

## Frequent Methylation of Vimentin in Well-differentiated Gastric Carcinoma

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**Abstract.** *Background:* Recently, it was shown that the vimentin gene, usually activated in mesenchymal cells, was highly methylated in colorectal carcinoma. *Materials and Methods:* The methylation status of the vimentin gene was examined in primary carcinomas and the corresponding normal tissues derived from 37 patients with gastric carcinoma using quantitative methylation-specific PCR (*qMSP*) and the correlation between the methylation status and the clinicopathological findings was evaluated. *Results:* Aberrant methylation of the vimentin gene was detected in 14 out of 37 (38%) primary gastric carcinomas. This result suggested that the aberrant methylation of the vimentin gene was frequent in gastric carcinomas. Subsequently, clinicopathological data were correlated with the methylation score. A significant difference was observed in histology ( $p=0.0429$ ). In addition, a trend was shown toward advancement of gastric carcinomas with vimentin methylation ( $p=0.0588$ ). *Conclusion:* In gastric carcinomas, well-differentiated adenocarcinoma was significantly methylated compared to poorly differentiated.

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). Aberrant methylation of CpG-rich sequences (CpG islands) is an epigenetic change that is common in human cancer (2). In gastric cancer, 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.

Recently, it was shown that the vimentin gene, usually activated in mesenchymal cells, was highly methylated in colorectal carcinoma (6). Several reports indicated that vimentin gene methylation was detected 53-84% of colorectal carcinomas (7-9). Moreover, vimentin methylation can be applied for screening or as a diagnostic tool of colorectal carcinoma in a fecal DNA test. These results prompted us to examine the methylation status of the vimentin gene in gastric carcinomas we surgically removed.

In the present study, we examined the methylation status of the vimentin gene in primary tumors derived from 37 patients with gastric carcinoma and evaluated the correlation between the methylation status and the clinicopathological findings.

### Materials and Methods

*Sample collection and DNA preparation.* Thirty-seven 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  $-80^{\circ}\text{C}$  until analysis. DNA was prepared as described elsewhere (10). The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

*Sodium bisulfate modification.* One  $\mu\text{g}$  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 (11).

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

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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 *vimentin* or β-actin primers (forward and reverse), and 12.5 µl of SYBR Premix Ex Taq II (Takara Bio Inc.), which consisted of Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for *vimentin* have been described elsewhere (6) and were: *vimentin* MS (sense), 5'-TCGTTTCGAGGTTTCGCGTTAGAGAC-3', and *vimentin* MAS (antisense), 5'-CGACTAAACTCGA CCGACTCG CGA-3'. The PCR amplification consisted of 40 cycles (95°C for 5 s and 55°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 *Sss*/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.

**Vimentin methylation scores.** The relative amounts of *vimentin* methylated DNA in the gastric carcinomas that were normalized to the internal control β-actin were calculated. The *vimentin* methylation score in each tissue was defined as follows: relative amount of *vimentin* in tumor/average relative amount of *vimentin* in all corresponding normal tissues. *vimentin* methylation was positive when the methylation score was more than 1.6.

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

## Results

We examined the methylation status of the *vimentin* in primary gastric carcinoma samples using qMSP. An aberrant methylation of the *vimentin* gene was detected in 14 out of 37 (38%) primary gastric carcinomas. Our results suggest that the aberrant methylation of the *vimentin* gene was frequent in gastric carcinomas.

Subsequently, clinicopathological data were tested for correlated with the methylation score. No significant correlations were observed between the presentation of aberrant methylation in the gastric carcinomas and patient gender, age, maximal tumor size, lymph node metastasis, or peritoneal dissemination (Table I). A significant difference was observed in histology (*p*=0.0429) (Table I). Well-differentiated adenocarcinoma was significantly methylated compared to poorly differentiated one. In addition, a trend was shown toward advancement of gastric carcinomas with *vimentin* methylation (*p*=0.0588).

Table I. Clinicopathological features and Vimentin methylation in gastric carcinomas.

Clinicopathological features	Variable	No. of cases	Vimentin methylation		<i>p</i> -Value
			+	-	
Gender	Male	30	12	18	0.568†
	Female	7	2	5	
Age (years, mean±S.D.)		37	71.8±9.7	67.7±9.6	0.227††
Maximal tumor size (mm, mean±S.D.)		37	62.1±26.4	65.5±28.9	0.359††
Histology	Well	16	9	7	0.0429†
	Poorly	21	5	16	
Lymph node	+	23	9	14	0.835†
	-	14	5	9	
Peritoneal	+	14	4	10	0.638†
	-	23	5	18	
TNM stage	I, II, IIIA	15	3	12	0.0588†
	IIIB, IV	22	11	11	
Total		37	14	23	

†Chi-square test; ††Student's *t*-test; Well, well-differentiated adenocarcinoma; Poorly, poorly differentiated adenocarcinoma.

## Discussion

Gastric cancer is one of the most common malignancies worldwide (13). In order to remove this fatal cancer from patients, we perform surgical operations 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.

*vimentin*, a member of the intermediate filament protein family, exhibits a complex pattern of gene expression that can be observed at several levels (14). *vimentin* is first expressed during development in mesoderm cells located between the primitive streak and the proximal endoderm. Many tissues differentiate from this origin and continue to express *vimentin*. Moreover, it has been suggested that *vimentin* can act as a signal transducer, relaying information from the extracellular matrix to the nucleus (14).

In our previous study, a significant difference was observed in Dukes' stage and a trend was shown towards preferentially developing liver metastasis and peritoneal dissemination in colorectal carcinomas with *vimentin* methylation (15). In this study, however, well-differentiated adenocarcinomas were significantly methylated compared to poorly differentiated ones whereas no significant correlations were observed between the presentation of aberrant methylation in the gastric carcinomas and TNM stage or peritoneal dissemination. These results suggest that the rate of *vimentin* methylation might be different depending on the primary carcinoma.

In conclusion, our results suggest that *vimentin* methylation occurs frequently in gastric carcinomas. Because of frequent methylation of the *vimentin* gene and the high sensitivity of qMSP, it can potentially be used for the detection and monitoring of gastric carcinoma by the detection of *vimentin* methylation in clinical samples such as serum and stool (16, 17).

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