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Original Article

Increased MT2-MMP expression in gastric cancer patients is associated with poor prognosis

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Abstract: Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that contribute to tumorigenesis and metastasis due to their ability to degrade the extracellular matrix (ECM) and basement membrane. In despite of many reports in other solid tumors, the role of membrane type-2 MMP (MT2-MMP) in gastric cancer (GC) remains to be elucidated. The aim of this study was to investigate MT2-MMP expression in human GC tissue microarray (TMA) samples using immunohistochemistry (IHC). We found that MT2-MMP expression in tumor tissues was significantly higher compared to peritumoral tissues ($P < 0.01$). However, there were no statistically significant differences between MT2-MMP expression and clinicopathological parameters. In addition, univariate and multivariate Cox regression analysis showed GC patients with high MT2-MMP expression have poor overall survival (OS) compared to patients with low MT2-MMP expression ($P = 0.013$, $P = 0.040$, respectively). In conclusion, MT2-MMP is involved in GC invasion and metastasis and may serve as an independent prognostic factor for GC patients.

Keywords: Membrane type-2 MMP, gastric cancer, prognosis

Introduction

In China, gastric cancer (GC) is the second most common type of cancer and the third leading cause of cancer deaths [1]. Despite surgery, chemotherapy, and radiotherapy have been demonstrated to improve the survival of GC patients, the invasive and metastatic potentials of GC cells are important indicators for poor prognosis. Therefore, it is urgent to figure out the underlying mechanisms of invasion and metastasis in the GC patients, which would help to find novel molecular therapeutic targets for GC treatments.

Matrix metalloproteinases (MMPs) are a family of enzymes that degrade the extracellular matrix (ECM), basement membrane, growth factors, etc. MMPs can act as oncogenes, which are frequently up-regulated in tumor progression and promote tumor invasion and metastasis [2, 3]. MMPs consist of more than 25 well-characterized secreted transmembrane proteins [4]. According to their structures and substrate specificity, MMPs are primarily classified into five subgroups: collagenases, gelatinases, stromelysins, membrane-type MMPs, and others [5]. As an important member of membrane-type MMPs, membrane type-2 MMP (MT2-MMP) was first characterized by Takino *et al*. in 1995 [6]. The full length of 3530 bp MT2-MMP was originally identified from a human lung cDNA library, which encodes 669 amino acids [7]. Moreover, MT2-MMP is identified as an anti-apoptotic factor in cancer cells [8].

In the present study, we investigated MT2-MMP expression in human GC tissue microarray (TMA) samples using immunohistochemistry (IHC). Additionally, the relationship between MT2-MMP expression and clinicopathological factors and its prognostic value were evaluated.

Materials and methods

Tissue microarray

The GC TMA was purchased from Shanghai Biochip Company (HStm-Ade180Sur-05), which

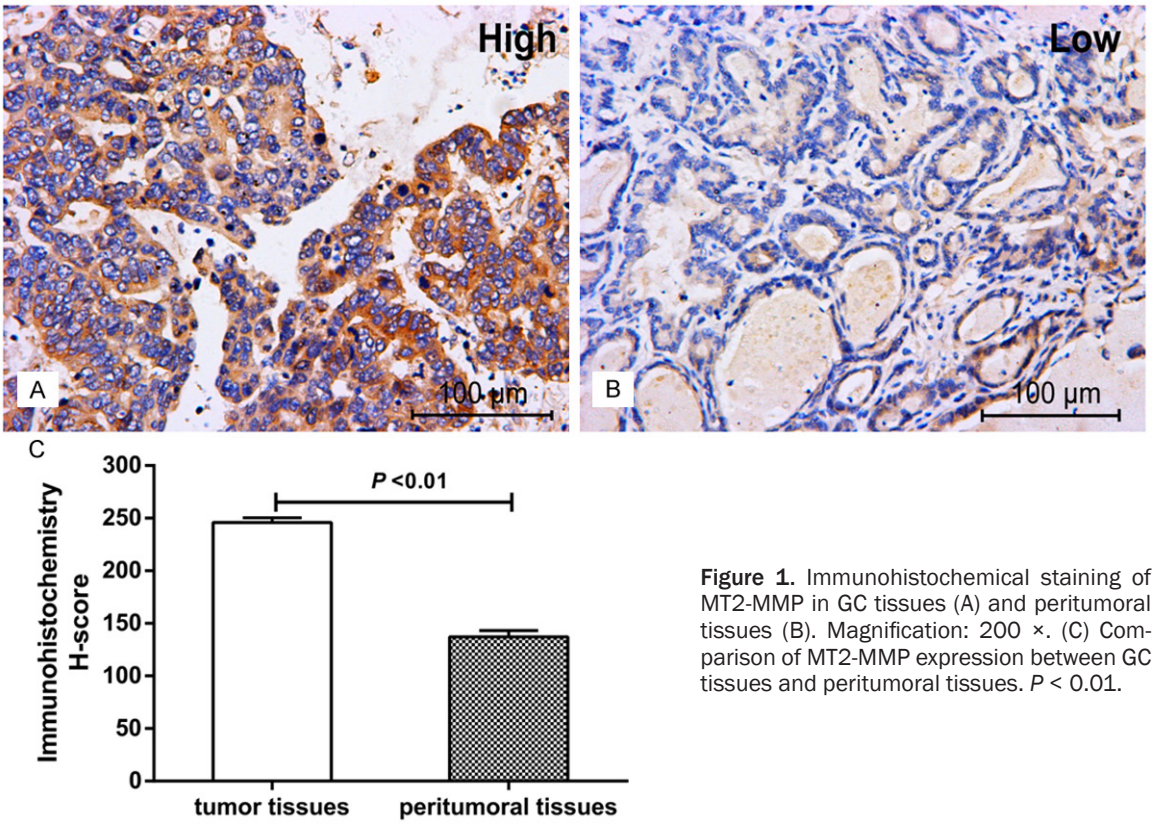

Figure 1. Immunohistochemical staining of MT2-MMP in GC tissues (A) and peritumoral tissues (B). Magnification: 200 ×. (C) Comparison of MT2-MMP expression between GC tissues and peritumoral tissues. $P < 0.01$.

included paired tumor and peritumoral tissues from 90 GC patients. All patients underwent surgical stomach resection between July 2006 and April 2007. Patients ranged in age from 34 to 83 years old (median age was 65.7 years). They were followed up for 6.3 to 7.1 years. None of these patients received pre-operative chemotherapy or radiotherapy. All cases were confirmed as gastric cancer by pathologists. Tumor-node-metastasis (TNM) stages were classified according to the American Joint Committee on Cancer Criteria [9].

Immunohistochemistry

Immunohistochemical staining was performed using the Envision™ method. TMA section was dewaxed in xylene and rehydrated in graded ethanol solutions. Antigen retrieval was performed at 100°C for 30 min in citrate solution (10 mmol/L, pH 6.0). TMA section was treated with 0.3% H_2O_2 solution for 15 min to block endogenous peroxidase activity, followed by overnight incubation with the polyclonal rabbit antibody against human MT2-MMP (1:500 dilution, RD Systems, Minneapolis, MN) in a humidified chamber at 4°C. After incubation for 30 min with the secondary antibody (Maxim Biotechnology Company, Fuzhou, China) at room temperature, TMA section was stained with DAB solution (Maxim Biotechnology Company) and counterstained with hematoxylin, and differentiated with 0.1% hydrochloric acid alcohol. Finally, the section was dehydrated, cleared and mounted. Negative control was treated similarly but without primary antibody. The section was observed under light microscope (Leica DM2500, Wetzlar, Germany) and some representative images were captured.

Evaluation of MT2-MMP expression

The staining of MT2-MMP in GC TMA section was assessed according to the H-score method as described by Hammes *et al.* [10] where H-score = (% tumor cells unstained × 0) + (% tumor cells stained weak × 1) + (% tumor cells stained moderate × 2) + (% tumor cells stained strong × 3). H-scores ranged from 0 (no staining) to 300 (highest staining).

Statistical analysis

Statistical analysis were performed using SPSS19.0 software to evaluate statistical dif-

Table 1. Correlation between MT2-MMP expression and patient clinicopathological parameters

Clinicopathological parameters	Case	MT2-MMP expression		*P* value
		Low, n (%)	High, n (%)	
Sex				
Male	24	9	15	0.682
Female	59	25	34	
Age (years)				
≤ 60	31	14	17	0.548
> 60	52	20	32	
Differentiation				
Well, moderate	14	6	8	0.874
Poor	69	28	41	
Tumor size (cm)				
≤ 5	46	19	27	0.944
> 5	37	15	22	
Depth of tumor invasion				
T1, 2	10	6	4	0.304
T3, 4	73	28	45	
Lymph node metastasis				
Negative	20	11	9	0.143
Positive	63	23	40	
TNM stage				
I-II	34	16	18	0.347
III-IV	49	18	31	

ferences. Chi-square tests were used to analyze the relationship between MT2-MMP expression and clinicopathological parameters. Overall survival (OS) of patients with different clinicopathological parameters was compared using Kaplan-Meier and log-rank tests. The Cox proportional hazards model was performed to estimate hazard risk (HR) with 95% confidence interval (CI) among different clinicopathological parameters, MT2-MMP expression, and death risks. A *P*-value of < 0.05 was considered statistically significant. All statistical tests were two-tailed.

Results

MT2-MMP expression levels in tumor and peritumoral tissues

As shown in **Figure 1**, MT2-MMP staining was predominantly observed on the membrane and in the cytoplasm of tumor cells (**Figure 1A**), while no or weak staining was observed in the peritumoral tissues (**Figure 1B**). The intensity of MT2-MMP staining in the tumor and peritumoral tissues was quantitated and compared, the expression level of MT2-MMP in the tumor tissues was significantly increased compared with peritumoral tissues ($P < 0.01$; **Figure 1C**).

Relationship of MT2-MMP expression and clinicopathological parameters

To determine the clinical relevance of MT2-MMP expression in GC tissues, we sub-grouped all patients into two groups according the staining intensity: H-score ≤ 245 (34 cases) and H-score > 245 (49 cases) because 7 paired tumor and peritumoral tissues were lost during the staining procedure. We evaluated the relationship of MT2-MMP expression and clinicopathological parameters, including sex, age, differentiation, tumor size, depth of tumor invasion, lymph node metastasis, and TNM stage. As shown in **Table 1**, we did not observe any significant correlation between MT2-MMP expression and clinicopathological parameters ($P > 0.05$).

Prognostic value of MT2-MMP expression in GC patients

Cox regression univariate analysis showed that late TNM stage and high MT2-MMP expression were negative prognostic factors for OS ($P = 0.006$, $P = 0.013$, respectively; **Figure 2**). However, other factors such as age, gender, and differentiation had no effect on patient survival ($P > 0.05$; **Table 2**). In addition, Cox regression multivariate analysis confirmed that TNM stage and MT2-MMP expression were independent prognostic factors [hazard ratio (HR) = 2.491, 95% CI = 1.305-4.755, $P = 0.006$ for TNM stage; (HR) = 1.888, 95% CI = 1.004-3.552, $P = 0.040$ for MT2-MMP expression, **Table 2**.

Discussion

Cancer invasion and metastasis are multi-factor, multi-step dynamic processes that involve in the degradation of extracellular matrix and basement membrane. MMPs are an important family of proteolytic enzymes that significantly

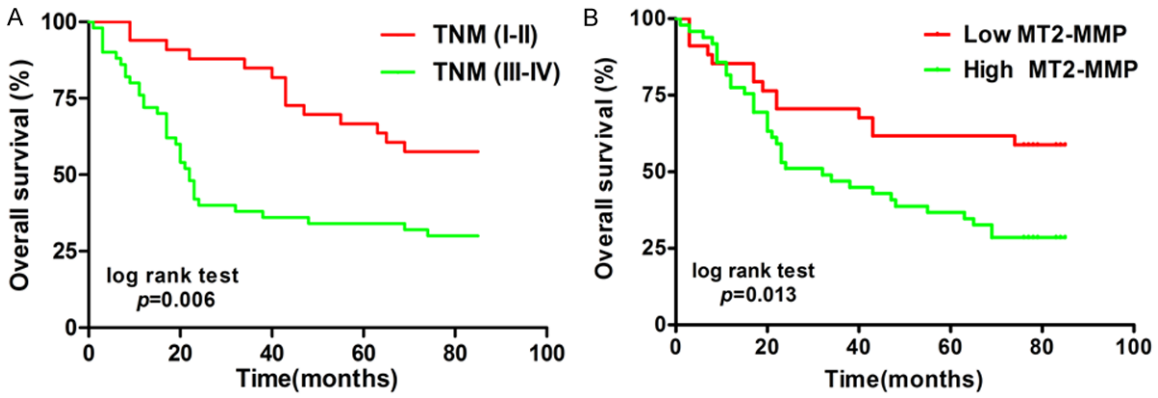

Figure 2. Kaplan-Meier survival curves of GC patients based on TNM stage and MT2-MMP expression. Patients with TNM (III-IV) showed significantly worse survival compared to those patients with TNM (I-II) (P = 0.006, log-rank test) (A). Patients with high MT2-MMP expression showed significantly worse survival compared to those patients with low MT2-MMP expression (P = 0.013, log-rank test) (B).

Table 2. Univariate and multivariate analysis of clinicopathological and molecular features for overall survival

Factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender				
Male *vs*. female	1.104 (0.601-2.027)	0.750	0.878 (0.472-1.635)	0.645
Age				
> 60 years *vs*. ≤ 60 years	1.387 (0.763-2.522)	0.283	1.670 (0.892-3.126)	0.109
Differentiation				
Poor *vs*. Well, moderate	1.684 (0.717-3.959)	0.232	1.379 (0.580-3.280)	0.468
TNM stage				
III-IV *vs*. I-II	2.351 (1.276-4.334)	0.006	2.491 (1.305-4.755)	0.006
MT2-MMP expression				
Higher *vs*. Lower	2.135 (1.145-3.980)	0.013	1.888 (1.004-3.552)	0.040

contribute to tumor microenvironment remodeling and associate with tumorigenesis and metastasis [11]. Various MMP family members correlate with invasion and metastasis, as well as with the poor prognosis of GC. Wang *et al*. reported that interleukin-17A promotes GC invasiveness via NF-kB-mediated MMP-2 and -9 expression [12]. Al-Batran *et al*. reported that MMP-9 is an important predictor of clinical outcomes in the patients with metastatic GC, which is further confirmed by meta-analysis data [13, 14]. MMP-12 and -21 are associated with OS of GC patients. In addition, MMP-14 is a negative prognostic marker for GC patients [15].

In the present study, we found that MT2-MMP expression in tumor tissues was significantly elevated compared to peritumoral tissues, suggesting that MT2-MMP may participate in GC tumorigenesis and progression. However, MT2-MMP expression levels were not significantly correlated to the clinic pathological parameters in GC patients. This may be due to the relatively small sample size. It has been demonstrated that MT2-MMP is highly expressed in many malignant tumors, including breast cancer, colorectal cancer, esophageal cancer and lung cancer [16-19], these evidence further supports that MT2-MMP is associated with cancer progression. Moreover, Kobayashi *et al*. reported that MT2-MMP could be a novel marker for molecular diagnosis and therapy of lung adenocarcinoma [20]. Tao *et al*. reported that MT2-MMP is a direct target of Snai1 during endothelial-to-mesenchymal transition, supporting that MT2-MMP plays an important role in tumor invasion and metastasis [21]. In addition, we

found that increased expression of MT2-MMP was significantly associated with poor OS by univariate and multivariate Cox regression analysis in GC patients. Therefore, our data suggest that MT2-MMP expression can serve as a prognostic marker for GC patients. However, this finding should be verified in a large cohort of GC patients and in prospective randomized clinical studies. Moreover, the underlying mechanisms of increased MT2-MMP expression during GC tumorigenesis and metastasis need further investigation.

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Disclosure of conflict of interest

None.

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