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Original Article

Increased expression of metastasis-associated in colon cancer-1 in renal cell carcinoma is associated with poor prognosis

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Abstract: Metastasis-associated in colon cancer-1 (MACC1) expression in tumor specimens is an independent prognostic indicator of metastasis, which has recently gained considerable attention in cancer research, due to its overexpression in several types of carcinoma. However, MACC1 expression patterns and its possible role in renal cell carcinoma remain unknown. This study aimed to investigate MACC1 expression in renal cell carcinoma via immunohistochemical analysis and determine the relationship between MACC1 expression and cancer prognosis. Positive MACC1 expression was found to significantly correlate with distant metastasis and TNM stage ($P < 0.05$). A Kaplan-Meier survival analysis revealed that patients with higher MACC1 expression had a significantly lower disease-free rate ($P < 0.05$). These results indicate that MACC1 expression is significantly associated with prognosis in patients with renal cell carcinoma. To the best of our knowledge, this is the first study on the significance of MACC1 as a prognostic marker in renal cell carcinoma. MACC1 expression may be a useful target for the development of new therapeutic approaches, including molecular targeted therapeutic agents, for renal cell carcinoma.

Keywords: MACC1, renal cell carcinoma, prognosis, biomarker

Introduction

Renal cell carcinoma (RCC) is one of the most common genitourinary tumors, with more than 84,400 new cases and approximately 34,700 related deaths recorded per year in the European Union [1]. Numerous renal masses remain asymptomatic until distant metastasis is detected at the time of diagnosis. Although some environmental and genetic factors have been found to be associated with RCC, the molecular mechanisms involved in the initiation and progression of RCC remain unclear. Therefore, a greater understanding of metastasis is required to develop better and more effective treatments.

Metastasis-associated in colon cancer-1 (MACC1) has been reported as a newly identified key regulator of the hepatocyte growth factor (HGF)-MET signaling pathway that can predict colon cancer invasiveness and metastasis [2]. The HGF-MET signaling pathway plays vital roles in angiogenesis, cell motility, cellular growth, invasiveness, and metastasis. Recent research has suggested that MET, an HGF receptor, is a transcriptional target of MACC1 [3, 4]. Overexpression of *MET*, which encodes the MET protein, can result in oncogenesis and cancer metastasis, as reported by Stein.

Although MACC1 expression has been extensively studied in several somatic cancers, including colon cancer, gastric cancer, lung cancer, and hepatocellular carcinoma [5-7], no previous studies of MACC1 expression in RCC have been reported. In the present study, MACC1 expression was detected in renal cell carcinoma tissues via immunohistochemistry, and the associations of MACC1 expression with clinicopathological characteristics and clinical patient outcomes were analyzed.

Materials and methods

Patients and tissue specimens

This study included a total of 112 paraffin-embedded RCC samples that had been histo-

Table 1. Characteristics of patients with renal cell carcinoma

Characteristics	Value
Total [No.]	112 (100)
Male (%)	77 (68.8)
Female (%)	35 (31.2)
Stage Classification [No.]	
T1 (%)	33 (29.5)
T2 (%)	40 (35.7)
T3 (%)	32 (28.6)
T4 (%)	7 (6.2)
Metastasis [No.]	
Negative (%)	67 (59.8)
Positive (%)	45 (40.2)
Mean follow-up (years)	3.5 ± 0.3

pathologically and clinically diagnosed at the Department of Urology, Zhujiang Hospital, Southern Medical University from January 2006 to December 2008. None of the patients had undergone chemotherapy and radiotherapy before operative treatment. All patients were classified according to the 1997 Union for International Cancer Control (UICC) TNM classification for pathologic staging and the 2002 American Joint Committee on Cancer (AJCC) staging system.

Written informed consent was obtained from all patients, and the study was approved by the institutional review board and ethical committee of Zhujiang Hospital.

Immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) tissue sections (4.5 ㎜) were de-paraffinized with xylene, rehydrated through graded alcohol washes, and subjected to endogenous peroxidase blocking with 3% H_2O_2 and antigen retrieval via heat treatment for 30 min in 10 mmol/L citrate buffer (pH 6.0). The slides were incubated for 10 min in 10% normal goat serum and subsequently incubated overnight at 4°C with primary polyclonal antibodies against MACC1 (1/200 dilution; PAB16755, Abnova, Taipei, Taiwan). Following primary antibody incubation, the tissue sections were incubated at room temperature for 2 h with the appropriate horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1/1000; Cell Signaling Technology, Beverly, MA, USA), followed by 3, 3'-diaminobenzidine (DAB) staining [8, 9]. All sections were counterstained with hematoxylin, dehydrated, mounted, and observed via light microscopy.

The immunostained sections were evaluated by two independent pathologists or urologists who were blinded to the clinicopathological data and clinical patient outcomes. MACC1 expression was assessed as follows: the numbers of tumor cells exhibiting immunoreactivity on intracellular organelles (positive staining) and negatively stained cells were counted in 10 representative microscopic fields, and the percentage of positive cells was calculated. Each tumor specimen was thus classified as negative (< 1%) or positive (> 1%). The staining intensities of the specimens were further classified into Weak, Moderate, and Strong categories as previously reported [9]; this was determined according to the most frequent staining intensity of 10 representative microscopic fields. The assessments (negative/positive; weak, moderate, or strong) made by the two evaluators were compared; to resolve discrepancies, the sections were reassessed by both researchers until consensus could be reached.

Statistical analysis

All statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard errors (SE). Fisher's exact test was used to evaluate the associations between MACC1 expression and clinicopathological parameters. The postoperative disease-free rate among patients with RCC was estimated using the Kaplan-Meier method. A P value of < 0.05 was considered statistically significant.

Result

Patient characteristics

The characteristics of the 112 included patients with RCC are listed in **Table 1**. The average age was 59.24 years (range: 29-86 years), and the male: female ratio was 2.20:1. The cases were classified according to pathological stage as follows: T1, N = 33; T2, N = 40; T3, N = 32; and T4, N = 7. Metastasis of RCC occurred in 45 (40.2%) of 112 patients.

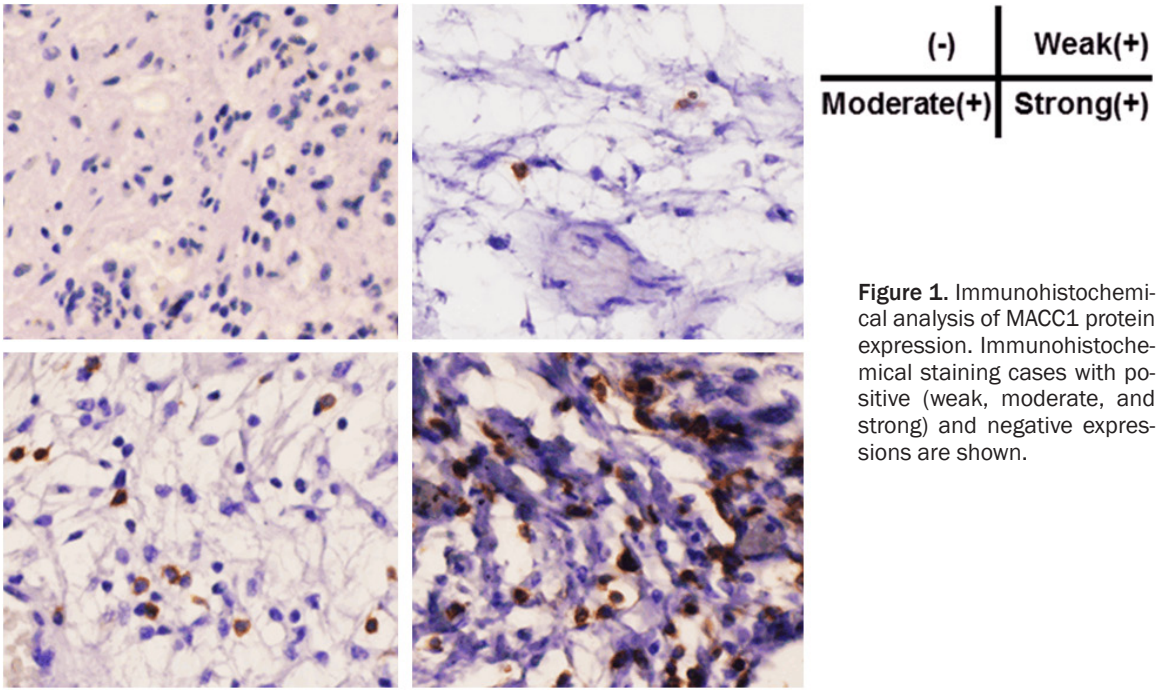

Figure 1. Immunohistochemical analysis of MACC1 protein expression. Immunohistochemical staining cases with positive (weak, moderate, and strong) and negative expressions are shown.

Table 2. Relationship between MACC1 expression and clinicopathological variables in renal cell carcinoma

Characteristics	Expression of MACC1 Protein		*p* value
	Negative (%)	Positive (%)	
Total [No.]	39 (34.8)	73 (65.2)	0.073
Male (%)	31 (40.3)	46 (59.7)	
Female (%)	8 (22.9)	27 (77.1)	
Stage Classification [No.]			0.012
T1 (%)	14 (42.4)	19 (57.6)	
T2 (%)	15 (37.5)	25 (62.5)	
T3 (%)	10 (31.3)	22 (68.7)	
T4 (%)	0 (0.0)	7 (100.0)	
Metastasis [No.]			0.007
Negative (%)	30 (44.8)	37 (52.2)	
Positive (%)	9 (20.0)	36 (80.0)	

MACC1 expression and cellular distribution

The expression and cellular distribution of MACC1 protein was determined in 112 paraffin-embedded RCC tissue sections by immunohistochemical staining. Several adjacent normal renal tissue specimens had been included in the paraffin blocks for comparison. Specific MACC1 signals were mainly localized in the cytoplasm and were indicated by brown staining (**Figure 1**). MACC1 protein expression was positive in 73 (65.2%) of the specimens from patients with RCC and exhibited the indicated staining patterns [e.g., negative or positive (weak, moderate or strong)].

Association of MACC1 expression with clinicopathological parameters of RCC

MACC1 immunoreactivity correlated positively with some of the investigated clinicopathological parameters, as shown in **Table 2**. Significant associations were observed between MACC1 expression, cancer stage, and metastasis of RCC [T1: 19/33, 57.6%; T2: 25/40, 62.5%; T3: 22/32, 68.7%; T4: 7/7: 100.0% (P = 0.012); metastasis negative: 37/67, 52.2%; and metastasis positive: 36/45, 80.0% (P = 0.007)].

Correlation between MACC1 expression and the disease-free rate among patients with RCC

The postoperative disease-free rate among patients with RCC was analyzed using the Kaplan-Meier method. The disease-free rate was determined from the date of surgery to the time of detection of RCC metastasis or the last follow-up. The 5-year overall disease-free rates of patients with RCC were 88.7% and 21.5% for the MACC1 negative and positive populations, respectively (**Figure 2A**). The association

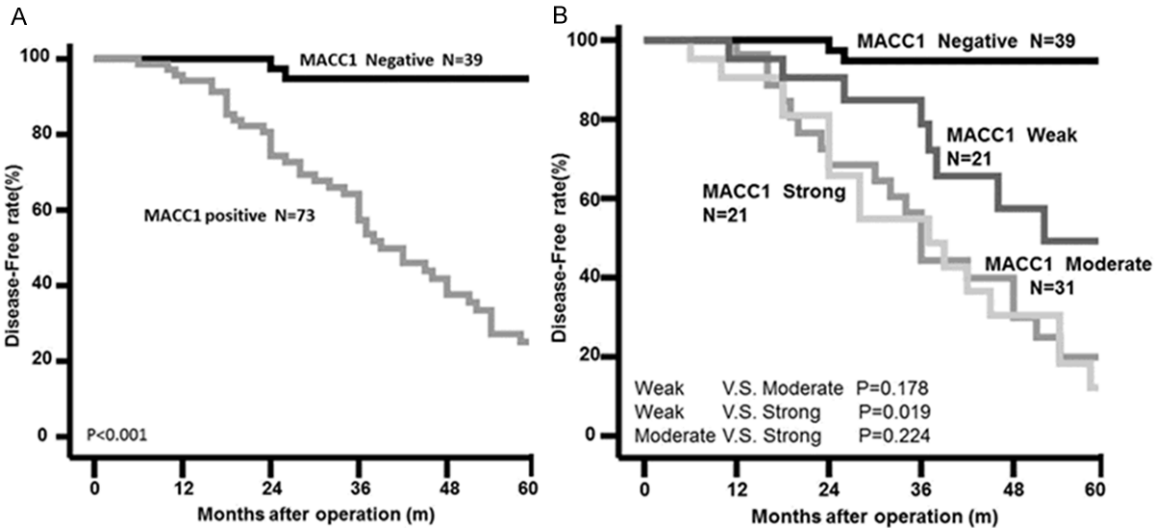

Figure 2. Survival analysis of renal cell carcinoma patients (n = 112) using the Kaplan-Meier method. Kaplan-Meier curves for cancer-associated disease-free rate in MACC1 negative and positive populations (A), and according to the intensity of MACC1 staining (B), are shown. The statistical significance was calculated by the log-rank test.

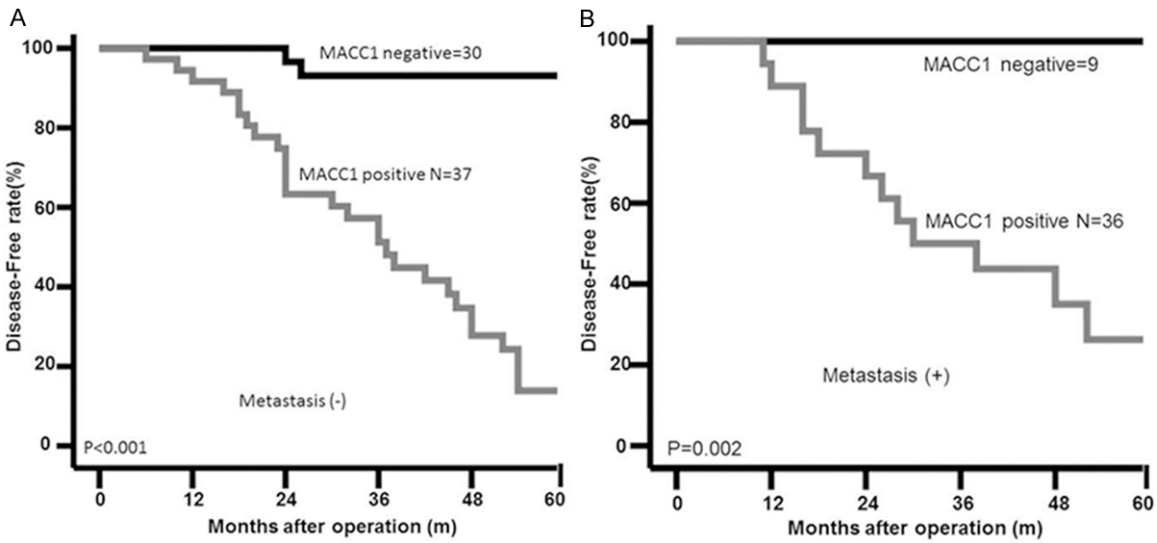

Figure 3. An analysis of renal cell carcinoma-associated disease after treatment was performed using the Kaplan-Meier method. Kaplan-Meier curves for cancer-associated disease-free rate, according to the MACC1 expression in metastasis (A) and metastasis-free (B) tumors, at diagnosis and after treatment are shown. The statistical significance was calculated by the log-rank tests.

between MACC1 expression intensity and the disease-free rate was further analyzed during the follow-up period, and association was revealed between strong MACC1 staining intensity and poor disease-free rate (**Figure 2B**). Log-rank tests revealed statistically significant differences between the negative/weak, weak/moderate, and weak/strong staining categories.

The impacts of tumor stage and metastasis on the disease-free rate were also investigated. The tumor stage and metastasis both correlated significantly with a poor disease-free rate (data not shown). A significant association was found between positive MACC1 staining and the disease free rate in each tumor category [metastasis (**Figure 3A**) and metastasis-free at diagnosis (**Figure 3B**) as well as stage T1

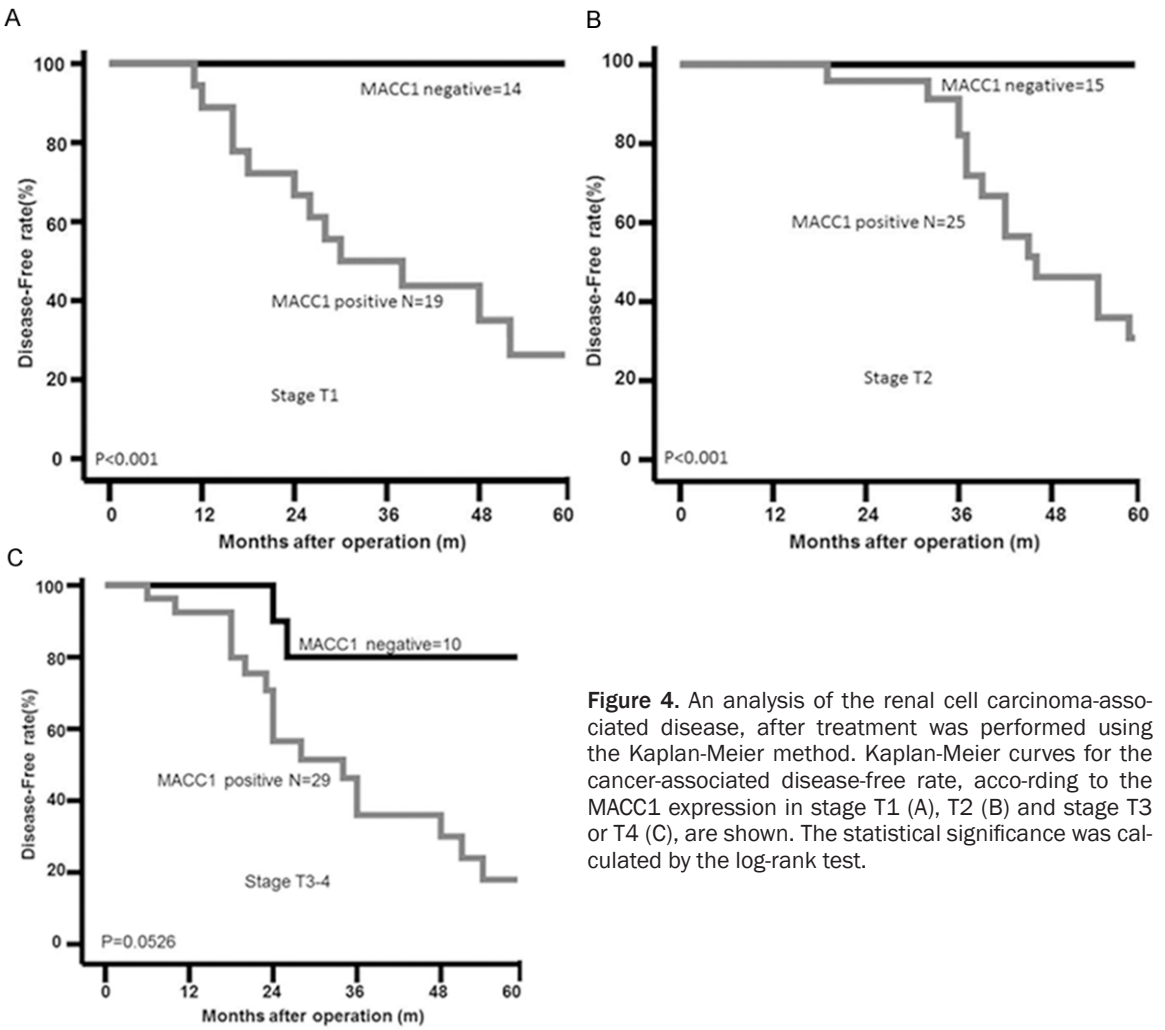

Figure 4. An analysis of the renal cell carcinoma-associated disease, after treatment was performed using the Kaplan-Meier method. Kaplan-Meier curves for the cancer-associated disease-free rate, acco-rding to the MACC1 expression in stage T1 (A), T2 (B) and stage T3 or T4 (C), are shown. The statistical significance was calculated by the log-rank test.

(**Figure 4A**), T2 (**Figure 4B**), and T3-4 (**Figure 4C**).

Discussion

Renal cell carcinoma is a common urological malignancy worldwide. Despite undergoing local or systemic therapy or immunotherapy, a majority of RCC patients exhibit progressive and metastatic disease [9, 10]. Metastatic RCC is resistant to chemotherapy and radiation therapy; however, recent advances in molecular biology have led to the development of novel molecular targeted therapies. Regarding these advances, previous studies of RCC have observed mutations in von Hippel-Lindau (VHL), hypoxia-inducible factor (HIF), vascular endothelial growth factor (VEGF), platelet-derived growth factor receptor (PDGF), and mammalian target of rapamycin [11, 12]. Regarding novel therapies, one example is the oral tyrosine kinase inhibitor sunitinib, which selectively inhibits VEGF and PDGF receptors and exhibits anti-tumor and anti-angiogenic activities [13, 14]. According to reports from the Guidelines on Renal Cell Carcinoma, phase II and III clinical trials of sunitinib as a second-line monotherapy in patients metastatic RCC, 34%-40% of patients achieved a partial response [10, 15].

In this study, we observed significant differences in MACC1 protein expression between RCC and normal renal tissue samples. Furthermore, a statistical analysis revealed associations of MACC1 expression with pathological stage, TNM stage, distant metastasis, and RCC prognosis. This suggests that strong MACC1 expression is associated with RCC aggressiveness and that MACC1 may play an important role in cancer development.

MACC1 overexpression has been observed in several tumor types relative to normal tissues and can therefore serve as a marker of poor prognosis and metastasis in patients with cancer [3, 15, 16]. MACC1 serves as a transcriptional activator of c-MET and also plays a key regulatory role in the metastasis-related HGF-MET signaling pathway [2, 10]. Interference with MACC1 might prove to be another strategy that could be exploited for therapeutic purposes, especially with respect to the identification of potential interacting proteins.

To the best of our knowledge, this study represents the first investigation of the clinical significance of MACC1 in RCC and is also the first study to evaluate the possibility of using MACC1 as a clinical and molecular indicator of tumor progression. The results clearly indicate that MACC1 overexpression may predict a high risk of RCC metastasis or recurrence. MACC1 expression can therefore be used not only as a prognostic marker in patients with RCC, but also as a target for the development of new therapeutic approaches for RCC, including molecular targeted therapeutic agents.

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Disclosure of conflict of interest

None.

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