MACC 1 as a Marker for Peritoneal-disseminated Gastric Carcinoma

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Abstract. *Background: Recently, metastasis associated with the colon cancer 1 (MACC1) gene was identified by genome-wide search for differentially expressed genes in human colon cancer tissues and metastases. Previously, the MACC1 expression levels were examined in colorectal carcinomas and it was found that MACC1 expression showed significant correlation with peritoneal dissemination and higher stage of TNM classification. Materials and Methods: In this study, MACC1 expression levels were analysed in 41 gastric cancer samples using quantitative real-time polymerase chain reaction (QRT-PCR). Results. Distribution of MACC1 expression scores in primary gastric carcinomas was between 0.01 and 4.36 (average±SD was 1.34±1.31). Subsequently, clinicopathological data were correlated with the MACC1 expression. It was found that MACC1 expression showed significant correlation with peritoneal dissemination (p=0.038). Conclusion: These results suggest that MACC1 is more frequently expressed in peritoneal-disseminated gastric carcinomas and may serve as a new parameter for the prognostic prediction of gastric cancer.*

Accumulating evidence indicates that gastric cancer is the result of various genetic and epigenetic alterations of oncogenes, tumour suppressor genes, DNA repair genes, cellcycle regulators and cell adhesion molecules (1). It has been reported that several genes are related to tumourigenic pathway of gastric cancer (2-5). There has been substantial interest in attempting to adapt such cancer-associated genetic disorders for clinical use.

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Recently, Stein *et al*. identified the metastasis associated with colon cancer 1 (*MACC1*) gene by a genome-wide search for differentially expressed genes in human colon cancer tissues and metastases (6). They also reported that *MACC1* mRNA expression in colorectal carcinoma might be an independent prognostic indicator of recurrence and disease-free survival. The hepatocyte growth factor (HGF) – mesenchymal-epithelial transition factor (MET) pathway plays a key role in the carcinogenic pathway (7). MET transmits intracellular signals *via* the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-Akt pathways, which promote migration, invasion, wound healing, and survival, and suppress apoptosis (7-9). The gene encoding the HGF receptor, MET, is a transcriptional target of *MACC1* (6). *MACC1* induces cell proliferation, motility, HGF-triggered scattering in cell cultures, tumour growth, and metastasis in xenograft models (10). These reports prompted an examination of the status of *MACC1* gene in gastric carcinomas that had been surgically removed.

In the present study, the expression of the *MACC1* gene was examined in primary tumours derived from 41 patients with gastric cancer and the correlation between the *MACC1* expression and the clinicopathological findings was evaluated.

Materials and Methods

Patients and tissue specimens. The study group consisted of 41 gastric cancer patients who underwent surgery at Showa University Fujigaoka Hospital, Japan. All tumours 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 gastric cancer and corresponding normal tissue samples with guanidinium thiocyanate as described elsewhere (11). The amount of RNA was measured spectrophotometrically by

Table I. *Clinicopathological features and MACC1 expression in gastric carcinoma.*

Clinicopathological feature	Variable	No. of cases	MACC1 expression score (mean±S.D.)	*p*-Value[a]
Gender	Male	31	1.20±1.25	0.245
	Female	10	1.76±1.45	
Age (years)	<70	16	1.35±1.21	0.958
	>70	25	1.33±1.39	
Maximal tumor size (mm)	<50	15	1.41±1.18	0.811
	>50	26	1.30±1.41	
Histology	Well differentiated	18	1.09±1.18	0.291
	Poorly differentiated	23	1.53±1.39	
Extent of tumor	<Mt	9	0.97±1.22	0.354
	Mt<	32	1.44±1.32	
Lymphatic invasion	+	29	1.43±1.38	0.489
	–	12	1.12±1.12	
Venous invasion	+	24	1.22±1.28	0.501
	–	17	1.50±1.37	
Lymphnode metastasis	+	25	1.35±1.38	0.968
	–	16	1.33±1.22	
Distant metastasis	+	5	0.78±0.63	0.321
	–	36	1.42±1.36	
Peritoneal dissemination	+	3	2.83±2.25	0.038
	–	38	1.22±1.17	
TNM stage	I A, I B, II, III A, III B	34	1.25±1.22	0.379
	IV	7	1.74±1.71	

[a]Student's *t*-test. Mt, Muscular tunic.

absorbance at 260 nm. First-strand cDNA was generated from RNA as described elsewhere (12).

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 *MACC1* 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 *MACC1* primers for quantitative PCR are described elsewhere (6). 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.

MACC1 expression score. The relative amount of *MACC1* in mRNA from gastric carcinomas (T) and corresponding normal tissues (N) that were normalized to an internal control (β-actin mRNA) was calculated. The *MACC1* expression score in each tissue was defined as follows: relative amount of T/relative amount of N that was the average value of normal tissue samples.

Statistical analysis. The associations between *MACC1* expression and clinicopathological parameters were analyzed using Student's *t*-tests. A *p*-value <0.05 indicated statistical significance.

Results

MACC1 expression levels were analyzed in 41 gastric cancer samples using QRT-PCR. Table I shows the distribution of *MACC1* expression score in primary gastric carcinomas, which was between 0.01 and 4.36 (the average±SD was 1.34±1.31) (Figure 1).

Subsequently, clinicopathological data were correlated with the *MACC1* expression. No significant correlations were observed between the *MACC1* expression in gastric carcinoma and patient gender, age, maximal tumour size, histology, extent of tumour, lymphatic invasion, venous invasion, lymph node metastasis, distant metastasis, or TNM stage (Table I). It was found that *MACC1* expression showed significant correlation with peritoneal dissemination (p=0.038) (Figure 2). These results suggested that *MACC1* was more frequently expressed in peritoneal-disseminated gastric carcinomas.

Discussion

Gastric cancer is one of the most common cancers worldwide, ranking fourth in overall frequency and accounting for over 650,000 deaths annually. The mortality of gastric cancer is exceeded only by lung cancer (13).

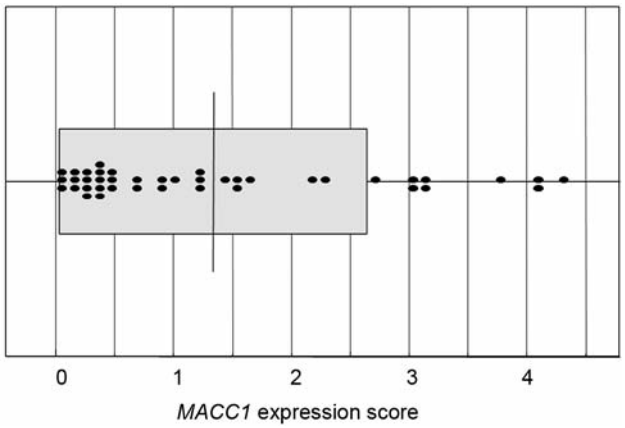

Figure 1. *MACC1 expression scores were distributed between 0.01 and 4.36 (the average±SD was 1.34±1.31).*

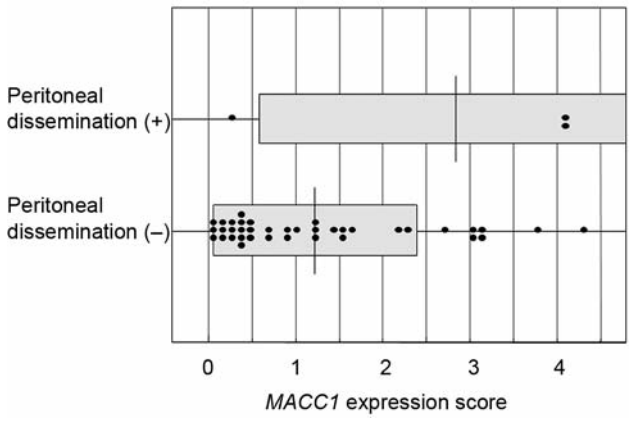

Figure 2. *MACC1 expression scores according to peritoneal dissemination. A significant increase in MACC1 expression scores was observed in cases with peritoneal dissemination (2.83±2.25) compared to those without (1.22±1.17) (p=0.038).*

Treatment of this fatal cancer is surgery and 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.

Stein *et al*. reported that *MACC1* mRNA expression in colorectal carcinoma might be an independent prognostic indicator of recurrence and disease-free survival (10). The survival rate for patients with colorectal carcinomas with low *MACC1* mRNA expression was 80% compared to 15% for those with high *MACC1* mRNA. Arlt *et al*. also reported that *MACC1* expression in colorectal carcinoma was significantly higher in primary tumours that later developed distant metastases, compared to those that did not metastasize within a 10-year follow-up period (14). Therefore, *MACC1* was a marker for metachronously metastasizing colorectal carcinomas, which was linked to a shorter metastasis-free survival. Previously, the *MACC1* expression levels was examined in primary carcinomas and the corresponding normal tissues derived from 52 patients with colorectal cancer using QRT-PCR and the correlation between the expression levels and the clinicopathological findings was evaluated (15). Significant correlations were observed between *MACC1* expression in colorectal carcinoma and high TNM stage or peritoneal dissemination. In the present study, *MACC1* expression in gastric carcinoma was compared with clinicopathological features and significant correlations between *MACC1* expression and peritoneal dissemination were demonstrated. Taken together, these results suggested that *MACC1* expression might be an indicator for peritoneal dissemination of digestive tract carcinoma.

This study demonstrated that *MACC1* expression was up-regulated along with the malignancy of gastric cancer such as peritoneal dissemination. Although the population used in this study was small, and further examination will be necessary in future, these results suggest that *MACC1* may serve as a new parameter for the prognostic prediction of gastric cancer.

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