Methylation of the DCC Gene Is Lost in Advanced Gastric Cancer

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Abstract. Background: Deleted in colorectal carcinoma (DCC), 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, we examined the methylation status of the DCC gene in colorectal carcinomas and found that aberrant methylation of the DCC gene was detected in 28 out of the 50 (56%) primary colon carcinomas. This result prompted us to examine the methylation status of the DCC gene in gastric carcinoma. Materials and Methods: The methylation status of the DCC 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 (qMSP) and the correlation between the methylation status and the clinicopathological findings was evaluated. Results. Aberrant methylation of the DCC gene was detected in 16 out of the 36 (44%) primary gastric carcinomas. A significant difference was observed in regard to the TNM stage (p=0.0093). Conclusion: DCC methylation was observed in the course of gastric carcinogenesis and disappeared in advanced gastric carcinoma.

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). The inactivation of human mutL homolog 1 (hMLH1), O-6-methylguanine-DNA methyltransferase (MGMT), tissue inhibitor of metalloproteinase (TIMP-3) and p16 by promoter hypermethylation has been demonstrated in gastric carcinoma (2-5). There has been substantial interest in attempting to adapt such cancer-associated aberrant gene methylation for clinical use.

Deleted in colorectal carcinoma (DCC), 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 (6, 7). Such a trait has been hypothesized to confer a tumor-suppressor activity (8). However, the molecular mechanisms responsible for the loss of DCC expression are poorly understood. A recent report indicated that DCC methylation was closely associated with loss of its expression in colorectal cancer (9). These results prompted us to examine the methylation status of the DCC gene in gastric carcinoma.

In the present study, the methylation status of the DCC gene was examined in primary carcinomas and corresponding normal tissues derived from 36 patients with gastric cancer and the correlation between the methylation status and the clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Thirty-six 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 (10). The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

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

Key Words: DCC, quantitative methylation-specific PCR, gastric cancer.
Quantitative methylation-specific polymerase chain reaction (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 carried out in a final volume of 25 μl containing 1.0 μl of the DNA sample, 100 nM each of the DCC or β-actin primers (forward and reverse), and 12.5 μl of SYBR Premix Ex Taq II (Takara Bio Inc., Otsu, Japan), which consists of Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for DCC have been described elsewhere (9) and were: DCC MS (sense) 5’-CGACTAAAACTTTACCGACGACG-3’ and DCC MAS (antisense) 5’-CGGGGGCGGGAGTACGTTCGTTC-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 that was 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. Each qMSP was repeated at least 2 times.

DCC methylation scores. The relative amounts of methylated DCC DNA in the gastric carcinomas and the corresponding normal tissues normalized to the internal control β-actin were calculated. The DCC methylation score in each tissue was defined as follows: relative amount of methylated DCC in tumor/average relative amount of methylated DCC in all corresponding normal tissues. DCC methylation was considered as being positive when the methylation score was more than 1.0.

Statistical analysis. The associations between DCC 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 DCC gene was detected in 16 out of the 36 (44%) primary gastric carcinomas, suggesting that the methylation of DCC was frequently observed in gastric carcinomas. To the best of our knowledge, this is the first report about DCC methylation in gastric cancer.

The clinicopathological data were tested for correlation with the methylation results. 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=0.0093) (Table I), indicating that the frequency of DCC methylation was significantly lower in advanced gastric carcinoma than earlier one.

Discussion

Gastric cancer is one of the most common malignancies worldwide (11). 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, DCC methylation was shown in gastric cancer and a significant difference was observed in regard to the TNM stage (p=0.0093), thus indicating that DCC methylation was observed in the course of gastric carcinogenic pathway and disappeared in advanced gastric carcinoma. We previously examined helicase-like transcription factor (HLTF) methylation in colorectal, gastric, and esophageal carcinomas (12). Twenty-five out of 76 colorectal (33%), 11 out of 65 gastric (17%), and 1 out of 40 esophageal (3%) carcinomas demonstrated abnormal methylation of the HLTF promoter. This result suggested that HLTF might play a role of varieties depending on the tissue type. Subsequently, we examined CDH13 methylation in esophageal and gastric carcinomas (13). Five out of 37 esophageal (14%) and 23 out of 66 gastric (35%) carcinomas demonstrated methylation of the CDH13 promoter. Methylation of CDH13 was frequently found in gastric cancer of patients at all clinical stages just as in E-cadherin, another of the cadherin family, suggesting that genes in these types of cancer could be methylated at an early stage. These results suggested that CDH13 might play a variety of roles depending on the tissue type.

In a recent study, we examined the methylation status of the DCC gene in primary carcinomas and the corresponding normal tissues derived from 50 patients with colorectal cancer (14). Methylation of the DCC gene was detected in 28 out of the 50 (56%) primary colon carcinomas, suggesting that the methylation of DCC was frequent in colorectal cancer. Although the clinicopathological data were then tested for correlation with this result, no significant correlation was detected. In the present study, DCC methylation in gastric cancer (44%) was less frequent than that in colorectal cancer (56%). Methylation of DCC was significantly higher in advanced TNM stages (p=0.0093), indicating that DCC methylation is lost with cancer progression. Taken together, these results suggested that the methylation status of DCC gene might depend on the type of primary carcinomas.

This study provides solid evidence in further studies of the molecular mechanism of netrin-1 receptor genes in gastric cancer and also suggests that netrin-1 receptor genes may play a role in the carcinogenic pathway in some patients with gastric cancer. These observations indicate the possibility that tumor formation in the stomach may thus be controlled by inducing the expression of silenced netrin-1 receptor genes using demethylating reagents.

References

Received June 22, 2009
Revised November 25, 2009
Accepted November 27, 2009