MT1-MMP promotes the proliferation and invasion of gastric carcinoma cells via regulating vimentin and E-cadherin

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Abstract. The present study aimed to explore the possible effects of membrane-type 1 matrix metalloproteinase (MT1-MMP) on gastric carcinoma cells proliferation and invasion. Immunohistochemistry analysis was conducted to measure MT1-MMP expression level in 15 patients with gastric carcinoma. Subsequently, recombinant short hairpin RNA (shRNA) vectors targeting MT1-MMP were constructed to silence the expression of MT1-MMP in gastric carcinoma cells. Then, the inhibitive efficiency was verified via reverse transcription quantitative polymerase chain reaction (RT-qPCR) and western blot analysis. The effects of MT1-MMP silencing on cell proliferation and invasion were detected through Cell Counting Kit-8 test and Transwell assays. The expression levels of vimentin and epithelial cadherin (E-cadherin) were detected by RT-qPCR. The immunohistochemistry analysis results revealed that MT1-MMP expression in gastric carcinoma tissues was markedly overexpressed compared with non-cancerous adjacent tissues. The MT1-MMP expression level in cancer-derived cell line AGS cells was also significantly increased compared with that in non-cancer-derived GES-1 cells. In addition, the MT1-MMP expression level in AGS cells was significantly decreased via shRNA transfection. Cell proliferation and invasion were markedly inhibited following knockdown of MT1-MMP level in AGS cells. These inhibitory effects were associated with the decreased expression of vimentin and increased expression of E-cadherin. MT1-MMP was overexpressed in gastric carcinoma cells, and silencing of MT1-MMP inhibited the proliferation and invasion of cells via regulating the expression of vimentin and E-cadherin.

Introduction

Gastric carcinoma, a major cause of cancer-associated mortality worldwide, remains a concern for clinicians and the scientific community (1). The majority of patients with gastric carcinoma are diagnosed at advanced stages and have poor outcomes, with susceptibility to invasion and metastasis (2,3). Unfortunately, the development of techniques for the treatment and diagnosis of gastric carcinoma in previous years have only made a modest contribution towards improving the prognosis of gastric carcinoma, in particular for patients with advanced-stage disease (4-6). Therefore, the exploration of novel approaches for the early diagnosis and management of gastric carcinoma is urgently required.

The matrix metalloproteinases (MMPs) family was demonstrated to be overexpressed in multiple types of cancer and to serve essential roles in the development and invasion of cancer (7-9). Accumulating evidence has indicated that several MMPs regulate the migration and invasion of cancer cells by promoting epithelial-mesenchymal transition (EMT) of cancer cells, including MMP-3, MMP-8 and MMP-14 (10-12). Membrane-type 1 matrix metalloproteinase (MT1-MMP) was demonstrated to be expressed at abnormally high levels in cancerous tissues from patients with gastric cancer (13). A previous study demonstrated that the overexpression of MT1-MMP was associated with poor prognoses of patients with gastric carcinoma, including increased tumor invasion, metastasis, advanced Tumor-Node-Metastasis stages and worse overall survival (14). Inhibition of MT1-MMP expression may inhibit cell migration, invasion, proliferation and angiogenesis (13,15). However, the specific mechanisms of MT1-MMP in gastric carcinoma remain unclear.

In the present study, the expression of MT1-MMP was determined in gastric carcinoma clinical samples. In addition, MT1-MMP expression was suppressed using a short hairpin RNA (shRNA) technique followed by proliferation and invasion assays. Using these analyses, the present study aimed to provide useful new information concerning the underlying mechanisms of gastric carcinoma.

Materials and methods

Tissue samples. Clinic tissue samples were obtained from 15 patients who underwent gastric carcinoma resection at
the First Affiliated Hospital of Suzhou University (Suzhou, China). Concomitantly, normal gastric tissue samples were collected to serve as controls, which were sourced from a site distant the cancerous lesion (≥5 cm) and blindly confirmed by two experienced pathologists. Among the 15 patients, 5 were females and 10 were males. The age of patients ranged from 49-81, and the average age was 61.267 years old. According to the Goseki classification (16), a total of 5 tumors were classed as moderately differentiated adenocarcinomas, 2 were moderately-poorly differentiated, and 8 were poorly differentiated. The present study was approved by the Research Ethics Committee of the First Affiliated Hospital of Suzhou University and was performed according to ethical standards of the Declaration of Helsinki. All participants provided written consent for their clinical information to be used for scientific research.

**Immunohistochemistry and hematoxylin-eosin staining (H&E).** Immunohistochemical staining and H&E staining were performed on 4% paraformaldehyde-fixed (for 24 h at room temperature), paraffin-embedded tissue sections. For immunohistochemistry, the sections were blocked with 10% goat serum (OriGene Technologies, Inc., Beijing, China) at 37°C for 1 h. MT1-MMP expression was detected with anti-MT1-MMP antibody (cat. no. ab51074; Abcam, Cambridge, UK; dilution, 1:100), followed by horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) (cat. no. 111-035-045; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) antibody at a dilution of 1:400. Assessment of immunohistochemical staining was performed as described previously by Pang et al (17) and Di Martino et al (18). For H&E staining, sections were stained with hematoxylin solution (0.2%) for 4 min, followed by eosin solution (0.5%) for 90 sec at room temperature.

**Cell culture.** The human gastric cancer AGS cell line and normal gastric epithelial GES-1 cell line were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). GES-1 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 1% streptomycin and 1% penicillin (Gibco; Thermo Fisher Scientific, Inc.). AGS cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, 1% streptomycin and 1% penicillin. All cells were maintained in a CO₂ incubator (Thermo Fisher Scientific, Inc.) with 5% CO₂ at 37°C.

**Construction of shRNA vector and cell transfection.** A total of 4 shRNA sequences against MT1-MMP were designed, synthesized and inserted (50 ng) into pLKO.1-puro vector (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) through Age I (ACCGGT) and Eco RI (GAATTC) restriction enzyme cutting sites. The sequences of the 4 oligonucleotides are summarized in Table I. A scrambled shRNA negative control (NC) sequence (shRNA-NC; Sangon Biotech Co., Ltd., Shanghai, China) was generated through complementary pairs of primers: shNC-forward, 5'-CCGGGTTTCTCCGACGGTG TCACGTCAAGAGATTACGTGACAGCGTTCGAGAATT-3' and shNC-reverse, 3'-CAAGAGGGCTTTC ACAGTGCGTCTCTAATGCACTGTGCAAGCCTTT-5' and used as the negative control. Different shMT1-MMP (3 µg) and negative control shRNA vectors (3 µg) were transduced into AGS cells by lentivirus. Briefly, the recombinant plasmids were transfected into 293T cells by lentiviruses using a Lipofectamine 2000 transfection kit (Invitrogen; Thermo Fisher Scientific, Inc.). Then, 293T cells were cultured in DMEM (Sigma-Aldrich; Merck KGaA) with 10% FBS for 24 h. Following replication, the viruses were harvested for the infection of the AGS cells. Subsequent experiments were then performed after 48 h transfection.

**Reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay.** Total RNA was isolated from GES-1 and AGS cells using the total RNA extraction reagent RNAiso Plus (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. cDNA was generated from RNA using PrimeScript RT Master Mix (Takara Biotechnology Co., Ltd.). RT-qPCR was performed with SYBR Premix EX Taq (Takara Biotechnology Co., Ltd.) as previously described (13). Briefly, reactions were performed with the following components: 5 µl 2X SYBR Premix EX Taq, 3.4 µl cDNA and 10 µM primers, in a final volume of 10 µl. β-actin was used as the control. The PCR thermocycler conditions were as follows: 50°C for 3 min and 95°C for 3 min, followed by 30 cycles of 95°C for 10 sec and 60°C for 30 sec, and finally 72°C for 5 min. The relative quantities of mRNAs were estimated using the 2-ΔΔCT method (19). The gene primers used are summarized in Table II.

**Western blot analysis.** GES-1 and AGS cells were lysed with radioimmunoprecipitation assay lysis buffer III (Sangon Biotech Co., Ltd., Shanghai, China), and concentration was quantified using a bicinchoninic acid protein assay kit (Pierce; Thermo Fisher Scientific, Inc.). A total of 20 µg protein was loaded in each lane of a 10% SDS-PAGE gel and separated by electrophoresis. Then, the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane followed by blocking with 5% skim milk for 1 h at room temperature. Following washing with PBS + 0.1% Tween-20 (PBST) buffer for 5 min, PVDF membranes were incubated with primary antibodies against MT1-MMP (dilution, 1:200; cat. no. ab51074; Abcam) and β-actin (dilution, 1:1,000; cat. no. 4967; Cell Signaling Technology, Inc., Danvers, MA, USA) at 4°C overnight. Then, membranes were incubated for 2 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) (cat. no. ab0101; ProteinTech Group, Inc., Chicago, IL, USA; dilution, 1:1,000) secondary antibodies. Subsequent to washing 3 times with PBST, proteins were detected by chemiluminescence (ECL; EMD Millipore, Billerica, MA, USA), and the expression was quantified by Image Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

**Proliferation analysis.** A Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Shanghai, China) was used to evaluate the proliferation ability and viability of AGS cells. The transfected AGS cells were resuspended, and 100 µl AGS cells were seeded in 96-well plates (2x10⁴ cells/well).
AGS cells were cultivated for 24, 48, 72 and 96 h, then 10 µl CCK-8 solution was added at each time point and cultivated for 2 h at 37˚C. The optical density values were evaluated at 450 nm by microplate reader (Epoch; BioTek Instruments, Inc., Winooski, VT, USA). All experiments were performed in quintuplicate.

**Transwell analysis.** Matrigel® (Corning Incorporated, Corning, NY, USA) was diluted 1:6 by serum-free culture media, added to the upper Transwell chamber and incubated for 1 h at 37°C prior to use. The transfected AGS cells were cultivated for 12 h and then the culture media was refreshed. After 48 h incubation, the cells were resuspended in serum-free media, counted and seeded into the upper chamber with 100 µl cell suspension for incubation. The bottom chamber was loaded with 500 µl DMEM with 20% FBS. After 24 h incubation at 37°C, media for AGS cells in the upper chamber was removed and washed twice by PBS. The cells were fixed in 4% formaldehyde at room temperature for 15 min, washed and stained by 0.01% crystal violet at room temperature for 20 min. Cells on the upper surface were removed by cotton swabs and the invasive cells were counted under an inverted microscope (IX73; Olympus Corporation, Tokyo, Japan) at x200 magnification.

**Statistical analysis.** SPSS statistical software 19.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Measurement data were presented as mean ± standard deviation. An unpaired Student's t-test was used to analyze the statistical significance between two groups. One- and two-way analysis of variance were used to compare data between three or more groups, followed by Bonferroni's post-hoc test. Enumeration data were presented as percentage, and analyzed by Chi-square test.
decreased the proliferation rate of AGS cells compared to the blank-transfected cells at 72 h (P<0.001) and 96 h (P<0.01; Fig. 5). These results suggested that MT1-MMP may promote the proliferation of gastric carcinoma cell.

**Inhibition of MT1-MMP resulted in reduced invasion ability.**

To assess the effect of MT1-MMP on gastric carcinoma cells, the invasion abilities of AGS cells were assessed using a Transwell invasion assay following silencing of MT1-MMP. As indicated in Fig. 6, the invasion ability of AGS cells was significantly inhibited following transfection with shRNA-2 (P<0.001). The results suggested that inhibition of MT1-MMP may suppress the invasion of cancer cells.

**Suppression of genes associated with invasion.**

The mRNA expression levels of EMT-associated genes, including vimentin and epithelial cadherin (E-cadherin), were examined by RT-qPCR. Following transfection of the AGS cells with shRNA-2, the mRNA expression level of vimentin (0.396±0.009) was significantly inhibited compared with the NC group (0.661±0.040; Fig. 7A; P<0.001). Conversely, the mRNA expression level of E-cadherin (0.774±0.038) was significantly increased following the transfection of AGS cells with shRNA-2 compared with the NC group (0.412±0.012; Fig. 7B) (P<0.001). In addition, it was observed that the mRNA levels of vimentin and E-cadherin were significantly decreased in the NC (vimentin and E-cadherin, P<0.001) and shRNA-2 group (vimentin, P<0.001; E-cadherin, P<0.01) compared with the blank group, which may due to a general cell response to transfection reagent toxicity. These results suggested that the suppression of MT1-MMP expression may decrease the invasion ability of gastric carcinoma cells.

**Discussion**

Gastric carcinoma, the second most common cause of tumor-associated mortality worldwide, contributes greatly to the global disease burden (20). Previously, with advances in surgical intervention and chemotherapy, the overall survival rate has been greatly improved. However, the 5-year mortality rate for advanced gastric carcinoma remains as high as 30-50% (21). Therefore, extensive investigations are required for elucidating crucial molecules that participate in the pathogenesis of gastric carcinoma.

MT1-MMP, also known as MMP14, belongs to the MMPs family, which is correlated with invasion and metastasis of cancer cell (22). At present, MT1-MMP has been identified to be overexpressed in a variety of cancer tissues, including...
A colorectal and breast cancer (23,24). A previous study demonstrated that MT1-MMP may promote breast tumor growth and angiogenesis through increasing the expression of vascular endothelial growth factor (VEGF) (25). Besides, the overexpression of MT1-MMP resulted in increased migration ability of esophageal squamous cell carcinomas (22). Corresponding results were also identified in the present study. In the present study, the tumor cell proliferation rate and invasion abilities were decreased by shRNA targeting MT1-MMP.

A previous study revealed that MT1-MMP was overexpressed in gastric carcinoma compared with that in adjacent tissues (26). The results from the present study also indicated that MT1-MMP was overexpressed in gastric carcinoma tissues through immunohistochemistry analysis. The overexpression of MT1-MMP was correlated with invasive lesions (22). Therefore, high expression levels of MT1-MMP may be associated with the invasion of gastric carcinoma. Furthermore, compared with the non-cancer-derived GES-1 cells, MT1-MMP was overexpressed in the cancer-derived cell line AGS cells. Subsequently, the present study screened an effective shRNA vector (shRNA-2) targeting MT1-MMP. Following transfection of the AGS cells with shRNA-2, the expression of MT1-MMP was markedly suppressed at mRNA and protein levels. Additionally, it was observed that inhibiting the expression of MT1-MMP was able to significantly decrease the proliferation rate and invasion ability of AGS cells. MT1-MMP is a critical protease that participates in the progress of cancer cell proliferation, migration and invasion (27). Tomari et al (28) revealed that the growth, invasion and metastasis of tumors was promoted by increasing MT1-MMP expression in tumor cells. Concomitantly, Pahwa et al (29) demonstrated that MT1-MMP was a crucial player in the growth and progression of melanoma. Therefore, these results indicated that MT1-MMP may promote gastric carcinoma cells growth and metastasis during the development of cancer.

In addition, the expression level of EMT-associated genes was examined, including vimentin and E-cadherin, to investigate the underlying mechanism of MT1-MMP in the progression of gastric carcinoma. Pang et al (22) suggested that MT1-MMP prompted esophageal squamous cell carcinoma invasion and metastasis by suppressing E-cadherin and subsequently inducing EMT. At present, a number of studies have demonstrated that EMT was associated with different types of tumors, including gastric, esophageal and hepatocellular carcinoma (22,30,31). Additionally, Sakamoto and Seiki (27) revealed that MT1-MMP was involved in the EMT progress of tumor development, by increasing the expression levels of hypoxia-inducible factors (32) and regulating the expression of

![Figure 1. Hematoxylin-eosin staining. Staining of a (A) moderately differentiated gastric carcinoma tissue sample and (B) non-cancerous adjacent tissue sample. Magnification, x200. Scale bar, 100 µm.](image1)

![Figure 2. Expression of MT1-MMP in gastric carcinoma and non-cancerous adjacent tissues samples. (A) Intense staining was observed in gastric carcinoma tissues. This image is representative of a sample with moderate differentiation. Magnification, x400. (B) MT1-MMP protein expression was not detected in non-cancerous adjacent tissues. Magnification, x400. Scale bar, 50 µm. MT1-MMP, membrane type 1 matrix metalloproteinase.](image2)
epithelial cell surface markers (22). The results of the present study suggested that the vimentin mRNA level was markedly decreased and the E-cadherin mRNA level was markedly increased following silencing of MT1-MMP. Concomitantly, differences in vimentin and E-cadherin expression between untreated AGS cells and empty pLKO.1-puro vector-treated AGS cells were observed in the present study, which may be due to a general cell response to transfection reagent toxicity, and require additional investigation. Taken together, we hypothesized that MT1-MMP was likely to mediate the invasion process via EMT, reflected by the altered expression of vimentin and E-cadherin. In summary, these results suggested that MT1-MMP may contribute to gastric carcinoma cell proliferation and invasion via regulating vimentin and E-cadherin expression. Future studies will investigate the molecular mechanisms underlying the effects of MT1-MMP on gastric cancer cell growth and invasion.

In conclusion, the present study confirmed that MT1-MMP was overexpressed in gastric carcinoma cells compared with non-cancerous adjacent tissues. Recombinant shRNA vectors targeting MT1-MMP successfully inhibited MT1-MMP expression in gastric carcinoma cells. In particular, silencing

Figure 3. Verification and selection of recombinant shRNA. The interference effects of 4 shRNAs were evaluated. (A) The effects of four shRNAs on the mRNA expression of MT1-MMP. (B) Quantitative data from western blot analysis. (C) Representative western blot analysis data of MT1-MMP expression in AGS cells transfected with shRNA-2. ***P<0.001 vs. blank group; ###P<0.001 vs. NC group (n=3). shRNA, short hairpin RNA; MT1-MMP, membrane type 1 matrix metalloproteinase; NC, negative control.

Figure 4. MT1-MMP expression levels in GES-1 and AGS cell lines. The expression level of MT1-MMP in GES-1 and AGS cells was evaluated. (A) Relative mRNA expression level of MT1-MMP in GES-1 and AGS cells. (B) Relative MT1-MMP protein expression level in GES-1 and AGS cells. (C) Representative western blot analysis of the protein expression of MT1-MMP in GES-1 and AGS cells. ***P<0.001 vs. GES-1 cells (n=3). MT1-MMP, membrane type 1 matrix metalloproteinase.

Figure 5. Cell Counting Kit-8 proliferation kit assay results of AGS cells transfected with shRNA-2. AGS were transfected with NC vectors (NC group) and shRNA-2. The proliferation rate of AGS was suppressed following transfection with shRNA-2. **P<0.01 and ***P<0.001 vs. blank group. #P<0.05 and ###P<0.001 vs. NC group (n=5). shRNA, short hairpin RNA; MT1-MMP, membrane type 1 matrix metalloproteinase; NC, negative control.
of MT1-MMP may inhibit cell proliferation and invasion via regulating the expression of EMT-associated genes, including vimentin and E-cadherin. In conclusion, the present study revealed that MT1-MMP may promote the proliferation and invasion of gastric carcinoma cells by regulating vimentin and E-cadherin expression.

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University and was performed according to ethical standards of the Declaration of Helsinki. All participants provided written consent for their clinical information to be used for scientific research.

Patient consent for publication

All participants provided written consent for their clinical information to be used for scientific research.

Competing interests

The authors declare that they have no competing interests.

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