Down-regulation of *Mus81* as a Potential Marker for the Malignancy of Gastric Cancer

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Abstract. *Background: The Mus81 gene encodes a critical endonuclease involved in DNA repair and tumor suppression. In the present study, the role of Mus81 in gastric cancer was explored. Materials and Methods: Mus81 expression in 53 cases of gastric cancer and the corresponding normal tissues was determined by quantitative real-time PCR. The correlations between Mus81 down-regulation and the clinicopathological data were also evaluated. Results: Mus81 expression was significantly lower in gastric cancer tissues than the corresponding normal tissues (p=0.018) and the down-regulation of Mus81 occurred in 51% (27/53) of the gastric carcinomas. More importantly, Mus81 down-regulation correlated significantly to invasion depth (p=0.015) and poorly-differentiated type (p=0.016) of gastric cancer. Conclusion: Mus81 might be a potential marker for the malignancy of gastric cancer.*

Gastric cancer is the fourth most common cancer worldwide and ranks first in incidence rate (age standardized) in Japan (1, 2). Though its prognosis has been improved in recent years, especially in Japan (2), gastric cancer remains the second most common cause of death from cancer in the world (1, 2). Accumulated evidence has indicated that gastric cancer results from various genetic and epigenetic alterations of oncogenes, tumor suppressor genes, cell cycle regulators, cell adhesion molecules and DNA repair genes (3). We have previously reported that the overexpression of the *PAI-1* (Plasminogen Activator Inhibitor-1) gene and the methylation or demethylation of other genes such as *DCC* (Deleted in Colorectal Cancer), *HACE1* (HECT domain and Ankyrin repeat Containing E3 ubiquitin-protein ligase 1) and *MGMT* (Methylguanine DNA Methyltransferase) were closely related to gastric cancer (4-7). However, further investigations to identify genetic alterations as new parameters for estimating the progression of gastric cancer are important in order to improve the success of treatment (8).

The *Mus81* (MMS and UV sensitive isolate number 81) gene, firstly identified in 2000 by Interthal *et al*. (9), encodes a structure-specific DNA endonuclease, which resolves holliday junctions (HJs) by constituting a heterodimer with Eme1 (essential meiotic endonuclease 1) and plays a critical role in the repair of double-strand breaks (DSBs) of DNA and the maintenance of chromosomal integrity (9-11). McPherson *et al*. have shown that 73% of *Mus81*$^{-/-}$ mice and 50% of *Mus81*$^{+/-}$ mice died of various spontaneous tumors such as lymphoma, breast cancer and prostate cancer, implicating *Mus81* as a potent tumor suppressor (12). Moreover, *Mus81* has also been found to interact with *p53* and checkpoint genes such as *Rad51*, which is also a critical DNA repair gene (13, 14). Recently, *Mus81* has been proved to be required for the survival of telomerase-negative cancer cells by the alternative lengthening of telomeres (ALT) pathway and single nucleotide polymorphisms (SNPs) of *Mus81* have been found to be associated with an increased risk of developing breast cancer (15, 16). Though all the above information indicates a potential role of *Mus81* in human malignancies, evidence showing the expression patterns of *Mus81* in human malignancies is still limited (17, 18). Based on these results, *Mus81* might be presumed to play a similar role in other human solid tumors such as gastric cancer.

The role of *Mus81* in gastric cancer remains unknown. Therefore, the expression pattern of *Mus81* in human gastric cancer tissues was investigated and its correlation with the clinicopathological characteristics of gastric cancer patients was explored in the present study.

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Table I. *Clinicopathological characteristics of gastric cancer and correlations with down-regulation of Mus81.*

Clinicopathological characteristics			Down-regulation of *Mus81*		
	Variable	n	Positive	Negative	*P*-Value
Gender	Male	41	23	18	0.165[1]
	Female	12	4	8	
Age (years)	51-82[a]	53	69.2±8.6[b]	70.5±9.6[b]	0.633[2]
Maximal tumor size (mm)	10-150[a]	53	58.9±30.0[b]	56.2±30.0[b]	0.738[2]
Invasion depth	≤Mt	11	2	9	0.015[1]
	>Mt	42	25	17	
Pathological type	Well-Mod	18	5	13	0.016[1]
	Poor	35	22	13	
Lymph node metastasis	Presence	31	17	14	0.501[1]
	Absence	22	10	12	
Peritoneal dissemination	Presence	8	4	4	0.954[1]
	Absence	45	23	22	
Distant metastasis	Presence	9	4	5	0.669[1]
	Absence	44	23	21	
TNM stage	I, II	26	12	14	0.494[1]
	III, IV	27	15	12	

[1]Chi-square test; [2]Student's *t*-test; [a]range (minimum-maximum); [b]mean±S.D; Mt, muscular tunic; Well, Mod, Poor, well, moderately or poorly-differentiated, mucinous or signet ring cell adenocarcinoma, according to General Rules for Gastric Cancer Study (13th ed., 1999). Down-regulation, Mus81 expression in cancer tissue <0.5 of that in corresponding normal tissue.

Patients and Methods

Patients and specimens. Specimens of gastric cancer tissues were obtained from 53 patients who had undergone operation at the Gastroenterological Surgery, Showa University Fujikaoka Hospital from April 2007 to August 2009. Matched cancer and normal specimens from all the patients were collected and frozen in liquid nitrogen immediately after surgery and then stored at –80˚C until analysis. The diagnoses of all the patients were confirmed by histopathological examination. Prior informed consent was obtained from all patients as required by the Institutional Review Board. The clinicopathological profiles of all the patients are shown in Table I.

RNA preparation and reverse transcription. The total RNA was extracted from the gastric cancer tissues and corresponding normal tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions as described previously (19). The quality of total RNA was measured by absorbance at 260nm with a U-2001 spectrophotometer (Hitachi Ltd., Chiyoda, Tokyo, Japan). First-strand cDNA was generated from RNA as described previously (20).

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, Shiga, Japan) using a SYBR Premix Ex Taq II kit (TaKaRa Bio Inc.). Thermocycling was conducted in a final volume of 25 μl containing 1.0 μl of cDNA sample, 0.5 μl of each primers (forward and reverse, 100 nM), 12.5 μl of SYBR Premix Ex Taq II (including Taq DNA polymerase, reacting buffer, and deoxynucleotide triphosphate mixture). The PCR amplification consisted of 40 cycles (95˚C for 5 sec, 55˚C for 30 sec after an initial denaturation step [95˚C for 10 sec]). The *Mus81* primers for PCR were as described previously (18): forward, 5'-TGTGGACATTGGCGAGAC-3'; reverse, 5'-GCTGAGGTTGTGG ACGGA-3'. To correct for differences in both quality and quantity of cDNA samples, *β-actin* gene was used as an internal control and measured in the same samples. All the PCR analyses were performed in duplicate.

Mus81 expression score. The relative expressions of Mus81 in the tissue samples were normalized to the internal control *β-actin* and calculated by the $2^{-\Delta Ct}$ method. The down-regulation of *Mus81* was defined to be positive when the relative expression of *Mus81* in the gastric cancer tissue was less then 0.5 of that in the corresponding normal tissue as described elsewhere (21).

Statistical analysis. The nonparametric Mann-Whitney *U*-test was applied to analyze the Mus81 expression levels in the gastric cancer tissues and the corresponding normal tissues. The associations between *Mus81* down-regulation and the clinicopathological characteristics were analyzed by Chi-square test (categorical data) and Student's *t*-test (continuous data). All the tests were two- sided and $p<0.05$ was considered statistically significant.

Results

Down-regulation of Mus81 in gastric cancer tissues. *Mus81* was detectable in all the gastric cancer tissue specimens and the corresponding normal tissue specimens. However, the relative expression of *Mus81* in the gastric cancer tissues was significantly lower than that in the corresponding normal tissues ($p=0.018$, Figure 1). *Mus81* down-regulation was positive in 51% (27/53) of gastric cancer patients.

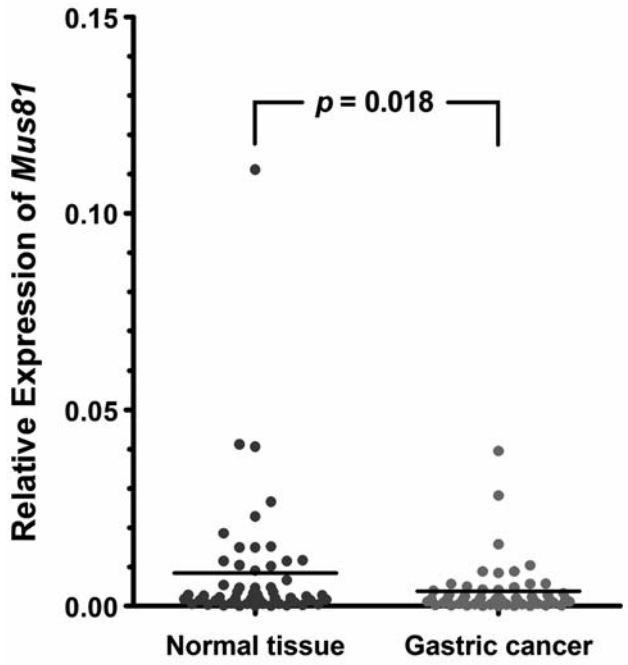

Figure 1. *Mus81 expression levels in gastric cancer tissues and corresponding normal tissues determined by quantitative real-time PCR and normalized to internal control β-actin gene. Bars, represent the means of relative expressions of Mus81 in normal tissues and cancer tissues, respectively.*

Correlations between Mus81 down-regulation and clinicopathological characteristics. The down-regulation of *Mus81* was significantly related to the invasion depth (p=0.015) and poorly-differentiated type (p=0.016) of gastric cancer (Table I). There was no significant association between the down-regulation of *Mus81* and the other clinicopathological characteristics such as gender, age, maximal tumor size, lymph node metastasis, peritoneal dissemination, distant metastasis and TNM stage.

Discussion

In a previous study, we found the aberrant methylation of *MGMT*, a critical DNA repair gene (22), was closely related to the invasion depth, lymph node metastasis and TNM stage of gastric cancer (7), implicating an important role of DNA repair genes in the progression of gastric cancer. Recently, we have documented the decrease of *Mus81* gene in human hepatocellular carcinoma (HCC) tissues and demonstrated its relevance to metastasis and poor prognosis, which provided the first evidence for the expression pattern and role of *Mus81* in human solid tumor (18). In the present study, the expression of *Mus81* was also reduced significantly in the human gastric cancer tissues (p=0.018), and the down-regulation of *Mus81* occurred in more than half (51%, 27 out of 53) of the gastric cancer specimens, which was in agreement with the data from the HCC tissues and further supported the presumption of *Mus81* as a human tumor suppressor (12). More importantly, these results indicated that the down-regulation of *Mus81* might be an important event during the carcinogenesis of gastric cancer.

Recently, Jiang *et al*. have found that the *Mus81* expression significantly decreased in astrocytoma stage III and IV (according to the WHO Grading System), the high malignancy stage of this common type of brain tumor (17). Our previous data also showed that the expression of *Mus81* was obviously reduced in the HCC with a high degree of malignancy (Edmondson-Steiner grade III-IV), multiple tumor nodes and venous invasion (18). In the present study, when the down-regulation of *Mus81* was correlated with the clinicopathological data, the *Mus81* down-regulation was closely related to invasion depth (p=0.015) and poorly-differentiated type (p=0.016) of gastric cancer. Invasion depth and differentiation type are well-accepted parameters of the degree of malignancy of gastric cancer (23, 24), thus the results suggested that *Mus81* might be down-regulated in line with the malignancy of gastric cancer.

In conclusion, *Mus81* expression is significantly down-regulated in human gastric cancer tissues and this decrease is related closely to invasion depth and poorly- differentiated cancer type, which suggests that *Mus81* might serve as a novel biomarker for the malignancy of gastric cancer. However, further studies are still needed to determine how and why *Mus81* is down-regulated in gastric cancer.

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