

MACC1 as a Marker for Advanced Colorectal Carcinoma

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Abstract. *Background:* Recently, Stein *et al.* identified the metastasis-associated in colon cancer 1 (*MACC1*) gene by genome-wide search for differentially expressed genes in human colon cancer tissues and metastases. *Patients and Methods:* We analyzed *MACC1* expression levels in 52 colorectal cancer samples using quantitative real-time polymerase chain reaction (QRT-PCR). *Results:* We found that *MACC1* expression showed significant correlation with peritoneal dissemination ($p=0.042$) and higher stage of TNM classification ($p=0.007$). *Conclusion:* These results suggest that *MACC1* is more frequently expressed in advanced colorectal carcinomas.

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).

Recently, Stein *et al.* identified the metastasis-associated in colon cancer 1 (*MACC1*) gene by genome-wide search for differentially expressed genes in human colon cancer tissues and metastases (10). The hepatocyte growth factor (HGF)-mesenchymal epithelial transition factor (MET) pathway plays a key part in carcinogenic pathway (11). 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 (11-13). The gene encoding the HGF receptor, MET, is a transcriptional target of *MACC1* (10). *MACC1* induces cell proliferation, motility, HGF-triggered scattering in cell cultures, tumor growth, and metastasis in xenograft models (14). These reports prompted us to examine the status of *MACC1* gene in colorectal carcinomas we surgically removed.

In the present study, we examined the expression of the *MACC1* gene in primary tumors derived from 52 patients with colorectal cancer and evaluated the correlation between the *MACC1* expression and the clinicopathological findings.

Patients and Methods

Patients and tissue specimens. The study group consisted of 52 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 (15).

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*

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

Clinicopathological feature	Variable	No. of cases	<i>MACC1</i> expression score (mean±SD)	<i>p</i> -Value ^a
Gender	Male	28	3.51±3.75	0.267
	Female	24	2.14±2.67	
Age (years)	<70	24	2.56±3.02	0.531
	>70	28	3.15±3.62	
Maximal tumor size (mm)	<45	25	2.56±2.93	0.761
	>45	27	2.85±3.63	
Histology	Well-differentiated	49	2.83±3.33	0.695
	Poorly differentiated	3	3.62±4.19	
Extent of tumor	<Mt	14	2.91±2.98	0.962
	<Mt	38	2.86±3.49	
Lymph node metastasis	+	23	2.94±3.95	0.861
	–	29	2.80±2.83	
Liver metastasis	+	7	4.77±4.91	0.107
	–	45	2.58±2.99	
Peritoneal dissemination	+	5	5.75±4.58	0.042
	–	47	2.57±3.09	
TNM stage	I, II, III	43	2.32±2.64	0.007
	IV	9	5.55±4.97	

^aStudent's *t*-test; Mt, muscular tunic.

primers for quantitative PCR are described elsewhere (10). 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.** We calculated the relative amount of *MACC1* in mRNA from colorectal carcinomas (T) and corresponding normal tissues (N) that were normalized to an internal control (*β-actin* mRNA). The *MACC1* expression score in each tissue was defined as follows: relative amount of T/relative amount of N that was average value of all 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

We analyzed *MACC1* expression levels in 52 colorectal cancer samples using QRT-PCR. Table I shows the distribution of *MACC1* expression score in primary colorectal carcinomas, which was between 0.11 and 14.26 (the average was 2.88±3.34) (Figure 1).

Subsequently, clinicopathological data were correlated with the *MACC1* expression. No significant correlations were observed between the *MACC1* expression in colorectal carcinoma and patient gender, age, maximal tumor size, histology, extent of tumor, lymph node metastasis, or liver metastasis (Table I). We found that *MACC1* expression showed significant correlation with peritoneal dissemination

(*p*=0.042), and higher stage of TNM classification (*p*=0.007). These results suggest that *MACC1* is more frequently expressed 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 (16, 17). 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.

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 tumors that later developed distant metastases compared to those that did not metastasize within a 10-year follow-up period (18). Therefore, *MACC1* appears to be a marker for metachronously metastasizing colorectal carcinomas, linked to a shorter metastasis-free survival. In the present study, significant correlations were observed between *MACC1* expression in colorectal carcinoma and TNM stage and peritoneal dissemination. Thus, our results suggest that *MACC1* expression is a prognostic indicator of metastasis formation.

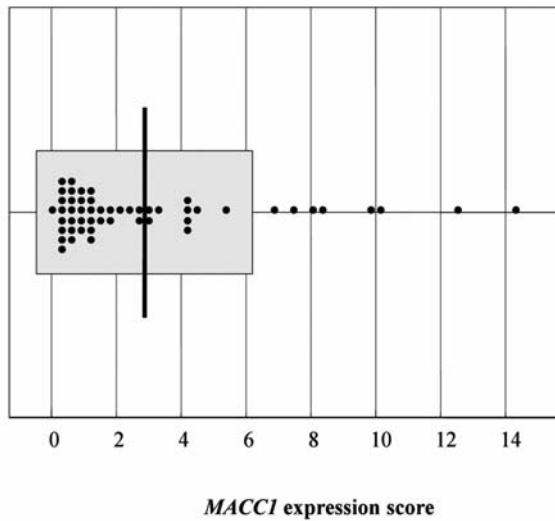


Figure 1. *MACC1* expression scores were distributed between 0.11 and 14.26 (the average was 2.88 ± 3.34).

We demonstrated that *MACC1* expression was up-regulated according to the malignancy of colorectal cancer. Although the population study here was small, and further examination will be necessary, these results suggest that *MACC1* might serve as a new parameter for the prognostic prediction of colorectal cancer.

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References

- 1 Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb AJ and Vogelstein B: Prevalence of *ras* gene mutations in human colorectal cancers. *Nature* 327: 293-297, 1987.
- 2 Baker SJ, Markowitz S, Fearon ER, Willson JK and Vogelstein B: Suppression of human colorectal carcinoma cell growth by wild-type *p53*. *Science* 249: 912-915, 1990.
- 3 Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, Utsunomiya J, Baba S and Hedge P: Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 253: 665-669, 1991.
- 4 Hibi K, Nakamura H, Hirai A, Fujikake Y, Kasai Y, Akiyama S, Ito K and Takagi H: Loss of H19 imprinting in esophageal cancer. *Cancer Res* 56: 480-482, 1996.
- 5 Hibi K, Taguchi M, Nakamura H, Hirai A, Fujikake Y, Matsui T, Kasai Y, Akiyama S, Ito K and Takagi H: Alternative splicing of the *FHIT* gene in colorectal cancers. *Jpn J Cancer Res* 88: 385-388, 1997.
- 6 Hibi K, Nakayama H, Koike M, Kasai Y, Ito K, Akiyama S and Nakao A: Colorectal cancers with both *p16* and *p14* methylation show invasive characteristics. *Jpn J Cancer Res* 93: 883-887, 2002.

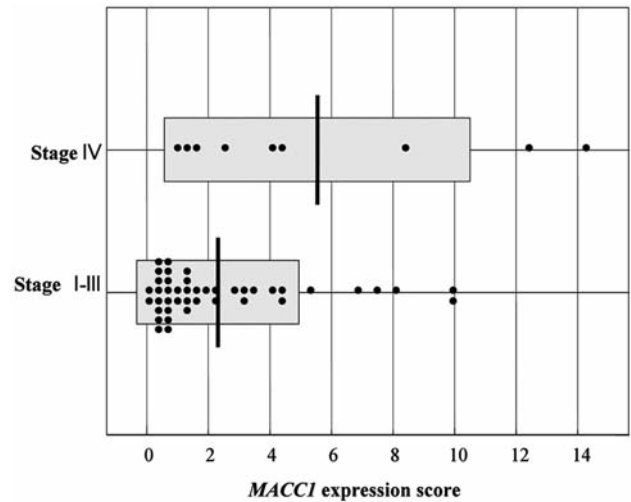


Figure 2. *MACC1* expression scores according to TNM stage. A significant increase in expression scores was observed in stage IV colorectal carcinomas (5.55 ± 4.97) compared to stage I, II and III colorectal carcinomas (2.32 ± 2.64) ($p=0.007$).

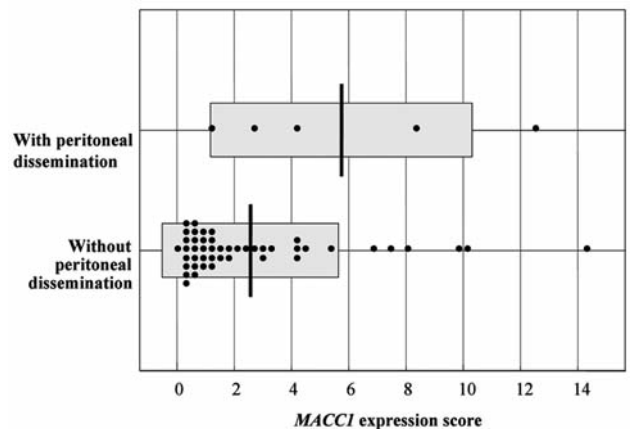


Figure 3. *MACC1* expression scores according to peritoneal dissemination. A significant increase in expression scores was observed in cases with peritoneal dissemination (5.75 ± 4.58) compared to those without (2.57 ± 3.09) ($p=0.042$).

- 7 Hibi K, Nakayama H, Kadera Y, Ito K, Akiyama S and Nakao A: CDH13 promoter region is specifically methylated in poorly differentiated colorectal cancer. *Br J Cancer* 90: 1030-1033, 2004.
- 8 Yamazaki T, Hibi K, Takase T, Tezel E, Nakayama H, Kasai Y, Ito K, Akiyama S, Nagasaka T and Nakao A: PGP9.5 as a marker for invasive colorectal cancer. *Clin Cancer Res* 8: 192-195, 2002.
- 9 Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL: Genetic alterations during colorectal tumor development. *N Engl J Med* 319: 525-532, 1988.

- 10 Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, Birchmeier W and Schlag PM: *MACC1*, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. *Nat Med* 15: 59-67, 2009.
- 11 Sattler M and Salgia R: C-MET and hepatocyte growth factor: potential as novel targets in cancer therapy. *Curr Oncol Rep* 9: 102-108, 2007.
- 12 Bottaro, DP, Rubin JS, Faletto DL, Chan AM, Kniecik TE, Vande Woude GF and Aaronson SA: Identification of the hepatocyte growth factor receptor as the *C-MET* proto-oncogene product. *Science* 251: 802-804, 1991.
- 13 Chmielowiec J, Borowiak M, Morkel M, Stradal T, Munz B, Werner S, Wehland J, Birchmeier C and Birchmeier W: C-MET is essential for wound healing in the skin. *J Cell Biol* 177: 151-162, 2007.
- 14 Stein U, Dahlmann M and Waither W: *MACC1*—more than metastasis; Facts and predictions about a novel gene. *J Mol Med* 88: 11-18, 2009.
- 15 Hibi K, Takahashi T, Sekido Y, Ueda R, Hida, T, Ariyoshi Y and Takagi H: Coexpression of the stem cell factor and the *C-KIT* genes in small cell lung cancer. *Oncogene* 6: 2291-2296, 1991.
- 16 Weitz J, Koch M, Debus J, Höhler T, Galle PR and Büchler MW: Colorectal cancer. *Lancet* 365: 153-165, 2005.
- 17 de Noo ME, Tollenaar RA, Deelder AM and Bouwman LH: Current status and prospects of clinical proteomics studies on detection of colorectal cancer: hopes and fears. *World J Gastroenterol* 12: 6594-6601, 2006.
- 18 Arlt F and Stein U: Colon cancer metastasis: *MACC1* and *MET* as metastatic pacemakers. *Int J Biochem Cell Biol* 41: 2356-2359, 2009.

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