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

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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).
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’-CGAATATACTAAAAACACCGCG-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 SsIII 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


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

<table>
<thead>
<tr>
<th>Clinicopathological feature</th>
<th>Variable</th>
<th>No. of cases</th>
<th>MGMT methylation</th>
<th>p-Value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>–</td>
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<tr>
<td>Gender</td>
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<td>4</td>
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<tr>
<td></td>
<td>Female</td>
<td>7</td>
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<tr>
<td>Age (years)</td>
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<td>38</td>
<td>72.3±6.9*</td>
<td>68.9±9.8*</td>
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<td>Maximal tumor size (mm)</td>
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<td>38</td>
<td>78.8±23.2*</td>
<td>58.7±24.9*</td>
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<td>Extent of tumor</td>
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<td>14</td>
</tr>
<tr>
<td></td>
<td>&gt;ss</td>
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<td>4</td>
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<td></td>
<td>Poor</td>
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<td>0</td>
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<td>TNM stage</td>
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<td></td>
<td>3.4</td>
<td>23</td>
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<tr>
<td>Total</td>
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<td>38</td>
<td>4</td>
<td>34</td>
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</table>

‡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.


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