Vimentin Methylation as a Marker for Advanced Colorectal 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. Moreover, Vimentin methylation can be applied for the screening or as a diagnostic tool of colorectal carcinomas in the fecal DNA test. Materials and Methods: The methylation status of the Vimentin gene was examined in primary carcinomas and the corresponding normal tissues derived from 48 patients with colorectal cancer 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 31 out of 48 (65%) primary colorectal carcinomas. This result suggested that the aberrant methylation of the Vimentin gene was frequent in colorectal carcinomas. Subsequently, clinicopathological data were correlated with the methylation score. A significant difference was observed in age and Dukes' stage (p=0.001 and p=0.034, respectively). Moreover, a trend was shown toward preferentially developing liver metastasis and peritoneal dissemination in colorectal carcinomas with Vimentin methylation (p=0.052 and p=0.080, respectively). Conclusion: Vimentin was frequently methylated in advanced colorectal carcinoma.*

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 carcinoma. The activation of oncogenes such as the *ras* gene, and the inactivation of tumor suppressor genes such as the *APC* and *p53* genes have been identified in colorectal carcinoma (1-3). In addition, we also found several other genes to be related to the pathogenesis of colorectal carcinoma (4, 5). An investigation of genetic changes is important in clarifying the tumorigenetic pathway of colorectal carcinoma (6).

Aberrant methylation of CpG-rich sequences (CpG islands) is an epigenetic change that is common in human cancer (7). In colorectal carcinomas, several genes are found to be methylated and silenced that are commonly unmethylated and expressed in normal colon mucosa (7). 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 (8). Several reports indicated that *Vimentin* gene methylation was detected 53-84% of colorectal carcinomas (9-11). 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 colorectal carcinomas we surgically removed.

In the present study, we examined the methylation status of the *Vimentin* gene in primary tumors derived from 48 patients with colorectal carcinoma and evaluated the correlation between the methylation status and the clinicopathological findings.

Materials and Methods

Sample collection and DNA preparation. Forty-eight primary tumor specimens were collected consecutively at Showa University Fujigaoka Hospital from colorectal carcinoma patients during colorectal 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 (12). The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

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Key Words: Vimentin, quantitative methylation-specific PCR, colorectal carcinoma.

Table I. *Clinicopathological features and Vimentin methylation in colorectal carcinomas.*

Clinicopathological feature	Variable	No. of cases	Vimentin methylation		*p*-Value
			+	−	
Gender	Male	26	18	8	0.464*
	Female	22	13	9	
Age (years, mean±S.D.)		48	63.2±9.8	73.4±9.2	0.001**
Maximal tumor size (mm, mean±S.D.)		48	48.2±26.5	45.6±18.1	0.666**
Extent of tumor	<Mt	8	6	2	0.500*
	Mt≤	40	25	15	
Lymph node metastasis	+	27	17	10	0.940*
	−	21	13	8	
Liver metastasis	+	6	6	0	0.052*
	−	42	25	17	
Peritoneal dissemination	+	5	5	0	0.080*
	−	43	26	17	
Duke's stage	A, B	24	12	12	0.034*
	C	24	19	5	
Total		48	31	17	

*Chi-square test; **Student's *t*-test; Mt, muscular tunic.

Sodium bisulfate modification. One μg of the genomic DNA extracted from the colorectal carcinoma specimens was subjected to bisulfite treatment using an Epitect Bisulfite Kit (Qiagen, Hilden, Germany) as described elsewhere (13).

Quantitative methylation-specific polymerase chain reaction (qMSP). The bisulfite-treated DNA was amplified with a 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 consists of Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for *Vimentin* have been described elsewhere (8) and were: *Vimentin* MS (sense), 5'-TCGTTTCGAGGTTTTCGCGTTAGAGAC-3', and *Vimentin* MAS (antisense), 5'-CGACTAAAACTCGACCGACTCGCGA-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 *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.

Vimentin methylation scores. The relative amounts of *Vimentin* methylated DNA in the colorectal 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 1.0.

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 colorectal carcinoma samples using qMSP. An aberrant methylation of the *Vimentin* gene was detected in 31 out of 48 (65%) primary colorectal carcinomas. Figure 1 shows the distribution of *Vimentin* methylation scores. Our results suggest that the aberrant methylation of the *Vimentin* gene was frequent in colorectal carcinomas.

Subsequently, clinicopathological data were tested for correlation with the methylation score. No significant correlations were observed between the presentation of aberrant methylation in the colorectal carcinomas and patient gender, maximal tumor size, extent of tumor, or lymph node metastasis (Table I). A significant difference was observed in age and Dukes' stage (p=0.001 and p=0.034, respectively) (Table I). Moreover, a trend was shown toward preferentially developing liver metastasis and peritoneal dissemination in colorectal carcinomas with *Vimentin* methylation (p=0.052 and p=0.080, respectively), thus indicating that *Vimentin* was frequently methylated in advanced colorectal carcinoma.

Discussion

Colorectal carcinoma is one of the most aggressive types of cancer and occurs at a high incidence in most countries (14). 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 (15). 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 (16).

In a previous study, early colorectal carcinomas that had not spread beyond the wall of the colon showed equal rates of *Vimentin* methylation as advanced colorectal carcinomas (7). Zou *et al*. also reported that *Vimentin* methylation in colorectal carcinomas was not associated with tumor size or Dukes' stage (9). In the present study, however, 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. These results suggest that the rate of

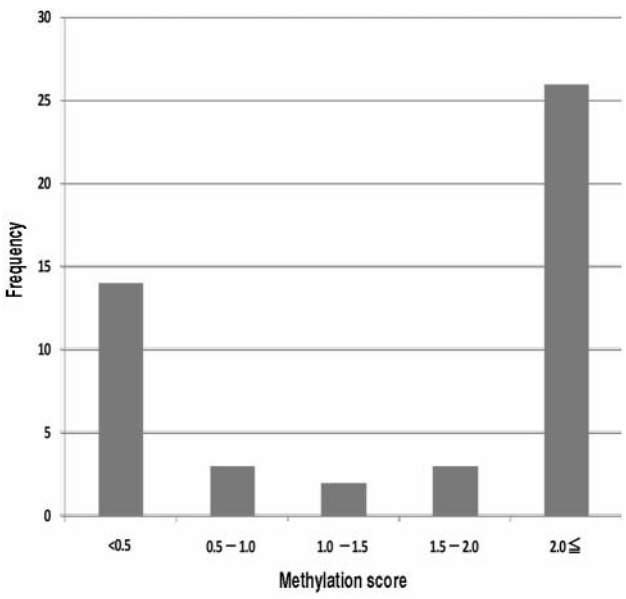

Figure 1. The distribution of Vimentin methylation scores in colorectal carcinomas (average was 7.85±13.50).

Vimentin methylation might be different depending on the ethnic group studied.

In conclusion, our results suggest that the *Vimentin* methylation occurs frequently in advanced colorectal carcinomas. Because of frequent methylation of the *Vimentin* gene and the high sensitivity of qMSP, the latter can potentially be used for the detection and monitoring of colorectal carcinoma by the detection of the *Vimentin* methylation in clinical samples such as serum and stool (17, 18).

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Received June 27, 2008
Revised November 25, 2008
Accepted December 2, 2008