Down-regulation of *Tip60* Gene as a Potential Marker for the Malignancy of Colorectal Cancer

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Abstract. *Background: Recently, it was shown that the loss of human Tip60 leads to an accumulation of double-strand DNA breaks and is linked to a growing number of cancer types. Patients and Methods: Tip60 expression levels were in examined in 38 colorectal cancer samples using a quantitative real-time polymerase chain reaction. Subsequently, clinicopathological data were correlated with the Tip60 expression score. Results: A down-regulation of the Tip60 gene was observed 5 out of 38 (13%) specimens of primary colorectal cancer. Tip60 down-regulation showed significant correlation with larger tumor size (p=0.0005), poorly differentiated type (p=0.0394), peritoneal dissemination (p=0.0053), distant metastasis (p=0.0394), and higher stage of TNM classification (p=0.0226). Conclusion: These results suggested that Tip60 was more frequently down-regulated in advanced colorectal carcinoma.*

There is now good evidence that a series of genetic alterations in both dominant oncogenes and tumor suppressor genes is involved in the pathogenesis of human colorectal cancer. Activation of oncogenes such as the *ras* gene, and inactivation of tumor suppressor genes such as the *APC* and *p53* genes, have been identified in colorectal cancer (1-3). In addition, we found that several other genes are related to the pathogenesis of this disease (4-8). An investigation of genetic changes is important in order to clarify the tumorigenic pathway of colorectal cancer (9).

The histone acetyl transferase Tip60, which shares many properties with p53, attracts attention (10). Tip60 and p53 proteins are involved in the cellular response to DNA damage, are subjected to proteosomal digestion following mdm2-mediated ubiquitination and accumulate after ultraviolet irradiation. Tip60 complexes have a role in chromatin double-strand break repair; the loss of human Tip60 leads to an accumulation of double-strand DNA breaks and is linked to a growing number of cancer types (11).

In both breast carcinoma and head and neck squamous cell carcinoma, the frequency of *Tip60* loss of heterozygosity (LOH) was higher in the subset of samples with *p53* mutations. Similarly, loss of nuclear Tip60 staining by immunohistochemistry was more frequent in breast tumors with strong (that is, mutant) p53 staining. Thus, down-regulation of Tip60 was most frequently associated with mutant rather than wild-type p53, as would have been expected if Tip60 acted mainly through the p53 pathway (12). Furthermore, *Tip60* mRNA and protein down-regulation was recently described in metastatic prostate cell lines when compared with normal or non-metastatic tumor cells (13).

These reports prompted us to examine the status of *Tip60* gene in colorectal carcinomas we surgically removed. In the present study, we examined the expression of the *Tip60* gene in primary tumors derived from 38 patients with colorectal cancer and evaluated the correlation between the *Tip60* expression and the clinicopathological findings.

Patients and Methods

Patients and tissue specimens. The study group consisted of 38 colorectal cancer patients who underwent surgery at Showa University Fujigaoka Hospital. All tumors and corresponding normal tissues were collected at surgical resection and stored immediately at –80˚C until analysis. All specimens were confirmed histologically. Written informed consent, as required by the Institutional Review Board, was obtained from all patients. The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

RNA preparation and reverse transcription. Total RNA was extracted from colorectal cancer and corresponding normal tissues with guanidinium thiocyanate as described elsewhere (4). The amount of RNA was measured spectrophotometrically by absorbance at 260 nm. First-strand cDNA was generated from RNA as described elsewhere (14).

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Table I. *Clinicopathological features and Tip60 expression in colorectal cancer.*

Clinicopathological feature	Variable	No. of cases	Down-regulation of Tip60 expression		*p*-Value
			+	–	
Gender	Male	22	2	20	0.3877[1]
	Female	16	3	13	
Age (years, mean±S.D.)		38	63.0±11.0	66.7±8.9	0.4002[2]
Maximal tumor size (mm, mean±S.D.)		38	112.0±102.8	42.2±17.7	0.0005[2]
Depth of tumor	<Mt invasion	7	1	6	0.9228[1]
	≥Mt	31	4	27	
Pathological type	Well	37	4	33	0.0394[1]
	Poorly	1	1	0	
Lymph node metastasis	+	15	2	13	0.9794[1]
	–	23	3	20	
Hepatic metastasis	+	5	2	3	0.0969[1]
	–	33	3	30	
Peritoneal dissemination	+	5	3	2	0.0053[1]
	–	33	2	31	
Distant metastasis	+	1	1	0	0.0394[1]
	–	37	4	33	
TNM stage	I, II, III	31	2	29	0.0226[1]
	IV	7	3	4	
Total		38	5	33	

[1]Chi-square test; [2]Student's *t*-test. Mt, muscular tunic; Well, well-differentiated adenocarcinoma; Poorly, poorly-differentiated adenocarcinoma.

Quantitative real-time polymerase chain reaction (QRT-PCR). QRT-PCR was performed in a Thermal Cycler Dice® Real-time System TP800 (Takara Bio Inc., Otsu, Japan) using SYBR Premix Ex Taq II (Takara Bio Inc.). Thermocycling was camed out in a final volume of 25 μl containing 1.0 μ l of the cDNA sample, 100 nM each of the *Tip60* or *β-actin* primers (forward and reverse), and 12.5 μ l of SYBR Premix Ex Taq II (including Taq DNA polymerase, reaction buffer, and deoxynucleotide triphosphate mixture). The *Tip60* primers for quantitative PCR were described elsewhere (12). The PCR amplification consisted of 40 cycles (95˚C for 5 s, 55˚C for 30 s after an initial denaturation step (95˚C for 10 s). 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 mRNA preparations.

Tip60 expression scores. The relative amounts of *Tip60* expressed cDNA in the colorectal tumors that were normalized to the internal control *β-actin* were calculated. The Tip60 expression score in each tissue was defined as follows: relative amount of *Tip60* in tumor/ average relative amount of *Tip60* in all corresponding normal tissues. *Tip60* down-regulation was considered positive when the *Tip60* expression score was less than 0.3.

Statistical analysis. The associations between *Tip60* down-regulation and clinicopathological parameters were analyzed using Chi-square tests or Student's *t*-tests. A *p*-value <0.05 indicated statistical significance.

Results

We analyzed *Tip60* expression levels in 38 colorectal cancer samples using QRT-PCR. A down-regulation of the *Tip60* gene was observed 5 out of 38 (13%) primary colorectal tumors.

Subsequently, clinicopathological data were correlated with the Tip60 expression score. No significant correlations were observed between the down-regulation of *Tip60* expression in colorectal tumor and patient gender, age, depth of tumor invasion, or lymph node metastasis (Table I). We found that *Tip60* down-regulation showed significant correlation with larger tumor size (p=0.0005), poorly differentiated type (p=0.0394), peritoneal dissemination (p=0.0053), distant metastasis (p=0.0394), and higher stage of TNM classification (p=0.0226). These results suggested that *Tip60* was more frequently down-regulated in advanced colorectal carcinomas.

Discussion

Colorectal cancer is the third most common type of cancer and the fourth most frequent cause of death worldwide. More than 945,000 new cases occur every year, and about 492,000 patients die (15, 16). 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.

We previously examined the methylation status of the *CDH13* gene and found significantly more frequent methylation (p=0.0053) comparing poorly differentiated colorectal carcinoma to differentiated carcinoma (17). We also investigated the methylation status of the *HACE1* gene in colorectal cancer. A significant increase was observed in the maximal tumor size in the methylated *HACE1* tumors (p=0.0304) (18). Recently, we examined the methylation status of the *UNC5C* gene in primary carcinomas and the corresponding normal tissues derived from 49 patients with colorectal cancer. A significantly greater proportion of cases with methylated *UNC5C* was found in Dukes' stage C (p=0.0380) than in earlier stages (19). The methylation status of the *Vimentin* gene was also examined in primary carcinomas and the corresponding normal tissues derived from 48 patients with colorectal cancer. A significant difference was observed in age and Dukes' stage (p=0.001 and p=0.034, respectively) (20). Taken together, all the results such as large tumor size and poor differentiation indicate that the methylated status, *i.e.* down-regulation of some genes, was significantly correlated with the malignant potential of colorectal cancer. In the present study, down-regulation of *Tip60* was more frequent in advanced colorectal cancer, suggesting that it would be important in the pathway of colorectal carcinogenesis.

In this study, we demonstrated that *Tip60* expression was down-regulated along with the malignancy of colorectal cancer. Although the population showed here was small, and further examination will be necessary in future, these results suggested that *Tip60* might serve as a new parameter for the prognostic prediction of colorectal cancer.

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