Changes in UNC5C Gene Methylation during Human Gastric Carcinogenesis

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Abstract. Background: UNC5C, one of the netrin-1 receptors, belongs to the functional dependence receptor family, members of which share the ability to induce apoptosis in the absence of their ligands. Recently, aberrant methylation of the UNC5C gene was found in 34 out of 49 (69%) primary colon carcinomas. Materials and Methods: The methylation status of the UNC5C gene was examined in primary carcinomas and the corresponding normal tissues derived from 36 patients with gastric cancer using quantitative methylation-specific polymerase chain reaction, and the correlation between the methylation status and the clinicopathological findings was evaluated. Results: Aberrant methylation of the UNC5C gene was detected in 9 out of the 36 (25%) primary gastric carcinomas. A significant difference was observed in regard to the TNM stage (p = 0.0455). Conclusion: UNC5C methylation was observed in the course of gastric carcinogenesis and disappeared in highly advanced gastric carcinomas.

Gastric cancer is one of the most common malignancies worldwide (1). This highly malignant type of cancer is usually treated with surgery and subsequent chemotherapy, and radiotherapy.

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 (2). Aberrant methylation of CpG-rich sequences (CpG islands) is an epigenetic change that is common in human cancer (3). In gastric cancer, the inactivation of hMLH1 (human mutL homolog 1), MGMT (O-6-methylguanine-DNA methyltransferase), TIMP-3 (tissue inhibitor of metalloproteinase 3) and p16 by promoter hypermethylation has been demonstrated (4-7). There has been substantial interest in attempting to adapt such cancer-associated aberrant gene methylation for clinical use.

UNC5C, one of the netrin-1 receptors, belongs to the functional dependence receptor family, members of which share the ability to induce apoptosis in the absence of their ligands (8-10). Such a trait has been hypothesized to confer tumor-suppressor activity. Indeed, the loss of UNC5C expression is particularly prominent in colorectal cancer (11). However, the molecular mechanisms responsible for the loss of UNC5C expression are poorly understood. Recently, two reports have indicated that UNC5C methylation was closely associated with loss of gene expression in colorectal cancer (12, 13). We have also examined the methylation status of the UNC5C gene (14), and aberrant methylation of the UNC5C gene was detected in 34 out of the 49 (69%) primary colon carcinomas investigated, suggesting that it was frequent in colorectal cancer. These results prompted us to examine the methylation status of the UNC5C gene in surgically removed primary gastric carcinomas.

The correlation between the methylation status and the clinicopathological findings was also evaluated.

Materials and Methods

Sample collection and DNA preparation. Thirty-six primary tumor and corresponding normal tissue specimens were collected 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 (15). The clinicopathological profiles of the patients enrolled in the study are shown in Table I.
shown in gastric cancer, and to the best of our knowledge, was observed in the course of gastric carcinogenesis and (difference was observed in regard to the TNM stage dissemination or distant metastasis (Table I). A significant and patient gender or age, maximal tumor size, tumor extent, presentation of abnormal methylation in the gastric carcinomas out of the 36 (25%) primary gastric carcinomas.

UNC5C methylation scores. The relative levels of UNC5C methylated DNA in the gastric carcinomas and the corresponding normal tissues normalized to the internal control β-actin were calculated. The UNC5C methylation score in each tissue was defined as: relative level of UNC5C in tumor/average relative level of UNC5C in all corresponding normal tissues. UNC5C methylation was considered as being positive when the methylation score was more than 1.5.

Statistical analysis. The associations between UNC5C methylation and clinicopathological parameters were analyzed using Chi-square test or Student’s t-test. A p-value <0.05 indicated statistical significance.

Results

Aberrant methylation of the UNC5C gene was detected in 9 out of the 36 (25%) primary gastric carcinomas. No significant correlations were observed between the presentation of abnormal methylation in the gastric carcinomas and patient gender or age, maximal tumor size, tumor extent, tumor histology, lymph node metastasis, peritoneal dissemination or distant metastasis (Table I). A significant difference was observed in regard to the TNM stage (p=0.0455) (Table I), thus indicating that UNC5C methylation was observed in the course of gastric carcinogenesis and disappeared in highly advanced gastric carcinomas.

Discussion

The identification of the genetic alterations as a new parameter to estimate the process of the neoplastic process is important to improve the success of treatment.

In the present study, aberrant methylation of UNC5C was shown in gastric cancer, and to the best of our knowledge, this was the first such report. Moreover, a significant difference was observed in regard to the TNM stage (p=0.0455), thus indicating that UNC5C methylation was observed in the course of gastric carcinogenesis and disappeared in highly advanced gastric carcinomas. In a previous study, 25 out of 76 colorectal (33%), 11 out of 65 gastric (17%) and 1 out of 40 esophageal (3%) carcinomas demonstrated abnormal methylation of the helicase-like transcription factor (HLTF) promoter (16). This result suggested that HLTF might play a variety of roles depending on the tissue type. Subsequently, we found 5 out of 37 esophageal (14%) and 23 out of 66 gastric (35%) carcinomas demonstrated abnormal methylation of the cadherin 13 (CDH13) promoter (17). Abnormal CDH13 methylation was frequently found in gastric cancer at all clinical stages just as E-cadherin methylation, another of the cadherin family, suggesting that these types of cancer could be methylated at an early stage. Thus CDH13 might also play a variety of roles depending on the tissue type.

In another previous study (14), aberrant methylation of the UNC5C gene was detected in 34 out of 49 (69%) primary colon carcinomas, suggesting that this was frequent in colorectal cancer. Furthermore, a significantly greater proportion of cases with methylated UNC5C was found in Dukes’ stage C (p=0.0380) than in earlier stages, indicating that UNC5C might act as a tumor suppressor and UNC5C methylation might provide a malignant potential in colorectal cancer. In the present study, UNC5C methylation in gastric cancer (25%) was less frequent than in colorectal cancer (69%). Additionally, although a significant difference was observed in TNM stage, UNC5C methylation disappeared in highly advanced gastric carcinomas. Taken together, these results suggested that the methylation status of the UNC5C gene might depend on the type of primary carcinomas.

Because of the frequent methylation of the UNC5C gene and the high sensitivity of qMSP, UNC5C methylation in clinical samples, serum could potentially be used for the detection and monitoring of gastric carcinoma as suggested in colorectal carcinoma (18, 19). In conclusion, the UNC5C methylation occurs in gastric carcinomas.

References

Table I. Clinicopathological features and UNC5C methylation in human gastric cancer.

<table>
<thead>
<tr>
<th>Clinicopathological feature</th>
<th>Variable</th>
<th>No. of cases</th>
<th>UNC5C methylation</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Gender</td>
<td>male</td>
<td>29</td>
<td>+ 7</td>
<td>0.8081</td>
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<tr>
<td></td>
<td>female</td>
<td>7</td>
<td>– 2</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>36</td>
<td>71.9±9.0</td>
<td>0.4292</td>
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<tr>
<td>Maximal tumor size (mm)</td>
<td></td>
<td>36</td>
<td>65.0±24.5</td>
<td>0.5952</td>
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<tr>
<td>Extent of tumor</td>
<td>smt4</td>
<td>5</td>
<td>+ 2</td>
<td>0.4041</td>
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<tr>
<td></td>
<td>mt&lt;</td>
<td>31</td>
<td>– 7</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>well5</td>
<td>14</td>
<td>+ 2</td>
<td>0.2361</td>
</tr>
<tr>
<td></td>
<td>mod6</td>
<td></td>
<td>– 13</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
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<td>23</td>
<td>+ 6</td>
<td>0.8411</td>
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<tr>
<td>Peritoneal dissemination</td>
<td></td>
<td>13</td>
<td>– 3</td>
<td>0.824</td>
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<td>Distant metastasis</td>
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<td>9</td>
<td>+ 2</td>
<td>0.164</td>
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<tr>
<td>TNM stage</td>
<td>1-3</td>
<td>27</td>
<td>– 9</td>
<td>0.04551</td>
</tr>
<tr>
<td></td>
<td>4</td>
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<tr>
<td>Total no. of cases</td>
<td></td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Chi-square test; 2Student’s t-test; 3mean±S.D; 4mt, muscular tunic. 5Well: well-differentiated adenocarcinoma, 6mod: moderately-differentiated adenocarcinoma, 7por: poorly-differentiuated mucinous or signet ring cell adenocarcinoma according to Japanese criteria.

4 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.