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RESEARCH

Overexpression of Metastasis-Associated in Colon Cancer-1 Associated with Poor Prognosis in Patients with Esophageal Cancer

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Abstract Recent studies have shown that expression of metastasis-associated in colon cancer-1(MACC1) is observed in different types of cancer and plays an important role in tumor metastasis. However, the expression of MACC1 and its possible role in esophageal cancer remains unknown. In this study, we determined the expression of MACC1 in esophageal cancer by utilizing immunohistochemistry and analyzed the relationship between the expression and esophageal cancer prognosis. Immunohistochemistry results showed that 47 of 85 cancer lesions (55.2 %) were stained positive, and high expression of MACC1 was correlated with the node metastasis and TNM stage ($P<0.05$). The Kaplan-Meier survival curve showed that patients with high MACC1 expression had significantly reduced overall 5-year survival rates ($P= 0.004$). Cox regression analysis revealed that high expression of MACC1 was associated with increased risk of death (hazard ratio [HR] =2.25) in patients with esophageal cancer. These findings suggested that high expression of MACC1 was correlated with progression and metastasis of esophageal cancer and might serve as a novel prognostic marker for patients with esophageal cancer.

Keywords MACC1 · Immunohistochemistry · Prognosis · Esophageal caner

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Abbreviations

HGF	Hepatocyte growth factor
HR	Hazard ratio
MACC1	Metastasis-associated in colon cancer-1
PBS	Phosphate buffered saline
pTNM	Pathological tumour-node-metastasis
TBS	Tris-buffered saline

Introduction

Esophageal cancer is the sixth leading cause of death from cancer worldwide for early local invasion and systemic metastasis [1]. Five-year survival rate for esophageal cancer in which no regional lymph nodes was involved, ranges from 30 to 95 % by virtue of combination therapy, while the 5-year survival rate decreases significantly (from 1 to 30 %) when the tumor cells spread to regional lymph nodes or distant organs [2]. Molecular markers are becoming more important to predict the prognosis and metastasis of esophageal cancer [3]. Although recent literatures have demonstrated many molecules involved in the invasion and metastasis of esophageal cancer [4–7], more molecular markers that help to predict early recurrence and clinical outcome are still needed.

MET encodes the tyrosine kinase that acts as a receptor for hepatocyte growth factor (HGF), which was originally known as an agent that induces cell scattering and acted as a mitogen of hepatocytes [8]. Currently, HGF/MET signaling pathway is shown to be involved in angiogenesis, growth, differentiation, cellular motility and invasion [9–13]. Aberrant activation of *MET* could cause oncogenesis and cancer metastasis by activating downstream signaling of the MAPK and/or PI3K/AKT pathways [14–17]. Recently, MACC1 was found to be a critical regulator of HGF/MET

signal pathway, and therefore was indispensable in the process of HGF-induced scattering in colon cancer [18]. Expression of MACC1 was determined in a wide spectrum of tumors, such as colon cancer, gastric carcinoma, lung adenocarcinoma, hepatocellular carcinoma and colorectal cancer [19–23]. Due to the direct relation between metastasis formation and survival, MACC1 expression may be useful both as a prognostic marker and possibly as a target for therapy [18, 23]. To date, the study of MACC1 expression in esophageal cancer has not been reported.

In the present work, we examined the expression frequency of MACC1 in 85 subjects with esophageal cancer by means of immunohistochemistry, in order to better understand the role of MACC1 expression in esophageal cancer and its clinicopathological implications.

Materials and Methods

Patients and Tissues Samples

Subjects were 85 patients with esophageal cancer who underwent esophagectomy with lymph node dissection between 2004 and 2005 at Jiangsu cancer hospital. All patients had a pathological diagnosis of esophageal cancer, and each patient was classified according to the pathological tumour-node-metastasis (pTNM) system. Specimens of cancer tissues and noncancerous adjacent tissues were collected from patients after informed consents had been obtained. The patients consisted of 64 men and 21 women. The cancer patients had an average age of 60 years (ranging from 42 to 79 years). Patients who received preoperative chemotherapy or radiotherapy were excluded from this study. Each sample had been fixed in formalin, routinely processed, and embedded in paraffin. Postoperative follow-up data were obtained from all patients, with a median follow-up period of 41.5 months (ranging from 5 to 65 months).

Immunohistochemistry

Consecutive 6 μm thick sections of formalin-fixed, paraffin-embedded tissue blocks were cut. Each slide was deparaffinized with xylene, rehydrated through a graded series of ethanol/water. All specimens were subjected to heat-induced antigen retrieval in 10 mM sodium citrate buffer (pH 6.0) and boiled in a water bathe for 10 min at 95 °C. To prevent unspecific binding, the slides were incubated with Tris-buffered saline (TBS) supplemented with 5 % goat serum. Sections were incubated at 37 °C with the primary antibodies anti-MACC1 (Abnova, PAB16755, 1: 200 dilution) for 2 h. After rinsing with phosphate buffered saline (PBS) for 15 min, sections were incubated with secondary antibody for 30 min and washed again with PBS for 10 min. EnVision™ Detection Systems (Dako) were used for visualization. Colon cancer tissue was included as a positive control. The negative controls consisted of sections treated with PBS instead of primary antibody.

Semiquantitative analysis of staining results of individual tissue array cores was carried out by two independent observers in a blinded manner without knowledge of the clinical data. The frequency of MACC1 positive cells was scored on the basis of the percentage of positive cells as 0 % = negative, 1–25 %=+1, 26–50 %=+2 and >51 %=+3. The intensity of MACC1 expression was

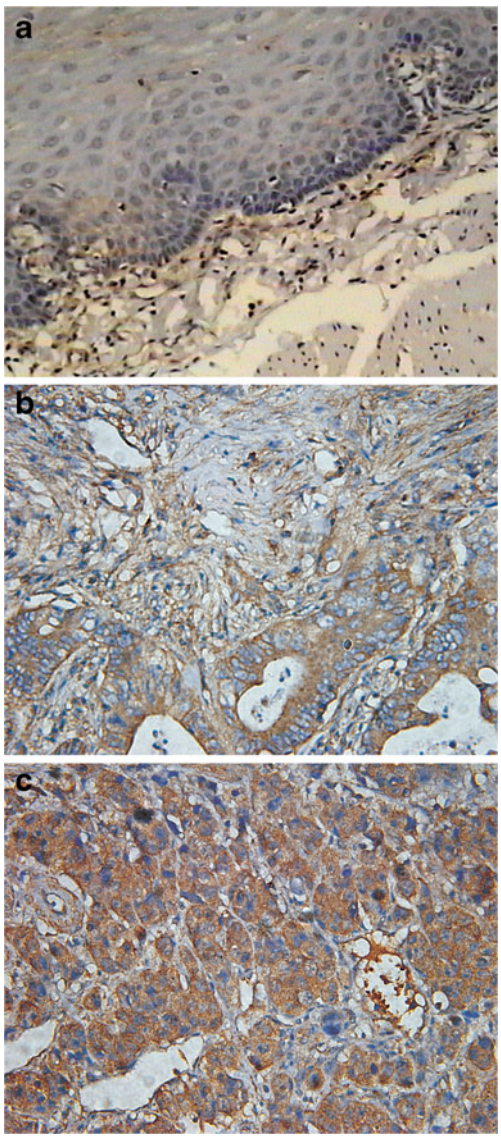

Fig. 1 Expression of MACC1 measured by immunohistochemistry. **a** Negative staining in normal esophageal eithelium (×400). **b** Control of positive staining of MACC1 on colorectal cancer (×400). **c** Positive staining in esophageal cancer (×400)

scored as weak = 1, moderate = 2 and strong = 3. The average MACC1 expression of each section was calculated as intensity multiplied by frequency and classified as low (≤2) or high (>2).

Statistical Analysis

Statistical calculations were performed using SPSS 16.0 (Statistical Package for the Social Sciences, USA). The association between MACC1 expression and clinicopathological characteristics of esophageal cancer was analyzed by χ^2 test. The Kaplan-Meier method was used for overall 5-year survival analysis, and differences in survival were estimated using the log rank test. Prognostic factors were examined by univariate and multivariate analyses (Cox proportional hazards regression model). A difference was considered significant at $P<0.05$.

Table 1 Correlation of MACC1 expression and clinicopathological features in esophageal cancer

Case		MACC1		*P*
	Total (n=85)	High (55.2 %)	Low (44.8 %)	
Age				
≤60	39	22	17	0.84
>60	46	25	21	
Gender				
Male	64	35	29	0.84
Female	21	12	9	
Differentiation				
Well	9	4	5	0.74
Moderate	56	31	25	
Poorly	20	12	8	
Location				
Upper	16	9	7	0.99
Middle	51	28	23	
Lower	18	10	8	
Tumor invasion				
T1	7	4	3	0.18
T2	28	15	13	
T3	41	20	21	
T4	9	8	1	
Lymph node metastasis				
N0	45	20	25	0.03
N1	40	27	13	
TNM stage				0.02
I	6	3	3	
II	48	21	27	
III	31	23	8	

Results

Overexpression of MACC1 in Esophageal Cancer

The expression and localization of MACC1 were detected by immunohistochemistry in samples from 85 patients with esophageal cancer. 47 of 85 cancer lesions (55.2 %) were stained positive. Strong staining was found mainly in cytoplasm. 33 cases were scored as strong expression and 14 as moderate expression. Representative results of MACC1 staining were shown in Fig. 1. The correlation between the MACC1 overexpression and clinicopathologic features of esophageal cancer including age, gender, differentiation, location, tumor invasion, lymph nodes metastasis and TNM stage was investigated (Table 1). The results showed that high expression was observed more frequently in patients with lymph node metastasis (P=0.03) and higher TNM stage (P=0.02). However, no correlation was observed between MACC1 overexpression and age (P=0.84), gender (P=0.84), tumor differentiation (P=0.74), tumor location (P=0.99) and tumor invasion (P=0.18).

MACC1 Expression and Esophageal Cancer Prognosis

The Kaplan-Meier and Cox regression analysis were used to determine the prognostic significance of clinicopathologic factors and MACC1 expression in esophageal cancer. The univariate analysis revealed that N status (P=0.04) and MACC1expression (P=0.005) were significantly associated with overall 5-year survival. However, the associations of gender, age, T status and TNM stage with prognosis were not significant in univariate analysis (Table 2). The 5-year survival rate was 32 % for esophageal cancer with low MACC1 expression and only 14 % for esophageal cancer with high MACC1 expression. The Kaplan-Meier survival curve showed that patients with high level of MACC1 intended to have shorter survival time than those with low level of MACC1 (Fig. 2). In the multivariate analysis,

Table 2 Results of univariate analysis of clinicopathologic parameters

	P-value	Relative risk	95.0 % CI for RR	
			Lower	Upper
Age (≤60 vs. >60)	0.43	1.23	0.73	2.01
Gender (male vs. female)	0.24	0.70	0.39	1.27
T status (3 and 4 vs. 1 and 2)	0.15	1.47	0.87	2.51
N status (1 vs. 0)	0.04	1.70	0.99	2.88
TNM stage (III vsIandII)	0.93	1.02	0.59	1.77
MACC1(high vs. low)	0.005	2.22	1.27	3.88

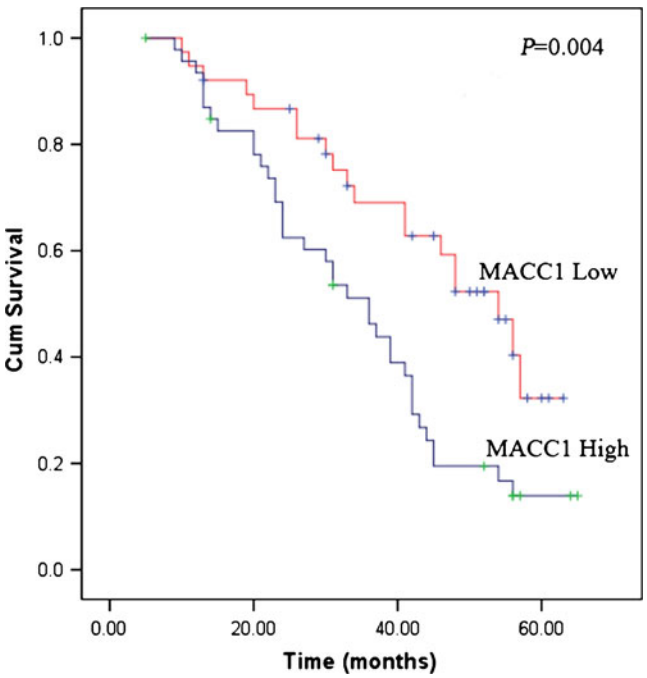

Fig. 2 Kaplan–Meier survival curves of patients with esophageal cancer. Patients with high MACC1 expression have a significantly worse overall 5-year survival

significant factors, including age, gender, T status, N status, TNM Stage, and the expression level of MACC1 were estimated. The results demonstrated that MACC1 expression was a useful prognostic factors (P=0.01). The expression of MACC1 was associated with significantly increased risk of death (HR=2.25) in patients with high level of MACC1 (Table 3).

Table 3 Multivariate cox regression analysis of overall survival in 85 patients with esophageal cancer

	P-value	Relative risk	95.0 % CI for RR	
			Lower	Upper
Age (≤60 vs. >60)	0.70	1.18	0.63	1.96
Gender (male vs. female)	0.95	1.01	0.50	1.94
T status (3 and 4 vs. 1 and 2)	0.29	1.30	0.79	2.14
N status (1 vs. 0)	0.12	1.70	0.88	3.27
TNM stage (III vsIandII)	0.96	1.01	0.62	1.66
MACC1(high vs. low)	0.01	2.25	1.18	4.28

Discussion

Metastasis, a result of cancer migration from primary site to distant site through the bloodstream or the lymph system [24, 25], was the major cause of death for cancer [26]. MACC1, first found in colon mucosa, primary tumors and metastases of subjects with colon cancer by differential display RT-PCR [27], plays a vital role in the process of tumour cell proliferation and distant metastases [18]. In the present study, we analyzed the expression of MACC1 in patients with esophageal cancer by immunohistochemistry assays and correlated it with clinicopathological variabilities and prognosis. We found that high expression was more frequently discovered in esophageal cancer. In the 85 cases, there was no differential frequency of MACC1 expression in different groups of age, gender, tumor differentiation, tumor location and tumor invasion. A significant association was found between MACC1 expression and TNM stage and lymph node metastasis. This suggests that high level of MACC1 is associated with aggressiveness of esophageal cancer and plays an important role in the process of metastasis.

MACC1 expression has been considered to be a prognostic marker in patients with cancer [21, 23]. However, the relationship between MACC1 expression and esophageal cancer prognosis remains unclear. A new finding in our study is that MACC1 expression is significantly associated with esophageal cancer overall survival. Patients with high MACC1 expression tends to have a lower overall survival rate compared with those with low expression.

As a transcriptional target of MACC1, the proto-oncogene *MET* is expressed mainly on the surface of epithelial cancer cells [28]. By activating downstream signaling of MAPK and/or PI3K/AKT pathways, MET could promote cell proliferation, invasion and metastasis [14]. Stein et al. has proved that upregulation of MACC1 induced downstream activation of HGF/c-MET and gave rise to metastasis of colon cancer, while blocking the expression of MACC1 led to reduce tumour proliferation, migration and new metastases [18]. Additionally, MACC1 was also found to be an independent factor for tumor recurrence and a prognostic factor for patients with malignant tumors [18]. These results imply the importance of MACC1 in the process of colon cancer malignant progression. However, the definite mechanism of esophageal cancer metastasis induced by MACC1 dysregulation remains unclear, which needs to be studied further.

In conclusion, our data indicates that MACC1 is expressed frequently in esophageal cancer and high expression is a significant indicator of poor overall survival. Because of the limitation of small amount of subjects, more researches on a larger population are needed to confirm the application of MACC1 in esophageal cancer diagnosis and therapy.

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