MACC 1 as a Marker for Vascular Invasive Hepatocellular Carcinoma

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Abstract. *Background: Recently, metastasis associated with colon cancer 1 (MACC1) gene was identified by genome-wide search for differentially expressed genes in human colon cancer tissues and metastases. Previously, MACC1 expression was examined in colorectal carcinomas and gastric carcinomas and was found to show significant correlation with peritoneal dissemination. Patients and Methods: In this study, MACC1 expression was analyzed in 60 samples (tumor and the surrounding non-tumorous liver tissue) collected from 30 patients with hepatocellular carcinoma (HCC) using quantitative real-time polymerase chain reaction (QRT-PCR). Results. MACC1 expression score (tumor:normal) in primary HCC was between 0.01 and 4.59 (average±SD=0.68±0.94). Subsequently, clinicopathological data were correlated with the MACC1 expression. It was found that MACC1 expression showed significant correlation with vascular invasion and α-fetoprotein level (p=0.034, p=0.0098, respectively). Conclusion: These results suggest that MACC1 is more frequently expressed in vascular invasive HCC and may serve as a new parameter for the prognostic prediction of HCC.*

Predisposing factors for hepatocellular carcinoma (HCC) include chronic hepatitis B and C virus infections, exposure to aflatoxin B1, chronic alcohol consumption, or any hepatic disease associated with cirrhosis (1). Nevertheless, the molecular pathogenesis of HCC remains largely unknown. Recognized abnormalities in HCC include aberrant signaling through the mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K)/AKT and mTOR pathways, and overactivation of several growth factor receptors (1-5). The number of genes that have been identified to date in the tumorigenic pathway is far fewer for HCC than for colon cancer or gastric cancer, which can be attributed to fewer attempts to perform genomic analysis compared with colon cancer or gastric cancer. Accordingly, an investigation of genetic change is important in clarifying the tumorigenetic pathway of HCC.

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 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 HCC that had been surgically removed.

In the present study, the expression of the *MACC1* gene was examined in 60 samples, tumor and the surrounding non-tumorous liver tissue, collected from 30 patients with HCC and the correlation between the *MACC1* expression and the clinicopathological findings was evaluated.

Patients and Methods

Patients and tissue specimens. The study group consisted of 30 HCC patients who underwent surgery at Showa University Fujigaoka Hospital, Japan. 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.

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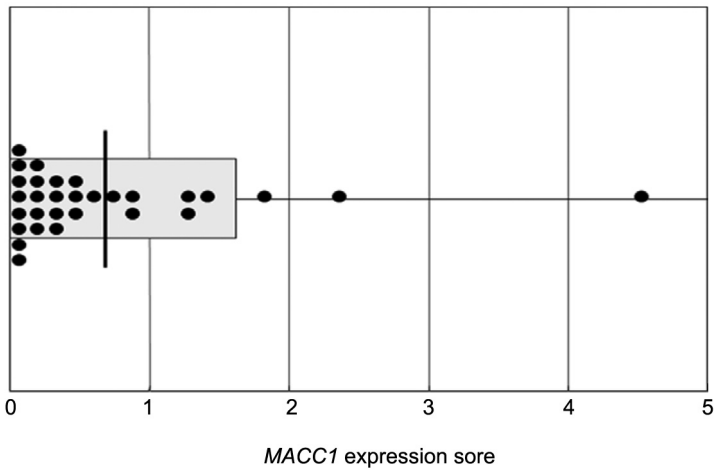

Figure 1. *MACC1 expression scores were distributed between 0.01 and 4.59 (average±SD=0.68±0.94).*

RNA preparation and reverse transcription. Total RNA was extracted from HCC and the surrounding non-tumorous liver tissue samples with guanidinium thiocyanate as described elsewhere (11). The amount of RNA was measured spectrophotometrically by 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 *ACTB* 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, *ACTB* 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 HCC (T) and the surrounding non-tumorous liver tissues (N) that were normalized to *ACTB* mRNA) was calculated. The *MACC1* expression score in each tissue was defined as: relative amount of *MACC1* in T/relative amount of *MACC1* in N.

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 60 samples (tumor and the surrounding non-tumorous liver tissue) collected from 30 patients with HCC using QRT-PCR. Figure 1 shows the distribution of *MACC1* expression score in primary HCCs, which was between 0.01 and 4.59 (average±SD=0.68±0.94) (Figure 1).

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

Clinicopatho-logical feature	Variable	No. of cases	*MACC1* expression score (mean±S.D.)	p-Value[a]
Gender	Male	24	0.68±1.01	0.982
	Female	6	0.69±0.67	
Age (years)	<70	13	0.52±0.58	0.418
	>70	17	0.81±1.15	
Background liver status	With cirrhosis	17	0.46±0.51	0.149
	Without cirrhosis	13	0.97±1.29	
Maximal tumor size (mm)	<50	24	0.52±0.62	0.06
	>50	6	1.33±1.66	
Tumor number	Single	15	0.82±1.25	0.434
	Multiple	15	0.55±0.49	
Histology	Well, Mod	22	0.77±1.06	0.399
	Poor	8	0.44±0.44	
Capsule formation	+	17	0.49±0.61	0.217
	–	13	0.93±1.24	
Vascular invasion	+	4	1.60±2.06	0.034
	–	26	0.54±0.61	
Intrahepatic metastasis	+	1	0.38	
	–	29	0.69±0.96	
α-Fetoprotein (ng/ml)	<100	21	0.40±0.45	0.0098
	>100	9	1.34±1.42	
TNM stage	I	13	0.66±0.77	0.907
	II, III, IV	17	0.70±1.08	

[a]Student's *t*-test; well, well-differentiated hepatocellular carcinoma; mod, moderately differentiated hepatocellular carcinoma; poor, poorly differentiated hepatocellular carcinoma.

Subsequently, clinicopathological data were correlated with the *MACC1* expression. No significant correlations were observed between the *MACC1* expression in HCC and patient gender, age, background liver status, tumor number,

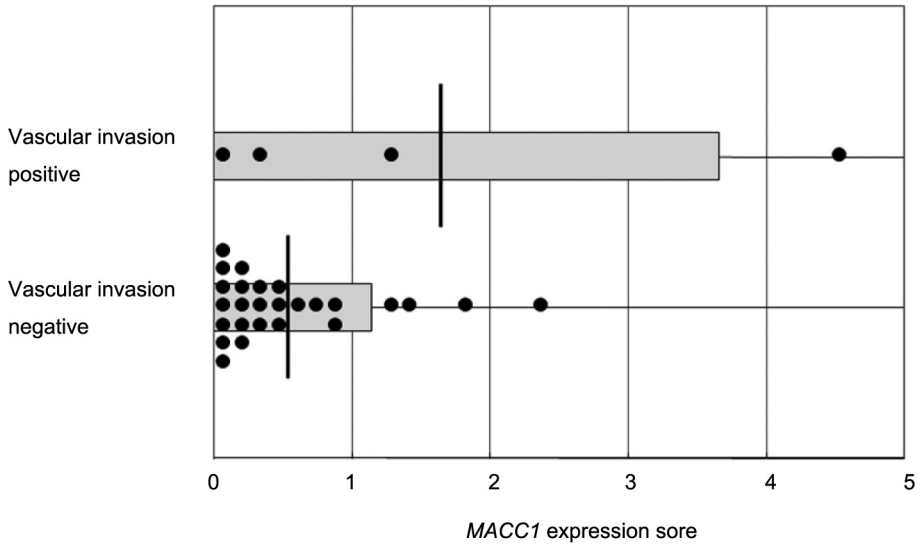

Figure 2. *MACC1 expression scores according to vascular invasion. A significant increase in MACC1 expression scores was observed in cases with vascular invasion (1.60±2.06) compared to those without (0.54±0.61) (p=0.034).*

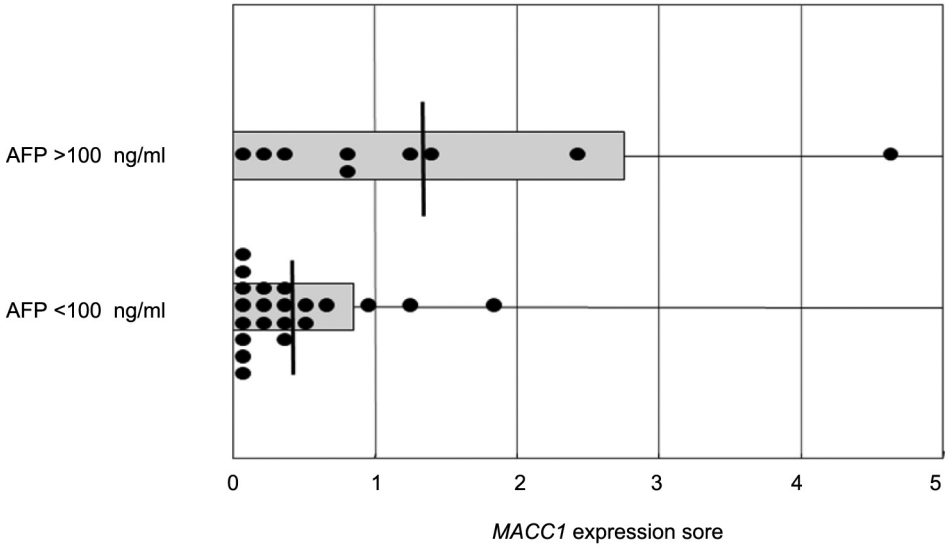

Figure 3. *MACC1 expression scores according to AFP level. A significant increase in MACC1 expression scores was observed with higher AFP level (p=0.0098).*

maximal tumour size, histology, capsule formation, and TNM stage (Table I). It was found that *MACC1* expression showed a significant correlation with vascular invasion (p=0.034) (Figure 2) and α-fetoprotein (AFP) level (p=0.0098) (Figure 3). These results suggest that *MACC1* is more frequently expressed in vascular-invasive HCC.

Discussion

HCC is the fifth most common solid malignancy worldwide and causes more than 600,000 deaths annually. Prognosis remains extremely poor, with a 5-year survival rate of less than 5% without treatment. Currently, the only curative therapeutic option for early-stage HCC is surgical intervention, including percutaneous ablation, hepatic resection, and liver transplantation. However, only 12% of diagnosed HCC patients are deemed eligible for curative therapy (13). 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 expression. Arlt and Stein 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 (14). Therefore, *MACC1* was a marker for metachronously metastasizing colorectal carcinoma, which was linked to a shorter metastasis-free survival. Previously, the *MACC1* expression level 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, and peritoneal dissemination. In addition, the *MACC1* expression levels were examined in 41 gastric carcinomas and it was found that *MACC1* expression also showed significant correlation with peritoneal dissemination (16). In the present study, *MACC1* expression in HCC was compared with clinicopathological features and significant correlations between *MACC1* expression and vascular invasion and AFP level were demonstrated. These results suggest that *MACC1* expression might be an indicator for vascular invasion of HCC. In addition, *MACC1* expression could be used as a tumor marker for the recurrence of HCC because the significance of the increased AFP measurement may lie in the early recognition of tumor recurrence of HCC after treatment (17).

This study demonstrated that *MACC1* expression was up-regulated along with a measure of the malignancy of HCC, namely vascular invasion. Although the population used in this study was small, and further examination is necessary, these results suggest that *MACC1* may serve as a new parameter for the prognostic prediction of HCC.

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