

Frequent CDH3 Demethylation in Advanced Gastric Carcinoma

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Abstract. *Background:* We recently found that *CDH3* was frequently demethylated in advanced colorectal carcinomas. This prompted us to examine the demethylation status of the *CDH3* gene in gastric carcinomas. *Materials and Methods:* The demethylation status of the *CDH3* gene was examined in primary tumors derived from 36 patients with gastric carcinoma using a quantitative methylation-specific PCR (*qMSP*) and was evaluated the correlation between the demethylation status and the clinicopathological findings. *Results:* Demethylation of the *CDH3* gene was detected in 25 out of the 36 (69%) primary gastric carcinomas, suggesting that the aberrant demethylation of *CDH3* is a frequent event in gastric carcinomas. Demethylation of *CDH3* was significantly associated with increasing TNM stage ($p=0.0261$). Moreover, a trend was shown toward infiltration beyond the serosa being associated with demethylation of *CDH3* ($p=0.0733$). *Conclusion:* *CDH3* was frequently demethylated 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). So far the inactivation of *hMLH1*, *MGMT*, *TIMP-3* and *p16* by promoter hypermethylation has been demonstrated (2-5). There has been substantial interest in attempting to adapt such cancer-associated aberrant gene methylation for clinical use.

CDH3 is usually expressed in stratified squamous epithelia such as that of the esophagus (6). However, *CDH3* overexpression has been reported in esophageal, pancreatic, bladder and breast cancer (7-11). Recently, Milicic *et al.* demonstrated that the *CDH3* promoter was hypomethylated

in colonic aberrant foci and colorectal cancer (12). 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. We then examined the demethylation status of the *CDH3* gene in primary carcinomas and the corresponding normal tissues derived from 53 patients with colorectal cancer (13). In the study, we found that *CDH3* was frequently demethylated in advanced colorectal carcinomas. These results prompted us to examine the demethylation status of the *CDH3* gene in surgically removed gastric carcinomas.

In the present study, the demethylation status of the *CDH3* gene was examined in primary carcinomas and the corresponding normal tissues derived from 36 patients with gastric carcinoma and the correlation between the demethylation status and the clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Thirty-six primary tumor specimens were collected consecutively at Showa University Fujigaoka Hospital from gastric carcinoma patients during gastric surgery. All specimens were confirmed histologically. Written informed consent, as required by the Institutional Review Board, was obtained from all patients. Collected samples were stored immediately at -80°C until analysis. DNA was prepared as described elsewhere (14). The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

Sodium bisulfate modification. One μg of the genomic DNA extracted from the gastric carcinoma specimens was subjected to bisulfite treatment using an Epitect Bisulfite Kit (Qiagen, Hilden, Germany) as described elsewhere (15).

*Quantitative methylation-specific polymerase chain reaction (*qMSP*).* The bisulfite-treated DNA was amplified with quantitative methylation-specific PCR (*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 *CDH3* 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

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Key Words: *CDH3*, quantitative methylation-specific PCR, gastric carcinoma.

Table I. Clinicopathological features and *CDH3* demethylation in gastric carcinomas.

Clinicopathological features	Variable	No. of cases	<i>CDH3</i> demethylation		<i>p</i> -Value
			+	-	
Gender	Male	29	22	7	0.0889†
	Female	7	3	4	
Age (years, mean±S.D.)		36	68.2±10.1	72.6±7.6	0.203††
		36	62.7±28.5	57.5±19.1	
Extent of tumor	<SE	12	6	6	0.0733†
	≥SE	24	19	5	
Histology	int	14	9	5	0.592†
	dif	22	16	6	
Lymph node metastasis	+	23	17	6	0.439†
	-	13	8	5	
TNM stage	I	8	3	5	0.0261†
	II, III, IV	28	22	6	
Total		36	25	11	

†Chi-square test; ††Student's *t*-test; SE, serosa; int, intestinal type; dif, diffuse type.

buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for *CDH3* have been described elsewhere (12) and were: *CDH3* MS (sense), 5'-CGAGGGGGCGGGATTCGTGGC-3', and *CDH3* MAS (antisense), 5'-ACAACTACCGCGACGACGAA CGGA-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 gastric carcinomas and the corresponding normal tissues as 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 considered positive when the methylation score was less than 1.0.

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

Results

Demethylation of the *CDH3* gene was detected in 25 out of the 36 (69%) primary gastric carcinomas, suggesting that the aberrant demethylation of *CDH3* is a frequent event in gastric carcinomas.

No significant associations were observed between the presentation of demethylation in the colorectal carcinomas and patient gender or age, maximal tumor size, histology or lymph node metastasis (Table I). A significant association

was observed between demethylation and TNM stage (*p*=0.0261) (Table I). Moreover, a trend was shown toward demethylation being associated with infiltration beyond the serosa (*p*=0.0733), thus indicating that *CDH3* is frequently demethylated in advanced gastric carcinomas.

Discussion

Gastric cancer is one of the most common malignancies worldwide (16). Therefore, it is important to identify the occurrence of genetic alterations as a new parameter to estimate the malignancy of the cancer.

We previously examined the demethylation status of the *CDH3* gene in primary carcinomas and the corresponding normal tissues derived from 53 patients with colorectal cancer using quantitative methylation-specific PCR (qMSP) and evaluated the correlation between the demethylation status and the clinicopathological findings (13). 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. Demethylation of *CDH3* is significantly associated with left-side colorectal cancer and advanced Dukes' stage (*p*=0.0187 and *p*=0.0192, respectively). Moreover, a trend was shown toward preferentially developing a larger tumor size (*p*=0.140). Thus, *CDH3* was found to be frequently demethylated in advanced colorectal carcinomas. In this study, we examined the demethylation status of the *CDH3* in primary gastric carcinomas and found that *CDH3* was frequently demethylated in advanced gastric carcinoma. Taken together, these results suggest that *CDH3* demethylation might play an important role in the malignant pathway of both colorectal and gastric carcinomas.

This study provides solid evidence of the demethylation of *CDH3* in gastric carcinoma and also suggests that *CDH3* may play a role in the carcinogenic pathway in some patients with gastric carcinoma. These observations indicate the possibility that tumor formation in the stomach may thus be controlled by reducing *CDH3* expression using methylating reagents.

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Received February 9, 2009

Revised June 30, 2009

Accepted July 14, 2009