Aberrant Methylation of the HACE1 Gene is Frequently Detected in Advanced Colorectal Cancer

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Abstract. Background: It has been recently reported that HACE1, the E3 ubiquitin ligase, is epigenetically inactivated in human Wilms’ tumors and HACE1 expression was also down-regulated in colon carcinomas. Materials and Methods: The methylation status of the HACE1 gene was examined in primary carcinomas and the corresponding normal tissues derived from 32 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 HACE1 gene was detected in 9 out of the 32 (28%) primary colon carcinomas, suggesting that the aberrant methylation of HACE1 was frequently observed in colorectal cancer. The clinicopathological data were then correlated with these results. A significant increase was observed in the maximal tumor size of the methylated HACE1 tumors (p=0.0304). Moreover, a trend was shown towards preferentially developing lymph node metastasis in the methylated HACE1 carcinomas (p=0.0612). Conclusion: HACE1 might act as a tumor suppressor in colorectal carcinomas and HACE1 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).

Loss of heterozygosity (LOH) on chromosome 6q has been detected in various human malignancies, including breast, ovarian and prostatic cancer, leukemia, and lymphoma (7-11). Recently, it has been reported that HACE1, the E3 ubiquitin ligase, is epigenetically inactivated in human Wilms’ tumors and maps to a region of chromosome 6q21 (12). Re-expression of HACE1 in human tumor cells directly abrogates tumor growth, whereas down-regulation of HACE1 via siRNA allows non-tumorigenic human cells to form tumors, suggesting that HACE1 is a tumor-suppressor gene in chromosome 6q. Additionally, HACE1 expression was also down-regulated in colon cancer (12). These results prompted us to examine the methylation status of the HACE1 gene in surgically-removed colorectal carcinomas.

In the present study, the methylation status of the HACE1 gene was examined in primary carcinomas and the corresponding normal tissues derived from 32 patients with colorectal cancer, and the correlation between the methylation status and clinicopathological findings was evaluated.

Materials and Methods

Sample collection and DNA preparation. Thirty-two primary tumor and corresponding normal tissue specimens were collected consecutively at Showa University Fujigaoka Hospital, Yokohama, Japan, 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 EpitectBisulfite Kit (QIAGEN, Hilden, Germany). qMSP. The bisulfite-treated DNA was amplified with quantitative methylation-specific PCR (qMSP), that was conducted in a Thermal Cycler Dice® Real Time System TP800 (Takara Bio Inc., Otsu, Japan). Thermocycling was done in a final volume of 25 μL containing 1.0 μL of the DNA sample, 100 nM each of the HACE1 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 deoxynucleotidetriphosphatemixture. The qPCR primer sequences for HACE1 has been described elsewhere (12) and were: HACE1 MS (sense), 5’-GAATGGAAGGTTAATTTCGC-3’, and HACE1 MAS (antisense), 5’-CTAAAACCCTACGTCAACCG-3’. The PCR amplification consisted of 40 cycles (95°C for 5 sec and 60°C for 30 sec) after an initial denaturation step (95°C for 10 sec). 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.

HACE1 methylation scores. The relative amounts of HACE1 methylated DNA in the colorectal carcinomas and the corresponding normal tissues that were normalized to the internal control β-actin were calculated. The HACE1 methylation score in each tissue was defined as follows: relative amount of HACE1 in tumor / relative amount of HACE1 in corresponding normal tissue. HACE1 methylation was positive when the methylation score was more than 1.5.

Statistical analysis. The associations between HACE1 methylation and clinicopathological parameters were analyzed using Chi-square test or Student’s t-test. A p-value <0.05 indicated statistical significance.

Results

Aberrant methylation of the HACE1 gene was detected in 9 out of the 32 (28%) primary colon carcinomas, suggesting that the aberrant methylation of HACE1 was frequently observed in colorectal carcinomas.

The clinicopathological data were correlated with the methylation results. No significant correlations were observed between the presentation of abnormal methylation in the colorectal carcinomas and patient gender or age, tumor extent, tumor site, histology or Dukes stage (Table I). A significant difference was observed in the maximal tumor size (p=0.0304) (Figure 1, Table I). Moreover, a trend was shown towards preferentially developing lymph node metastasis (p=0.0612), thus indicating that HACE1 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). The treatment option for 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 methylation of HACE1 was observed in colorectal cancer. A significant increase was observed in the maximal tumor size in the methylated HACE1 tumors (p=0.0304). Moreover, a trend was shown toward the development of lymph node metastasis in these carcinomas (p=0.0612). We have previously examined the methylation status of the p16 and p14 genes in 86 primary colorectal carcinomas using methylation-specific PCR (MSP) and found a significant difference in maximal tumor size (p=0.022) when patients with both p16 and p14 methylation were compared to other patients (15). We also investigated the methylation status of the CDH13 gene and found a significant difference in histology (p=0.0053) compared the CDH13 methylation of poorly-differentiated colorectal carcinomas was compared to that of 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. A significant difference in histology (p=0.0041) was found when we compared the number of methylated genes of poorly-differentiated colorectal carcinomas was compared to that of differentiated ones (17). Taken together, all the results such as large tumor size and poor differentiation indicated that the methylated status of colorectal carcinomas was significantly correlated with malignant potential.

This study provides solid evidence that could be used in further studies of the molecular mechanism of HACE1 in colorectal cancer and also suggests that HACE1 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 be controlled by inducing the expression of silenced HACE1 using demethylating reagents.

References


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