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, two reports indicated that UNC5C methylation was closely associated with loss of gene expression in colorectal cancer. Materials and Methods: The methylation status of the UNC5C gene was examined in primary carcinomas and the corresponding normal tissues derived from 49 patients with colorectal 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 UNC5C gene was detected in 34 out of the 49 (69%) primary colon carcinomas, suggesting that the aberrant methylation of UNC5C was frequent in colorectal cancer. The clinicopathological data were then tested for correlation with this result. A significantly greater proportion of cases with methylated UNC5C was found in Dukes' stage C (p=0.0380) than in earlier stages. Conclusion: UNC5C might act as a tumor suppressor and UNC5C methylation might present a malignant potential in colorectal cancer.

There is now solid evidence that a series of genetic alterations in both dominant oncogenes and tumor suppressor genes are involved in the pathogenesis of human colorectal cancer. The activation of oncogenes such as the ras gene and the inactivation of tumor suppressor genes such as the APC (adenomatous polyposis coli) and p53 genes have been identified in colorectal cancer (1-3). In addition, we have also found several other genes to be related to the pathogenesis of colorectal cancer (4, 5). An investigation of genetic changes is important in clarifying the tumorigenic pathway of colorectal cancer (6).

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 (7-9). Such a trait has been hypothesized to confer a tumor-suppressor activity. Indeed, the loss of UNC5C expression is particularly prominent in colorectal cancer (10). However, the molecular mechanisms responsible for the loss of UNC5C expression are poorly understood. Recently, two reports indicated that UNC5C methylation was closely associated with loss of gene expression in colorectal cancer (11, 12). These results prompted us to examine the methylation status of the UNC5C gene in the colorectal carcinomas we surgically removed.

In the present study, the methylation status of the UNC5C gene was examined in primary carcinomas and corresponding normal tissues derived from 49 patients with colorectal cancer and the correlation between the methylation status and the clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Forty-nine primary tumor and corresponding normal tissue specimens were collected consecutively at Showa University Fujigaoka Hospital from colorectal cancer patients during colorectal 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 (13). 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 colorectal tissue specimens was subjected to bisulfite treatment using an Epitect Bisulfite Kit (Qiagen, Hilden, Germany).
The bisulfite-treated DNA was amplified with quantitative methylation-specific polymerase chain reaction (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 UNC5C 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 UNC5C has been described elsewhere (12) and were: UNC5C MS (sense), 5’-CGACTAAAACTTTACCGACGCGACG-3’ and UNC5C 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 Ssis 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.

**Results**

Aberrant methylation of the UNC5C gene was detected in 34 out of the 49 (69%) primary colon carcinomas, suggesting that the aberrant methylation of UNC5C was frequent in colorectal carcinomas.

The clinicopathological data were tested for correlation with the methylation results. No significant correlations were observed between the presentation of abnormal methylation in the colorectal carcinomas and patient gender or age, maximal tumor size, tumor extent, tumor site or histology (Table I). A significantly difference was observed in regard to the Dukes’ stage (p=0.0380) (Table I), thus indicating that UNC5C was more frequently methylated in advanced colorectal carcinomas.

**Discussion**

Colorectal cancer is one of the most aggressive malignancies and occurs at a high incidence in most countries (14). Only treatment of this fatal cancer is surgery 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.

In the present study, frequent methylation of UNC5C was observed in colorectal cancer. Moreover, a significant difference was observed in regard to the Dukes’ stage (p=0.0380), thus indicating that UNC5C was more frequently methylated in advanced colorectal carcinomas. We
previously examined the methylation status of the p16 and p14 genes in 86 primary colorectal carcinomas using methylation-specific PCR (MSP) and found that patients with both p16 and p14 methylation presented a significantly greater maximal tumor size ($p=0.022$) when compared to other patients (15). We also investigated the methylation status of the CDH13 gene and found that poorly-differentiated colorectal carcinomas significantly showed the CDH13 methylation ($p=0.0053$) when compared to differentiated ones (16). Finally, we examined the combined methylation status of p16, p14, HLTF (helicase-like transcription factor), SOCS-1 (suppressor of cytokine signaling-1), CDH13, RUNX3 (a member of the human runt-related transcription factor family) and CHFR (checkpoint with FHA and RING finger) in 58 resected primary colorectal carcinomas and correlated it with the clinicopathological features of the affected patients. Poorly-differentiated colorectal carcinomas significantly showed the more number of methylated genes ($p=0.0041$) when compared to differentiated ones (17). Taken together, the results, in particular large tumor size and poor differentiation, indicated that the methylated status of UNC5C in colorectal carcinomas was significantly correlated with malignant potential.

This study provides solid evidence in further studies of the molecular mechanism of UNC5C in colorectal cancer and also suggests that UNC5C may play a role in the carcinogenic pathway in some patients with colorectal cancer. These observations indicate the possibility that tumor formation in the colorectum may thus be controlled by inducing the expression of silenced UNC5C via demethylation using demethylating agents.

References


Received May 27, 2008
Revised July 15, 2008
Accepted November 20, 2008