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RESEARCH

Prognostic Value and Clinical Pathology of MACC-1 and c-MET Expression in Gastric Carcinoma

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Abstract This study was to assess the expression of MACC-1 and c-MET in gastric cancer, and to correlate this expression with clinicohistological parameters and patient prognosis. Total RNA was extracted from cancer tissue and adjacent normal mucosa from frozen biopsy specimens of 30 patients with gastric cancer, and MACC-1 expression was assessed by RT-PCR. MACC-1 and c-MET protein expression were also assessed in paraffin-embedded tissues obtained from 436 tumor mucosa and 92 normal mucosa specimens by immunohistochemistry. The correlation between MACC-1 and c-MET expression and clinicopathological factors (age, sex, histology, tumor depth, lymph node status and vessel invasion) were also evaluated. RT-PCR analysis revealed that *MACC-1* expression was significantly higher in cancerous mucosa compared with normal tissue. Immunohistochemical analysis indicated that MACC-1 and c-MET were moderately or strongly expressed in gastric cancer tissue, whereas expression was weak or absent in non-cancer tissue. Expression of MACC-1 or c-MET was significantly associated with larger tumor size, deeper tumor invasion, presence of lymph node metastasis, lymphatic involvement, venous invasion, distant metastasis and advanced clinical stage. However, only MACC-1 exhibited significantly greater expression in carcinomas from the higher age group. The intensity of MACC-1 and c-MET expression was also positively correlated. Survival analysis of the 436 gastric cancer patients revealed that patients in clinical stages I, II and III exhibiting lower MACC-1 and c-MET expression had a higher 5-year survival rate compared with patients expressing high levels of these proteins. Multivariate analysis revealed that MACC-1 and c-MET may be independent prognostic indexes of gastric carcinoma ($P<0.01$). Our findings confirm that MACC-1 and c-MET expression is strongly related to gastric cancer stage and degree of malignancy, and is inversely correlated to patient prognosis. Thus, MACC-1 and c-MET may interact to promote tumorigenesis and their expression may be used as independent prognostic markers in gastric cancer.

Keywords Gastric cancer · MACC-1 · c-MET · Progression · Prognosis

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Introduction

Gastric cancer is one of the most common cancers, accounting for approximately 17.2 % of all malignant tumors. In China alone, the crude mortality rate of gastric cancer is 2.5 per 1000, which accounts for 23.2 % of all cancer deaths in the same period, making gastric cancer the leading cause of cancer death. Gastric cancer is characterized by invasion and metastasis, which are major causes contributing to the lethality of this disease [1, 2]. The process of tumor invasion and metastasis involves cell adhesion, cell movement, degradation of the extracellular matrix and the formation of the new vessels [3, 4]. The molecular mechanisms underlying these processes involve changes in the expression of various metastasis-related genes, oncogenes and tumor suppressors, and research in this area is becoming increasingly popular. A major focus in the field of clinical gastric cancer research has been to discuss molecular mechanisms underlying invasion and metastasis, and to identify biological markers associated with these processes.

Aberrant activation of the hepatocyte growth factor (HGF)/HGF receptor(MET) signaling pathway is associated with both malignant transformation and metastatic potential of tumors [5]. Metastasis-associated with colon cancer-1 (MACC-1), a newly identified key regulator of HGF/MET signaling,

strongly induces MET expression and promotes tumor cell invasion and metastasis [6]. High expression of MACC-1 has been shown to be associated with an increased risk of metastasis and decreased patient survival in numerous cancers. However, the significance of MACC-1 expression and its effect on the HGF/MET pathway in the gastric cancer biology and prognosis are not well understood. In the current study, we attempted to clarify the clinical features of MACC-1 and MET expression in gastric cancer and to determine their clinical implications and related mechanisms.

Materials and Methods

Patients and Frozen Tissue Samples

Frozen tissue samples were collected from 30 gastric cancer patients (18 male, 12 female; median age 60 years, range 26–88 years) who had received total gastrectomy between January 2010 and January 2011, from the Department of Surgery, Zhejiang Provincial People's Hospital. None of the patients were treated with radiotherapy or perioperative chemotherapy. Resected specimens were examined according to the criteria described in the p tumor node metastasis classification system of the Union for International Cancer Control (2002). The study items included age, sex, tumor location, tumor size, gross (Borrmann) type, gastric wall invasion, resection margin, histological type, lymph node metastasis, vascular invasion, lymphatic invasion and perineural invasion. Fresh samples of tumor tissue, matched adjacent normal mucosa (>5 cm), and lymph node and peritoneal metastases were obtained immediately after gastric resection. The samples were dissected carefully from resected specimens by a pathologist, and immediately snap-frozen in separate vials using liquid nitrogen. Frozen specimens were stored at −80 °C in a tumor bank before use.

Archived Surgical Specimens and Clinicopathological Records of Patients

Gastric cancer tissues were collected from gastrectomy specimens from 436 patients (311 male, 125 female; median age 60 years, range 17–91 years) between January 1998 and January 2004, from the Department of Surgery, Zhejiang Provincial People's Hospital. Tissues had been formalin-fixed, paraffin-embedded and diagnosed clinically and histopathologically at the Departments of Gastrointestinal Surgery and Pathology. All patients had follow-up records for >5 years. The follow-up deadline was December 2008. The survival time was calculated from the date of surgery to the follow-up deadline or date of death, which was predominantly due to carcinoma recurrence or metastasis. Non-cancerous human gastric tissues (n=92) were obtained from adjacent gastric cancer margins (>5 cm). Routine chemotherapy was given to the patients with advanced-stage disease after operation, but no radiation treatment was administered to any of the patients included in our study.

Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

The expression of *MACC-1* was assessed by qRT-PCR in 30 tumor tissue samples with matched normal gastric mucosa and lymph node metastases, and in nine tumor tissue samples with matched normal gastric mucosa and peritoneal metastases. Total RNA was extracted using TRIzol and cDNA was reverse-transcribed using RevertAid™ reverse transcriptase. Real-time PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Cycling parameters were: 94 °C for 5 min, followed by 45 cycles of 94 °C for 30 s, 56 °C for 40 s, and 72 °C for 15 s, with a final extension of 72 °C for 10 min. The primers used for amplification of *MACC-1* were 5′-GACCAGGCAATCATTACGGC-3′ (sense) and 5′-CCCAGCAGTCTGTTTCACCAAG-3′ (antisense). Primers for *GAPDH* were 5′-CGATTGGATG GTTTAGTGAGG-3′ (sense) and 5′-AGTTCGACCGTCTT CTCAGC-3′ (antisense). The expression of *GAPDH* was used to normalize that of candidate genes. Assays were performed in triplicate, and *MACC-1* expression was calculated using the following formula: $2^{-\Delta\Delta Ct}$, $\Delta Ct = Ct(MACC-1) - Ct(GAPDH)$.

Tissue Microarray Analysis

Blocks containing a total of 436 tumor tissue samples and 92 normal gastric mucosa specimens were prepared as previously described [7, 8]. Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded gastric tumors (donor blocks) and arranged in recipient paraffin blocks (tissue array blocks) using a trephine. Based on previous studies which showed that staining results obtained from different intratumoral areas in various tumors correlate well [9], one core was sampled in each case. An adequate case was defined as a tumor occupying >10 % of the core area [14]. Each tissue array block contained more than three internal controls that consisted of non-neoplastic gastric mucosa. Sections (4 μm) were cut from each tissue array block, deparaffinized and dehydrated.

Immunohistochemistry

Immunohistochemical analysis was performed to study altered protein expression in 436 human gastric cancer and 92 adjacent normal tissue samples, as previously described [10, 11]. Paraffin-embedded formalin-fixed tissue slides (4 μm) were baked at 60 °C for 2 h, followed by deparaffinization with xylene, and rehydrated. Antigen retrieval was performed by submerging sections in EDTA antigenic retrieval buffer, and microwaving for x min. Sections were then treated with 3 %

(v/v) hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 1 % (w/v) bovine serum albumin to block non-specific binding. Sections were incubated with mouse monoclonal antibody against MACC-1 and MET (Santa Cruz Biotechnology, 1:50 dilution) overnight at 4 °C. Normal goat serum was used as a negative control. After washing, tissue sections were treated with secondary antibody. Tissue sections were then counterstained with hematoxylin, dehydrated and mounted. A negative control slide was processed without primary antibody to detect any background staining or false-positive results.

Evaluation of Results

MACC-1 and MET were stained as buffy colored in the cytoplasm and membrane. Protein expression was assessed by two expert pathologists blinded to the clinical details. Scoring was performed according to the percentage of stained cells observed (0=≤ 5 % of cells; 1=6–25 %; 2=26–50 %; 3=51–100 %). An intensity score of "0–3" was also determined (0=none, 1=light yellow, 2=yellow brown, 3=brown) [12, 13]. The final score was the product of the two former values (0–1=−, 2–3=+, 4–6=++, >6=+++). Patients with a score of "−/+" were classified as the low expression group, whereas those with "++/+++" were classified as the high expression group.

Statistical Analysis

All statistical analyses were performed using SPSS13.0 software. Measurement data were analyzed using the Student's *t* test, while categorical data were studied using $\chi 2$ or Fisher exact tests. Survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used to compute differences between the curves. Multivariate analysis using the Cox proportional hazards regression model was performed to assess the prognostic values of protein expression. Correlation coefficients between protein expression and clinicopathological findings were estimated using the Pearson correlation method. Statistical significance was set at $P<0.05$.

Results

Analysis of MACC-1 mRNA Expression in Human Gastric Cancer Samples by qRT-PCR

The expression of *MACC-1* was analyzed in paired normal and gastric cancer specimens from 30 patients by qRT-PCR. The study revealed significantly higher expression in gastric carcinoma specimens compared with normal tissue ($P<0.001$). The average expression of *MACC-1* in gastric tumor tissue samples compared with matched, normal gastric mucosa was 0.0131±0.00163 versus 0.0069±0.00055, respectively (Table 1).

Immunohistochemical Analysis of MACC-1 and C-MET Expression in Paired Gastric Tumor and Normal Tissues

MACC-1 and c-MET were predominantly localized in the cytoplasm or membrane of primary cancer cells. In accordance with our qRT-PCR results, IHC analysis revealed a significant increase in the intensity of MACC-1 and c-MET expression and in the percentage of positively stained cells in gastric cancer specimens compared with normal samples. Indeed, MACC-1 was expressed in 57.8 % (252/436) of tumor samples, whereas expression was not detected in normal mucosa ($P<0.01$) (Fig. 1). High expression of MACC-1 was observed in 34.8 % of gastric cancer cases (152/436). We also found that c-MET was expressed at weak, moderate or strong levels in 70.41 % (307/436) of carcinoma samples, whereas only very few stained cells were observed in non-cancer tissues ($P<0.01$). High expression of c-MET was observed in 43.8 % (191/436) of gastric cancer specimens (Fig. 2).

Correlation Between MACC-1 Expression, C-MET up-Regulation and Clinical Features of Gastric Cancer

Analysis of MACC-1 and c-MET expression with respect to tumor stage revealed weak expression (−/+) in the majority of stage I and II samples, whereas >40 % or 50 % of the specimens in stage III and IV exhibited strong (++/+++) expression of MACC-1 and c-MET, respectively ($P<0.01$).

Positive expression of MACC-1 was correlated with age, tumor location, size, depth of invasion, lymph node and distant metastases, regional lymph node stage and TNM stage ($P<0.05$) (Table 2). In contrast, MACC-1 expression was not correlated with gender, Lauren's classification, differentiation or histological classification ($P>0.05$) (Table 2).

Analysis of c-MET expression with respect to clinicopathological features revealed that expression increased robustly in tumors of larger size or deeper invasion, or with nodal involvement, venous/lymphatic invasion or with distant metastasis ($P<0.01$). Similarly, patients exhibiting diffuse type gastric carcinoma, or clinical progression to the lymph node or TNM stage, tended to exhibit high frequency and intensity

Table 1 M*ACC-1* mRNA expression in normal gastric and cancerous mucosa

Specimen	Case (*n*)	Relative expression (2-Δ(ΔCt))	*p*-value
Non-cancer mucosa	30	0.0069±0.00055	0.000
Cancerous mucosa	30	0.0131±0.00163	

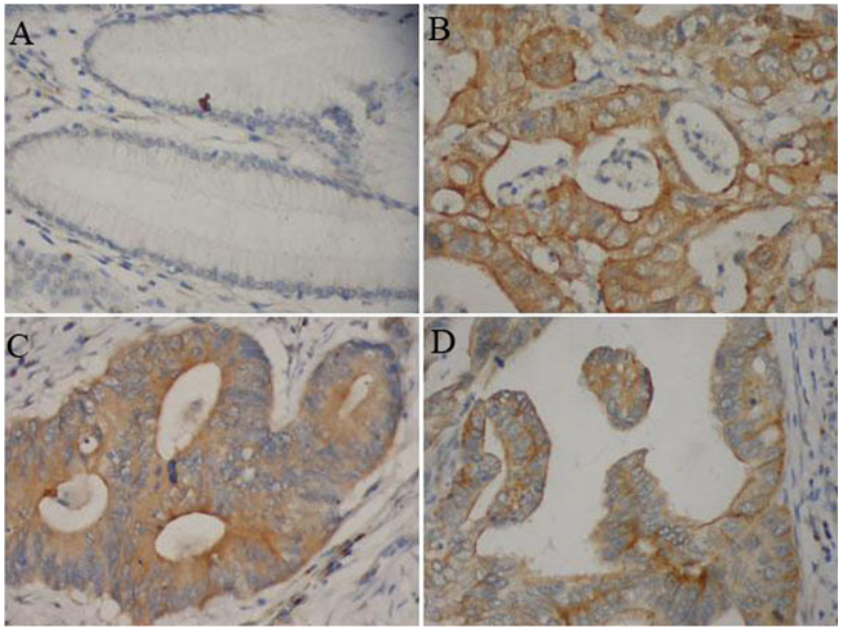

Fig. 1 Representative images of immunohistochemical staining for MACC-1 in gastric cancer tissue or normal mucosa. **a** normal mucosa (−) 400×; **b** poorly differentiated adenocarcinoma (+++)400×; **c** moderately differentiated adenocarcinoma (+++)400×; D: poorly differentiated carcinoma (+++) 400×

of c-MET staining ($P<0.01$). Although MET levels displayed a trend toward increased expression at lower stages of differentiation, this was not statistically significant. We observed no significant correlation between c-MET expression and gender, age, tumor location and histological type ($P>0.05$) (Table 3).

Correlation Between MACC-1, C-MET Expression and Patient Prognosis

Correlation analysis of MACC-1 or c-MET protein expression and patient prognosis revealed that the 5-year survival rates of patients with stage I, II and III tumors expressing high levels of MACC-1 or c-MET were significantly poorer than those expressing low levels of these proteins ($P<0.01$) (Figs. 3, 4, 5, 6, and 7). While patient prognosis also declined when MACC-1 or c-MET expression increased in stage IV tumors, this was not statistically significant ($P=0.235$, $p=0.388$).

Associations Between MACC-1 and C-MET and the Impact of Their co-Expression on Patient Prognosis

As described above, MACC-1 was expressed at low levels in 284 cases of gastric carcinoma, and among these, 235 cases simultaneously expressed low levels of c-MET. Of the tumors

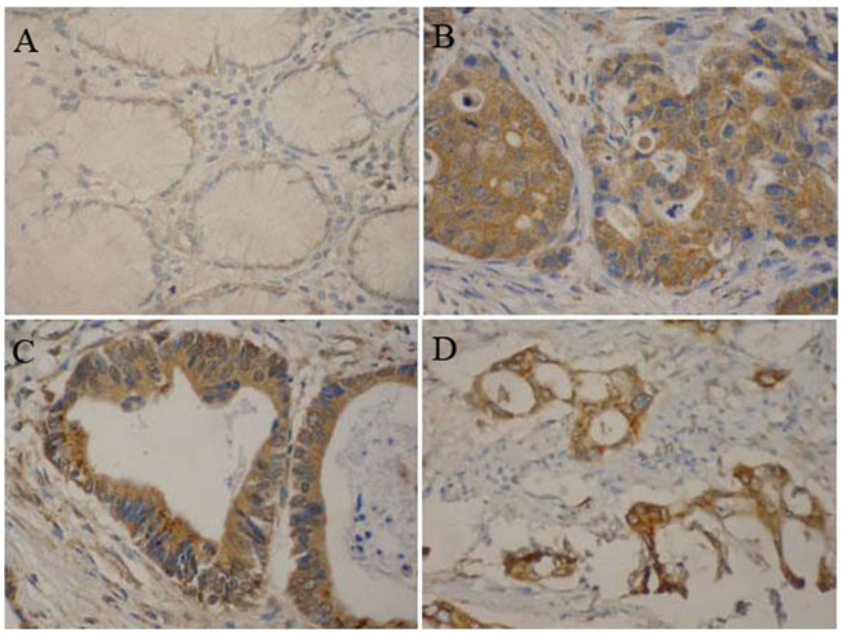

Fig. 2 IHC analysis of MET in gastric cancer tissue and normal mucosa. **a** normal mucosa (−) 400×; **b** poorly differentiated adenocarcinoma (+++) 400×; **c** moderately differentiated adenocarcinoma (+++) 400×; **d** poorly differentiated carcinoma (+++) 400×

Table 2 Correlation of MACC-1 expression with clinico-pathological parameters of the gastric carcinoma patients

Clinico-pathological parameters	MACC1			
	Low	High	t/χ^2	*P*-value
Age (years)	57.73±12.10	61.53±11.82	3.15	0.002
Gender			0.009	0.925
Male	203 (65.3 %)	108(34.7 %)		
Female	81 (64.8 %)	44(35.2 %)		
Location			9.04	0.011
Cardia	28(50.9 %)	27(49.1 %)		
Body	101(62.0 %)	62(38.0 %)		
Antrum	155(71.1 %)	63(28.9 %)		
Tumor size			26.56	0.0001
<5 cm	192(75.0 %)	64(25.0 %)		
≥5 cm	92(51.1 %)	88(48.9 %)		
Lauren classification			130.1	0.0001
Intestinal	202(90.6 %)	21(9.4 %)		
Difuse	82(38.5 %)	131(61.5 %)		
Histology classification			1.513	0.679
Papillary adenocarcinoma	9(56.2 %)	7(43.8 %)		
Tubular adenocarcinoma	217(66.6 %)	109(33.4 %)		
Mucinous adenocarcinoma	17(58.6 %)	12(41.4 %)		
Signet-ring cell carcinoma	41(63.1 %)	24(36.9 %)		
Differentiation			5.902	0.116
Well	12(92.3 %)	1(7.7 %)		
Moderate	85(66.4 %)	43(33.6 %)		
Poor	185(63.1 %)	108(36.9 %)		
Undifferentiated	2(100 %)	0(0 %)		
Invasion depth			45.702	0.0001
T1	52(91.2 %)	5(8.8 %)		
T2	85(78.0 %)	24(22.0 %)		
T3	139(57.0 %)	105(43.0 %)		
T4	8(30.8 %)	18(69.2 %)		
TNM stages			118.0	0.0001
I	84(93.3 %)	6(6.7 %)		
II	89(85.6 %)	15(14.4 %)		
III	97(56.1 %)	76(43.9 %)		
IV	14(20.3 %)	55(79.7 %)		
Node status			61.44	0.0001
No	146(88.0 %)	20(12.0 %)		
Yes	138(51.1 %)	132(48.9 %)		
Regional lymph nodes			98.503	0.0001
PN0	146(88.0 %)	20(12.0 %)		
PN1	90(66.2 %)	46(33.8 %)		
PN2	43(43.4 %)	56(56.6 %)		
PN3	5(14.3 %)	30(85.7 %)		
Distant metastasis			55.58	0.0001
No	270(72.0 %)	105(28.0 %)		
Yes	14(23.0 %)	47(77.0 %)		

Table 3 Correlation of c-Met expression with clinico-pathological parameters of the gastric carcinoma patients

clinico-pathological parameters	C-met			
	Low	High	t/χ^2	*P*
Age (years)	58.26±11.78	60.07±12.51	1.55	0.122
Gender			0.479	0.489
Male	178(57.2 %)	133(42.8 %)		
Female	67(53.6 %)	58(46.4 %)		
Location			1.154	0.562
Cardia	29(52.7 %)	26(47.3 %)		
Body	88(54.0 %)	75(46.0 %)		
Antrum	128(58.7 %)	90(41.3 %)		
Tumor size			20.59	0.0001
<5 cm	192(75.0 %)	89(34.8 %)		
≥5 cm	92(51.1 %)	102(56.7 %)		
Lauren classification			115.6	0.0001
Intestinal		42(18.8 %)		
Difuse		149(70.0 %)		
Histology classification			2.337	0.505
Papillary adenocarcinoma		8(50.0 %)		
Tubular adenocarcinoma		137(42.0 %)		
Mucinous adenocarcinoma		16(55.2 %)		
Signet-ring cell carcinoma		30(46.2 %)		
Differentiation			4.633	0.201
Well		2(15.4 %)		
Moderate		55(43.0 %)		
Poor		133(45.4 %)		
Undifferentiated		1(50.0 %)		
Invasion depth			30.822	0.0001
T1		11(19.3 %)		
T2		38(34.9 %)		
T3		123(50.4 %)		
T4		19(73.1 %)		
TNM stages			88.88	0.0001
I		14(15.6 %)		
II		24(23.1 %)		
III		102(59.0 %)		
IV		51(73.9 %)		
Node status			68.78	0.0001
No		31(18.7 %)		
Yes		160(59.3 %)		
Regional lymph nodes			97.26	0.0001
PN0		31(18.7 %)		
PN1		59(43.4 %)		
PN2		73(73.7 %)		
PN3		28(80.0 %)		
Distant metastasis			28.77	0.0001
No		145(38.7 %)		
Yes		46(75.4 %)		

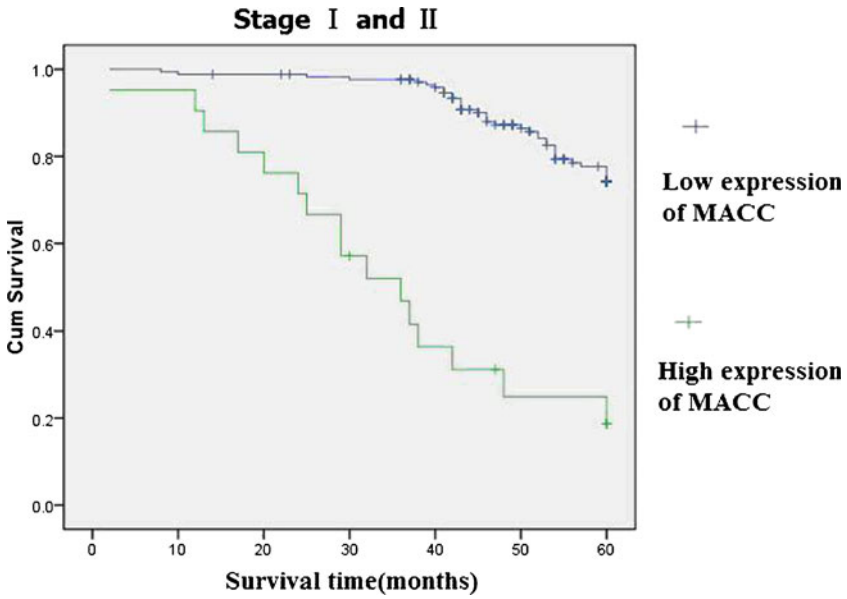

Fig. 3 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low MACC-1 expression versus high MACC-1 expression tumors in all gastric cancer in stage I–II. The cumulative 5-y survival rate was 23.8 % in the low MACC-1 protein expression group but was only 79.2 % in the high expression group($P<0.05$)

expressing high levels of c-MET, 152 also expressed high levels of MACC-1. We observed a positive linear relationship between MACC-1 and MET protein expression ($\chi 2=186.6.0$, $P=0.0001$). We next performed Kaplan-Meier survival analysis to establish the correlation between MACC-1 and c-MET co-expression and patient outcome. This analysis revealed that patients with tumors exhibiting high expression of both MACC-1 and c-MET protein had a significantly worse prognosis compared with those co-expressing these proteins at low levels (Fig. 8).

Multivariate Analysis of Clinicopathological Parameters and Patient Prognosis

We next analyzed factors with possible prognostic effects in gastric carcinoma by Cox regression analysis. This analysis indicated that tumor TMN stage and MACC-1 or MET expression were independent prognostic factors in patients with gastric carcinoma ($P < 0.05$). In contrast, tumor location and size, Lauren's classification, histological classification, tumor differentiation, nodal status, venous invasion, distant metasta-

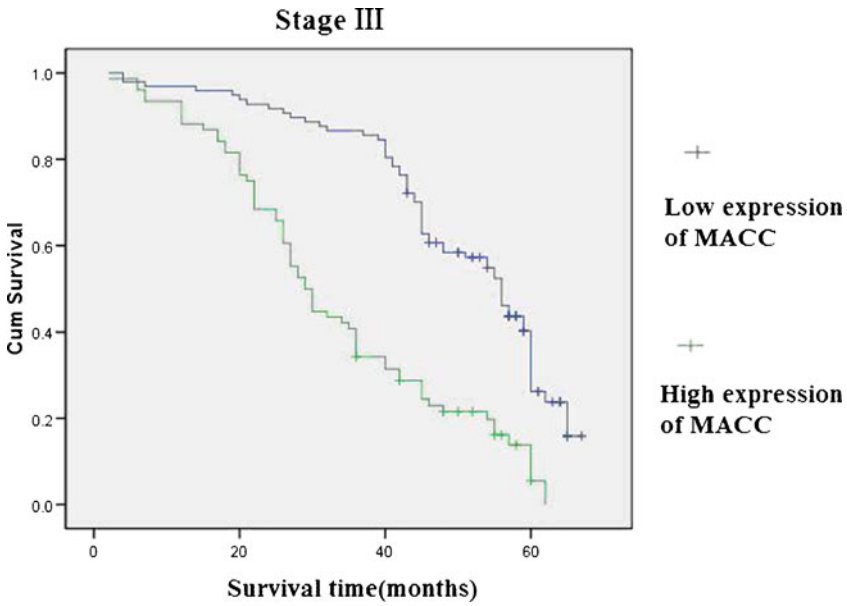

Fig. 4 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low MACC-1 expression versus high MACC-1 expression tumors in all gastric cancer in stage III. The cumulative 5-y survival rate was 11.8 % in the low MACC-1 protein expression group but was only 34.0 % in the high expression group($P<0.05$)

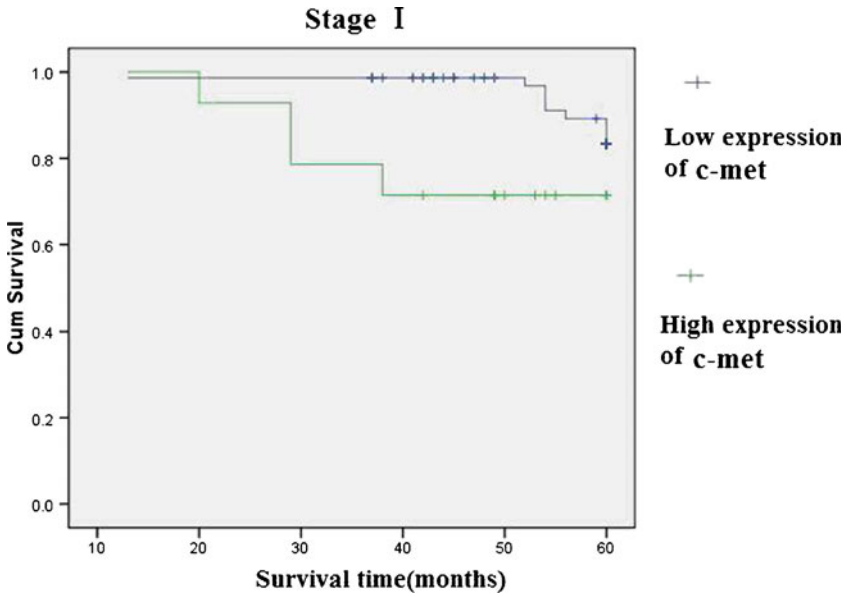

Fig. 5 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low MACC-1 expression versus high MACC-1 expression tumors in all gastric cancer in stage III. The cumulative 5-y survival rate was 11.8 % in the low MACC-1 protein expression group but was only 34.0 % in the high expression group($P<0.05$)

ses and regional lymph node stage were not independently associated with a reduction in overall survival ($P > 0.05$).

Discussion

Previous studies have shown that tumor development, invasion and metastasis are associated with distinct gene expression profiles [14]. In a recent study to identify genes differentially expressed in gastric cancer, we profiled gene expression changes in gastric cancer specimens and healthy tissues using microarrays. We identified 434 genes and 169 expressed sequence tags (ESTs) up-regulated (≥ 2-fold, $p<$xxx) in primary gastric carcinomas compared with normal tissue, and of these, *MACC-1* was shown to be one of the mostly highly up-regulated genes. In the present study, we verified this microarray data by qRT-PCR and immunohistochemical analysis. We found that *MACC-1* expression was significantly higher in gastric carcinoma compared with non-malignant tissue. Importantly, high MACC-1 expression was significantly correlated with more aggressive clinicopathological features related to tumor location, size, depth of invasion, lymph node and distant metastases. Furthermore, patients with tumors expressing abnormal MACC-1 levels, exhibited more likeness

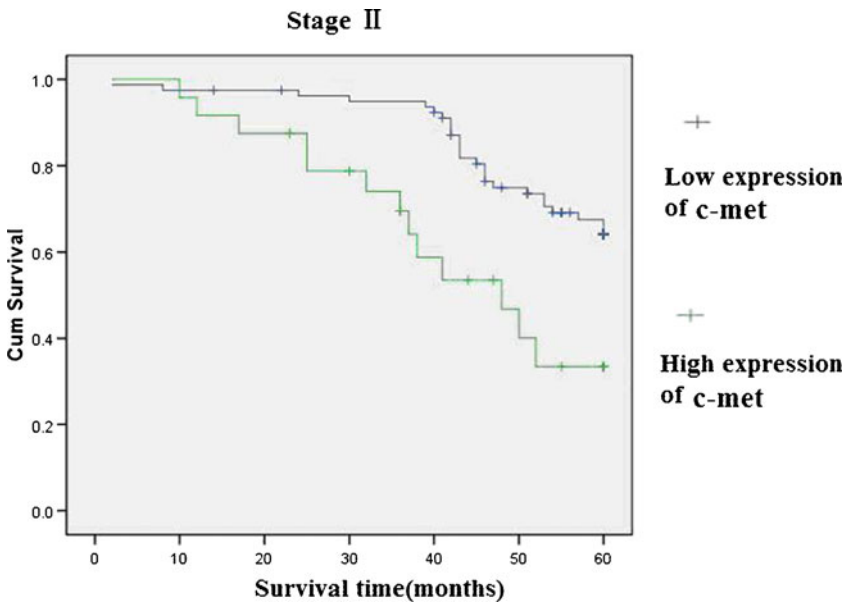

Fig. 6 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low c-met expression versus high c-met expression tumors in all gastric cancer in stage I. The cumulative 5-y survival rate was 71.4 % in the low c-met protein expression group but was only 88.2 % in the high expression group($P<0.05$)

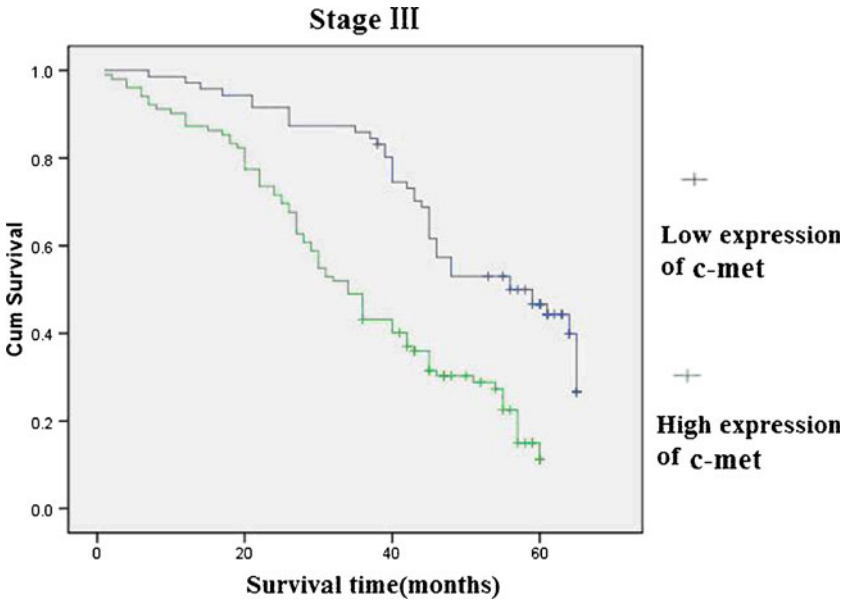

Fig. 7 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low c-met expression versus high c-met expression tumors in all gastric cancer in stage II. The cumulative 5-y survival rate was 45.8 % in the low c-met protein expression group but was only 67.5 % in the high expression group($P<0.05$)

in later regional lymph node stage and TNM stage and shorter survival time.

The *MACC-1* gene, located on human chromosome 7 (7p21.1), contains seven exons and six introns and encodes a protein that regulates injury response and tissue growth via the HGF/MET signaling pathway [15, 16]. Chromosome 7 polysomy and gain of the p-arm were observed in 21 % and 8 % of liver metastases from colorectal carcinoma (CRC), respectively, and were significantly correlated with higher expression of both MET and MACC-1 [17]. The induction of MACC-1 expression occurs at a crucial step in the transition of cells from the benign to the malignant phenotype, and also confers properties that potentiate metastasis [18]. Several distinct functional regions of MACC-1, including the ZU5, SH3 and death domains are thought to participate in vital steps of the malignant process such as protein-protein interactions, invasion-dependent signal transduction and regulation of apoptosis [19]. To further investigate the mechanisms by which MACC-1 contributes to tumor progression, Lang et al. recently investigated the link between the MACC-1 tagging polymorphisms and prognosis in patients with CRC. Among the single nucleotide polymorphismsinvestigated, variant rs1990172 was shown to be the only one linked to a potentially functional region, and it was associated with significantly decreased overall survival in CRC patients [20].

MACC-1 was first identified by Stein et al. in a genome-wide search for genes differentially expressed in human colon cancer [6]. According to this study, the 5-year-survival of

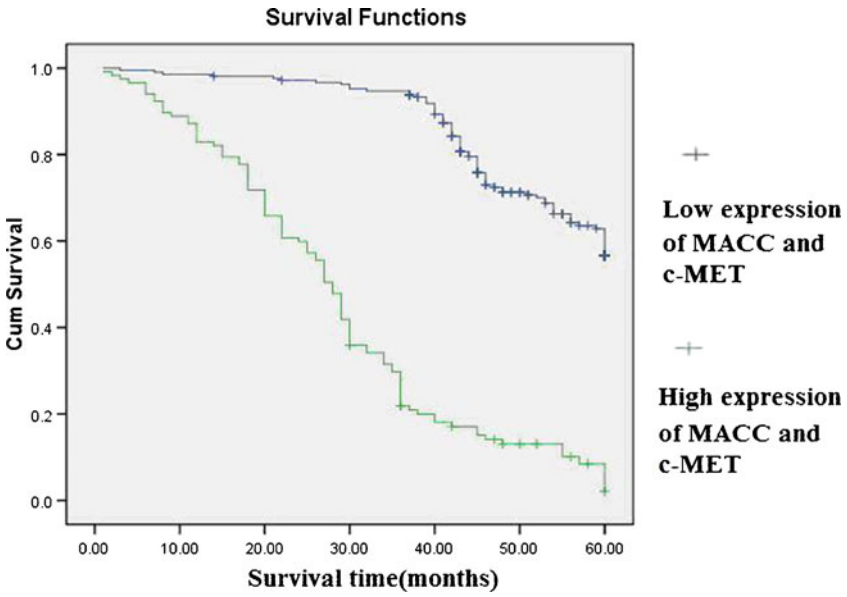

Fig. 8 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low c-met expression versus high c-met expression tumors in all gastric cancer in stage III. The cumulative 5-y survival rate was 21.6 % in the low c-met protein expression group but was only 42.3 % in the high expression group($P<0.05$)

CRC patients expressing low levels of MACC-1 was 80 %; however, this was significantly lower (15 %) for individuals expressing high levels of MACC-1. These conclusions were also supported by a series of cell culture and mouse model experiments, which revealed accelerated tumor cell proliferation, invasion and HGF-induced scattering of colon cancer cells following up-regulation of MACC-1 [21]. Since then, a number of studies have been conducted to evaluate the influence of MACC-1 expression on the clinical outcome of other cancers including hepatocellular, lung, gastric and ovarian cancers [22–25]. Research by Qiu et al. demonstrated that the 5-year disease free survival rate of HGC patients with low-level expression of MACC-1 was 54.5 %, compared with 33.5 % in the high-level group (P=0.008). The 5-year overall survival rate of patients expressing low levels of MACC-1 was 61.9 %, which was significantly higher than patients expressing high levels of MACC-1 (37.6 %, P=0.003) [22]. Similar survival disadvantages associated with high MACC-1 expression were also detected in lung cancer, indicating that MACC-1 may be a useful marker for predicting post-operative recurrence in patients with lung adenocarcinoma following surgery [23]. In the context of gastric cancer, analysis of MACC-1 expression in 41 patients by qRT-PCR revealed that MACC-1 was overexpressed more frequently in peritoneal-disseminated versus metastatic gastric carcinoma. Notably, MACC-1 expression was localized in the nuclei of tumors that later progressed to distant metastases, whereas expression was almost exclusively localized to the cytoplasm of non-metastasizing tumors [26]. While our study did not address the localization of MACC-1 in metastatic tumor cells, we comprehensively defined the role of MACC-1 in a large cohort of patients with gastric cancer. By stratifying the survival data according to TNM stage, we demonstrated a decline in the 5-year survival rates of patients with stage I-II, stage III and stage IV gastric cancer from 79.2 %, 34 % and 3.6 % in low MACC-1 expressors, to 23.8 %, 11.8 % and 0.0 % in high MACC-1 expressors. Thus, elevated MACC-1 expression is significantly linked to aggressive phenotype and an unfavorable prognosis in gastric cancer.

MACC-1 is a crucial regulator of MET. The *MET* gene encodes a tyrosine kinase that serves as a cell surface receptor for HGF/scatter factor (SF), one member of a family of soluble proteins that regulates invasive cell growth [27]. HGF/SF is essential for the development of several epithelial organs. Under physiological conditions, mice lacking HGF/SF failed to develop completely and died in utero, owing to impaired embryonic liver and placental development [28–30]. In the context of disease pathology, binding of MACC-1 to the MET promoter leads to strong transcriptional activation of MET and elicits multiple cellular responses regulating cell morphogenesis, migration or breakdown of the extracellular matrix [6]. Dysregulation of MET is common in liver, breast, colon and thyroid cancers [31–34]. Zhang et al. reported that MACC-1, HGF and MET were expressed in 73.1 %, 63.5 % and 78.8 % of epithelial ovarian cancers, respectively, and expression of these proteins was significantly different to that observed in normal ovarian tissue and benign ovarian tumors [35]. A study by Di Renzo et al. revealed that *MET* expression was increased 5–50-fold in approximately 50 % of tumors at any stage of progression, and in 70 % of liver metastases. Overexpression was associated with amplification of the *MET* gene in only 10 % of carcinomas, but in eight of nine metastases examined. This implies that overexpression of *MET* may confer selective growth advantage to neoplastic colorectal cells at any stage of tumor development, and its amplification may increase the chance for acquisition of metastatic potential [36]. Amplification of *MET* and high expression was also observed in 46.1 % and 10.2 % of gastric carcinoma cases, respectively, and may account for the depth of tumor invasion and the lymph node metastasis [37]. In our study, we demonstrate that MET is expressed at high levels in gastric cancer patients, and these patients displayed a trend towards shorter overall survival time. A finding of considerable clinical significance was the increased expression of MET in patients with larger tumors (>5 cm), higher TNM stage (III–IV), vessel invasion, lymph node involvement, or with liver peritoneum metastasis. Furthermore, Cox multivariate analysis indicated that MET and its upstream signaling molecule, MACC-1, are independent predictors of gastric carcinoma prognosis.

In conclusion, our study contributes to a growing body of evidence that MACC-1 and MET play an important role in tumorigenesis. Because MACC-1 regulates HGF/MET signaling via interactions with the intracellular region of the MET receptor, the co-expression of these two proteins may have a stronger adverse effect on survival in patients with gastric carcinoma. Indeed, this hypothesis was validated in our analysis, which revealed that patients with high expression of both MET and MACC-1 had a 5-year survival rate of 9.4 % compared with 62.9 % for patients with low expression of these proteins. Given the negative prognostic significance of MACC-1 and MET expression, these proteins may represent attractive targets for therapeutic inhibition in patients with gastric cancer. Targeting either of these proteins may benefit patients by disrupting aberrant signaling by HGF/MET. In one study by Ma et al. the viability of MET-expressing non-small cell lung cancer cells was effectively inhibited using the selective small molecule inhibitor of MET, SU11274, and this coincided with abrogation of HGF-induced MET phosphorylation and downstream signaling pathways [38]. Recently, a feedback loop between miR-1 and MET was proposed by Migliore et al., who showed that enforced expression of miR-1 led to a decrease in MET levels and impaired MET-induced invasive growth in colon cancer cells in vitro [39]. Taken together, these studies indicate that the interactions between MACC-1 and MET signaling and the biological outcomes of these interactions are far more complicated than previously

anticipated. In the future, the identification of additional factors involved in the activation of MACC-1 and MET signaling will likely provide us with more selective ways to treat the gastric cancer, via targeted inhibition [40–43].

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