24	4	20	0.0470†
	–	14	0	14	
TNM stage	1.2	15	0	15	0.0377†
	3.4	23	4	19	
Total		38	4	34	

†Chi-square test; ‡Student's *t*-test; *mean±S.D; ss, subserosa; Well, well- or moderately differentiated adenocarcinoma according to Japanese criteria; Poor, poorly-differentiated, mucinous, or signet ring cell adenocarcinoma according to Japanese criteria.

6 Shim YH, Kang GH and Ro JY: Correlation of *p16* hypermethylation with p16 protein loss in sporadic gastric carcinomas. Lab Invest *80*: 689-695, 2000.

7 Citron M, Graver M, Schoenhaus M, Chen S, Decker R, Kleynerman L, Kahn LB, White A, Fornace AJ Jr and Yarosh D: Detection of messenger RNA from O^6-methylguanine-DNA methyltransferase gene *MGMT* in human normal and tumor tissues. J Natl Cancer Inst *84*: 337-340, 1992.

8 Herfarth KK, Brent TP, Danam RP, Remack JS, Kodner IJ, Wells SA Jr and Goodfellow PJ: A specific CpG methylation pattern of the *MGMT* promoter region associated with reduced MGMT expression in primary colorectal cancers. Mol Carcinogenesis *24*: 90-98, 1999.

9 Hibi K, Taguchi M, Nakayama H, Takase T, Kasai Y, Ito K, Akiyama S and Nakao A: Molecular detection of *p16* promoter methylation in the serum of patients with esophageal squamous cell carcinoma. Clin Cancer Res *7*: 3135-3138, 2001.

10 Fuchs CS and Mayer RJ: Gastric carcinoma. N Engl J Med *333*: 32-41, 1995.

11 Hibi K, Goto T, Mizukami H, Kitamura Y, Sakata M, Saito M, Ishibashi K, Kigawa G, Nemoto H and Sanada Y: *MGMT* gene is aberrantly methylated from the early stages of colorectal cancer. Hepato-gastroenterol in press.

12 Hibi K, Robinson CR, Booker S, Wu L, Hamilton SR, Sidransky D and Jen J: Molecular detection of genetic alterations in the serum of colorectal cancer patients. Cancer Res *58*: 1405-1407, 1998.

13 Koike M, Hibi K, Kasai Y, Ito K, Yamazaki T, Akiyama S and Nakao A: Molecular detection of circulating esophageal squamous cell cancer cells in the peripheral blood. Clin Cancer Res *8*: 2879-2882, 2002.

14 Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, Hibi K, Goodman SN, D'allessio M, Paty P, Hamilton SR, Sidransky D, Barany F, Levin B, Shuber A, Kinzler KW, Vogelstein B and Jen J: Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst *93*: 858-865, 2001.

Received July 29, 2009
Revised October 6, 2009
Accepted October 7, 2009