Abstract. Background: Recently, it has been shown that the loss of the human histone acetyl transferase, TIP60, led to an accumulation of double-strand DNA breaks and has been linked to a growing number of cancer types. Materials and Methods: TIP60 expression levels were examined in 46 gastric cancer samples using a quantitative real-time polymerase chain reaction (QRT-PCR). Subsequently, clinicopathological data were correlated with the TIP60 expression score. Results: A down-regulation of the TIP60 gene was observed in 28 out of 46 (61%) specimens of primary gastric cancer. TIP60 down-regulation showed significant correlation with patient age (p=0.0224), depth of tumor invasion (p=0.0401) and lymph node metastasis (p=0.0481). Conclusion: The down-regulation of TIP60 is important for the malignant pathway of gastric carcinogenesis.

Gastric cancer is one of the most common malignancies worldwide (1). Although the prognosis of this cancer has improved in recent years, many patients still die from it. Treatment consists of surgery and subsequent chemotherapy and radiotherapy, and the identification of precise prognostic markers and effective therapeutic targets is pivotal for estimating the malignancy and improving treatment outcomes in gastric cancer. 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 (2). DNA hypermethylation has been particularly well studied and is found in the CpG islands of several genes. Inactivation of human mutl homolog 1 (hMLH1), O-6-methylguanine-DNA methyltransferase (MGMT), tissue inhibitor of metalloproteinase 3 (TIMP-3) and p16 by promoter hypermethylation has been demonstrated (3–6). In addition, we have found that several other genes are related to the pathogenesis of this disease (7–10). There has been substantial interest in attempting to adapt such cancer-associated genetic disorder for clinical use.

The histone acetyl transferase TIP60, which shares many properties with p53 has attracted attention (11). TIP60 and p53 proteins are involved in the cellular response to DNA damage, are subjected to proteosomal digestion following MDM2 (murine double minute)-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 has been linked to a growing number of cancer types (12).

In the present study, the expression of the TIP60 gene in primary tumors derived from patients with gastric cancer was examined and the correlation between the TIP60 expression and the clinicopathological findings was evaluated.

Materials and Methods

Patients and tissue specimens. The study group consisted of 46 gastric cancer patients who underwent surgery at Showa University Fujigaoka Hospital from 2007 to 2009. All the tumors and corresponding normal tissues were collected at surgical resection and stored immediately at –80˚C until analysis. All the specimens were confirmed histologically. Written informed consent, as required by the Institutional Review Board, was obtained from all the patients. The clinicopathological profiles of the patients enrolled in the study are shown in Table I.

RNA preparation and reverse transcription. The total RNA was extracted from the gastric cancer and the corresponding normal tissues with guanidinium thiocyanate as described elsewhere (13). The quantity of RNA was measured spectrophotometrically by absorbance at 260 nm. First-strand cDNA was generated from RNA as described elsewhere (14).

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 carried out in a final volume
of 25 μl containing 1.0 μl of the cDNA sample, 100 nM each of the TIP60 or β-actin primers (forward and reverse) and 12.5 μl of SYBR Premix Ex Taq II (including Taq DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture). The TIP60 primers for quantitative PCR have been described elsewhere (15). The PCR amplification consisted of 40 cycles (95˚C for 5 s, 55˚C for 30 sec 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 levels of TIP60 expressed cDNA in the gastric tumors that were normalized to the internal control β-actin were calculated. The TIP60 expression score in each tissue was defined as follows: relative level of TIP60 in tumor/average relative level of TIP60 in all the corresponding normal tissues. TIP60 down-regulation was considered positive when the TIP60 expression score was less than 0.6.

Statistical analysis. The associations between TIP60 down-regulation and the clinicopathological parameters were analyzed using Chi-square test or Student’s t-test. A p-value <0.05 indicated statistical significance.

Results

Twenty-eight out of the 46 (61%) primary gastric carcinomas presented down-regulation of the TIP60 gene (Table I), suggesting that the down-regulation of TIP60 was frequently observed in the gastric carcinomas.

No significant correlations were observed between the down-regulation of TIP60 expression in the gastric tumor and patient gender, tumor size, pathological type, peritoneal dissemination, distant metastasis or Japanese Classification of Gastric Carcinoma (13th Edition) (16) (Table I). TIP60 down-regulation showed significant correlation with patient age (p=0.0224), depth of tumor invasion (p=0.0401) and lymph node metastasis (p=0.0481). These results suggest that TIP60 was more frequently down-regulated in the advanced gastric carcinomas.

Discussion

TIP60 is a histone acetyl transferase of the MYST (named for members MOZ, YBF2/SAS3, SAS2, and TIP60) family, which modulate DNA-damage response (DDR) signaling (17), and a DDR triggered by oncogenes can counteract tumor progression (18, 19). Recently, some reports have suggested that the failure of an oncogene-induced DDR that was caused by loss of heterozygosity (LOH) at the TIP60 gene, which was a frequent event in the human tumors analyzed, might synergize with p53 mutation towards tumor progression (20-23).

In our previous study, a significant down-regulation of TIP60 was observed in colorectal carcinomas with larger tumor size (p=0.0005), poorly differentiated type (p=0.0394), peritoneal dissemination (p=0.0053), distant metastasis...
References


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