Demethylation of the CDH3 Gene Is Frequently Detected in Advanced Colorectal Cancer

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Abstract. Background: Recently, it has been proven that the CDH3 promoter was hypomethylated in colonic aberrant foci and colorectal cancer. The hypomethylation was also associated with induction of CDH3 expression in colorectal cancer. These results indicated that epigenetic demethylation of the CDH3 promoter in the human intestine permits its ectopic expression in colorectal cancer. Materials and Methods: The demethylation status of the CDH3 gene was examined in primary carcinomas and the corresponding normal tissues derived from 53 patients with colorectal cancer using quantitative methylation-specific PCR (qMSP) and the correlation between the demethylation status and the clinicopathological findings was evaluated. Results. Aberrant demethylation of the CDH3 gene was detected in 41 out of the 53 (77%) primary colon carcinomas. The clinicopathological data were correlated with the demethylation results. A significant difference was observed in the tumor site and Dukes' stage (p=0.0187 and p=0.0192, respectively). Moreover, a trend was shown toward preferentially developing tumor size (p=0.140). Conclusion: These results indicated that CDH3 was more frequently demethylated in advanced colorectal carcinomas.

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, several other genes have also been found 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).

CDH3 is usually expressed in the stratified squamous epithelia such as esophagus (7). However, CDH3 overexpression has been reported in esophageal, pancreatic, bladder and breast cancers (8-12). Recently, Milicic et al. demonstrated that the CDH3 promoter was hypomethylated in colonic aberrant foci and colorectal cancer (13). The hypomethylation was also associated with induction of CDH3 expression in colorectal cancer. Their results indicated that epigenetic demethylation of the CDH3 promoter in the human intestine permits its ectopic expression in colorectal cancer. These results prompted the examination of the demethylation status of the CDH3 gene in surgically removed colorectal cancers.

In the present study, the demethylation status of the CDH3 gene was examined in primary carcinomas and the corresponding normal tissues derived from 53 patients with colorectal cancer and the correlation between the demethylation status and the clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Fifty-three 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 (14). 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).
Quantitative methylation-specific PCR (qMSP). The bisulfite-treated DNA was amplified with qMSP, that was 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 CDH3 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 CDH3 have been described elsewhere (13) and were: CDH3 MS (sense), 5’-CGAGGGGGCGGGATTTCGTGGC-3’, and CDH3 MAS (antisense), 5’-ACAACTACCGCGACGACGACGCAGA-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.

**CDH3 methylation scores.** The relative amounts of CDH3 methylated DNA in the colorectal carcinomas and the corresponding normal tissues that were normalized to the internal control β-actin were calculated. The CDH3 methylation score in each tissue was defined as follows: relative amount of CDH3 in tumor/relative amount of CDH3 in corresponding normal tissue. CDH3 demethylation was positive when the methylation score was less than 0.5.

**Statistical analysis.** The associations between CDH3 demethylation and clinicopathological parameters were analyzed using Chi-square tests or Student’s t-tests. A p-value <0.05 indicated statistical significance.

**Results**

Aberrant demethylation of the CDH3 gene was detected in 41 out of the 53 (77%) primary colon carcinomas, suggesting that the aberrant demethylation of CDH3 was frequently observed in colorectal carcinomas.

The clinicopathological data were correlated with the demethylation results. No significant correlations were observed between the presentation of demethylation in the colorectal carcinomas and patient gender or age, tumor extent, histology, or lymph node metastasis (Table I). A significant difference was observed with the tumor site and Dukes’ stage (p=0.0187 and p=0.0192, respectively) (Table I). Moreover, a trend was shown toward more frequent demethylation with increasing tumor size (p=0.140), thus indicating that CDH3 was more frequently demethylated in advanced colorectal carcinomas.

**Discussion**

Colorectal cancer is one of the most aggressive malignancies and occurs at a high incidence in most countries (15). One 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, the frequent demethylation of CDH3 was observed in colorectal cancer. Demethylation was significantly associated with more advanced Duke’s stage \((p=0.0192)\). Moreover, there was a trend for more frequent demethylation with increasing tumor size \((p=0.140)\). The expression of PGP9.5 in primary colorectal cancer has been previously examined using immunohisto-chemistry and it was found that PGP9.5 expression is related to tumor progression and may be useful as a marker for invasive colorectal cancer \((16)\). This was followed by examination of the methylation status of the PGP9.5 gene in primary tumors derived from 49 patients with colorectal cancer using qMSP and the association between the methylation status and the clinicopathological findings was evaluated \((17)\). An aberrant methylation of the PGP9.5 gene was detected in 36 out of 49 (73\%) primary colon cancer samples. Subsequently, clinicopathological data were tested for their association with the methylation results. Lymph node metastasis was significantly associated with a lower frequency of methylation \((p=0.029)\). These findings indicated that PGP9.5 was frequently demethylated in metastatic colorectal cancer, suggesting that PGP9.5 hypomethylation might play an important role in re-expression of the PGP9.5 gene in colorectal cancer. This result also indicated that the demethylated status of colorectal carcinomas was significantly correlated with malignant potential.

This study provides solid evidence of the molecular mechanism of CDH3 in colorectal cancer and also suggests that CDH3 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 reducing the CDH3 expression using methylating reagents.

References


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