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Tumor Discovery

ORIGINAL RESEARCH ARTICLE

Matrix metalloproteinase-1 as a potential biomarker for early gastric cancer detection and its effect on gastric cancer cell proliferation and migration

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Abstract

The present study aimed to investigate the association between matrix metalloproteinase-1 (MMP-1) and early gastric cancer (EGC), while also evaluating the effect of MMP-1 on gastric cancer cell proliferation and migration. Transcriptome RNA sequencing and database analysis were conducted to assess the relationship between MMP-1 expression and EGC. Differences in MMP-1 expression between clinical EGC samples and paracancerous tissues were detected using fluorescence quantitative polymerase chain reaction (PCR). In N87 gastric cancer cells, changes in proliferation- and migration-related indicator expression were determined. Gene sequencing revealed differential expression of MMP-1 in early and advanced gastric cancers. Furthermore, enhanced MMP-1 expression was observed in early and advanced gastric cancer tissues, exhibiting a positive correlation with the malignant phenotype in gastric cancer cell lines. Fluorescence quantitative PCR revealed considerably higher MMP-1 expression in EGC tissues than in paracancerous tissues. CCK8 and EdU assays demonstrated a significant increase in N87 cell proliferation on MMP-1 upregulation and a decrease on its downregulation. The scratch assay results demonstrated a corresponding enhancement in N87 cell migratory capacity with MMP-1 upregulation, which was attenuated on its downregulation. Western blot experiments revealed a decrease in the expression of the epithelial-mesenchymal transition-related protein E-cadherin after MMP-1 upregulation, while vimentin expression significantly increased. Conversely, the downregulation of MMP-1 led to opposite outcomes. Overall, MMP-1 emerges as a potential biomarker for EGC diagnosis and plays a crucial role in the regulation of N87 gastric cancer cell proliferation and migration.

Keywords: Proliferation; Gastric cancer; MMP-1; Early diagnosis; Migration

1. Introduction

Gastric cancer is among the most prevalent cancers worldwide, ranking as the third most common cancer after breast and lung cancers, and represents the second leading cause of

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cancer-related deaths in Asia.[1] Gastric cancer encompasses two main classifications: early and advanced stages. In early gastric cancer (EGC), lesions are confined to the mucosal and submucosal layers of the gastric wall. EGC patients typically exhibit a 5-year survival rate exceeding 90%, whereas, for advanced gastric cancer patients, this rate drops below 30%. The incidence rate of gastric cancer increases progressively with age, with a median age of 70 years at diagnosis, and the prognosis is usually poor. Approximately 10% of gastric cancers are detected in patients aged 45 years or younger. Therefore, early diagnosis of gastric cancer plays a vital role in improving patient prognosis and reducing mortality, which are keys to improving survival rates.[2,3]

Matrix metalloproteinases (MMPs), produced by both tumor cells and normal cells, are also known as interstitial collagenases. Among the MMPs, matrix metalloproteinase-1 (MMP-1) is one of the most commonly expressed types. The overexpression of MMP-1 is correlated with metastasis, inflammation, and tumor invasion,[4,5] highlighting its significant role in the pathogenesis of gastric cancer.[6,7] A comprehensive study involving 1,377 cases and 1,543 controls showed that the MMP1-1607 1G > 2G polymorphism was associated with a significantly elevated risk of gastric cancer.[8] Furthermore, in tumors, MMP-1 overexpression has been reported to be correlated with increased invasive and migratory capacities of hepatocellular carcinoma cells.[9]

In the present study, gene sequencing revealed that the enhanced expression of MMP-1 is correlated with the malignant properties of gastric cells in both early and advanced gastric cancer. Furthermore, an analysis of 20 pairs of EGC and paracancerous tissue samples demonstrated that elevated MMP-1 expression levels exhibited high sensitivity and specificity in identifying EGC. Therefore, MMP-1 holds promise as a potential diagnostic marker for EGC and plays a regulatory role in gastric cancer cells.

2. Materials and methods

2.1. Materials

The NCI-N87 human gastric cancer cell line utilized in the present research was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences, China. The human-derived MMP-1 gene shRNA downregulation vector, overexpression vector, and pLent-U6-GFP-Puro negative control plasmid with a nonsense sequence were constructed by Shandong WZ Biosciences Inc., China. The specific shRNA sequences used were as follows: sh1: 5'-GCTAGCTCAGGATGACATTGATT C A A G A G A T C A A T G T C A T C C T G A G C T A G CTTTTTT-3' and sh2:5'-GCGTGTG ACAGTAAGCTA A C C T T C A A G A G A G G T T A G C T T A C T G T C A CACGCTTTTTT-3'. Other reagents employed in this study included: 1640 medium (Hyclone, USA); fetal bovine serum (BI, Zhejiang Sorfa Life Science Research Co., Ltd., China); Lipofectamine 3000 (Thermo Fisher Scientific, USA); Penicillin-Streptomycin solution (Shanghai Beyotime Biotechnology Co., Ltd., China); trypsin ((Shanghai Beyotime Biotechnology Co., Ltd., China); SDS-PAGE assay kit (Shanghai Beyotime Biotechnology Co., Ltd., China); ECL assay kit ((Shanghai Beyotime Biotechnology Co., Ltd., China); RIPA lysis buffer (Shanghai Beyotime Biotechnology Co., Ltd., China); BCA assay kit (Shanghai Beyotime Biotechnology Co., Ltd., China); CCK8 assay kit (MCE, China); and an EDU assay kit (Guangzhou RiboBio Co., Ltd., China).

2.2. Cell culture, cell transfection, and grouping

The 1640 culture medium, consisting of 10% fetal bovine serum and 1% double antibodies, was used to culture NCI-N87 human gastric cancer cells (hereafter referred to as N87) under culture conditions of 5% CO_2 at 37°C. During the logarithmic growth phase, good-condition N87 cells were cultured into 6-well plates and transfected when they reached an approximately 70% growth rate. The MMP-1-overexpression plasmid (Ad-MMP-1), two RNA-interference plasmids, and corresponding controls were transfected using Lipofectamine 3000, and the cells were further cultured.

2.3. Cell proliferation

Transfected N87 cells were inoculated into 96-well plates at a density of 5000 cells/well, with each well containing 100 μL of medium. Six replicate wells were prepared for each experiment to ensure experimental accuracy.

2.3.1. CCK8 assays

After incubation for 24, 48, and 72 h, 10 μL of CCK8 reagent was added to each well and incubated for an hour. The optical density values were measured at 450 nm using a multifunction microplate reader, and the data were subsequently analyzed.

2.3.2. EdU assays

After 24 h of incubation, the cells were treated with the EdU reagent and incubated for an additional 2 h for labeling. Subsequently, the cells were fixed with 4% paraformaldehyde and treated with 0.5% Triton X-100, followed by Apollo dye for 30 min. Cells were washed three times with PBS and then stained with Hoechst nuclear dye at a ratio of 1:100 for 10 min. After washing, cells were imaged and counted for further analysis.

2.4. Cell migration

During the logarithmic growth phase, N87 cells were inoculated into a 6-well plate. When the cell growth rate reached 80%, Ad-MMP-1, the two interference plasmids and the corresponding controls were transfected with Lipofectamine 3000 at specific concentrations. Subsequently, a 200-μL pipette tip was used to make scratches, and the medium was replaced with a new medium containing 1% serum. Images were captured to document the initial (0-h) scratch data. Following incubation under low-serum culture conditions for 48 h, the scratch was imaged to record the 48-h data. The changes in the scratch-wound area and the differences between the groups were calculated and compared using the ImageJ software.

2.5. Western blotting

Total protein was extracted from transfected N87 cells using RIPA lysis buffer, and their concentrations were measured using a BCA kit. Then, the cells were mixed with SDS buffer for 5 min at 100°C to prepare the samples. After SDS-PAGE electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes using electrophoresis equipment. Immunoreactivity was measured after blocking with 5% skim milk. Images were captured using a chemiluminescence imaging system, and any differences observed were analyzed.

2.6. Statistical methods

GraphPad 9.0 software was utilized to conduct all statistical analyses of the data. The mean values of the two groups were compared using an independent-sample *t*-test. A $P < 0.05$ indicated a significant difference.

3. Results

3.1. MMP-1 as the potential diagnostic marker for EGC

After whole-transcriptome RNA sequencing and subsequent bioinformatics analysis, three pairs of early and advanced gastric cancer cells, along with their paracancerous tissues, were derived using clinical endoscopy. Pathological examination confirmed the samples, which were then subjected to analysis. Nearly 1,831 differentially expressed coding genes were present in advanced gastric cancer, whereas there were only 121 differentially expressed coding genes in EGC (Figure 1A). A heat map revealed 44 differentially expressed genes in both early and advanced gastric cancer, of which 17 were upregulated, including *MMP-1* (Figure 1B). The membrane surface protein molecule, MMP-1, was initially screened due to its higher expression levels in both early and advanced gastric cancer tissues. Its expression level was correlated with the malignant phenotype of gastric cancer cell lines (Figure 1). A search of The Cancer Genome Altlas database revealed the presence of enhanced MMP-1 expression or mutations in various malignancies. Based on 170 gastric cancer-related studies involving 4067 patients, MMP-1 polymorphisms were found to be associated with gastric cancer. Previous studies have linked elevated MMP-1 expression levels with patient prognosis across different malignancies (Figure 2A). In the present study, fluorescence quantitative polymerase chain reaction (PCR) assays were performed on collected EGC and control tissue samples. MMP-1 expression was significantly higher in EGC tissues than in their paracancerous counterparts (Figure 2B). Further analysis indicated that high MMP-1 expression has a sensitivity and specificity of 0.67 and 0.8, respectively, for detecting EGC, suggesting its potential as a biomarker for EGC diagnosis (Figure 2C).

3.2. Effect of MMP-1 on gastric cancer cell proliferation

The upregulation of MMP1 in N87 cells was verified by western blotting (Figure 3A). EdU experiments were performed on N87 cells transfected with Ad-MMP-1 and the respective control groups. The ratio of EdU-positive cells was significantly higher in the MMP-1 overexpression group than in the control group (Figure 3B), indicating a significant improvement in the proliferative ability of N87 cells by MMP-1 overexpression. In addition, MMP1 downregulation was verified by western blot analysis (Figure 4A). Simultaneously, N87 cells with MMP-1 knockdown were examined using EdU experiments, revealing a decreased proportion of EdU-positive cells and a significantly diminished proliferative ability, particularly evident in the sh2 group ($P < 0.05$; Figure 4B).

3.3. Effect of MMP-1 on gastric cancer cell migration

Results of the scratch experiment analysis revealed that the scratch wound area of N87 cells with upregulated MMP-1 expression was reduced by 62.3% compared to the initial scratch area at 48 h. This represented a significant increase compared to that in the control group; specifically, a 44% reduction in the scratch wound area was observed with a *P*-value of 0.0047 (Figure 5A). In contrast, the scratch wound areas in the sh1 and sh2 (RNA interference) groups were reduced by 46.8% and 48.6%, respectively. This represented a significant decrease compared with the percentage reduction in the corresponding control group (60.6%). These differences were statistically significant, with *P*-values of 0.0009 and 0.004, respectively (Figure 5B), suggesting that MMP-1 promoted cell migration.

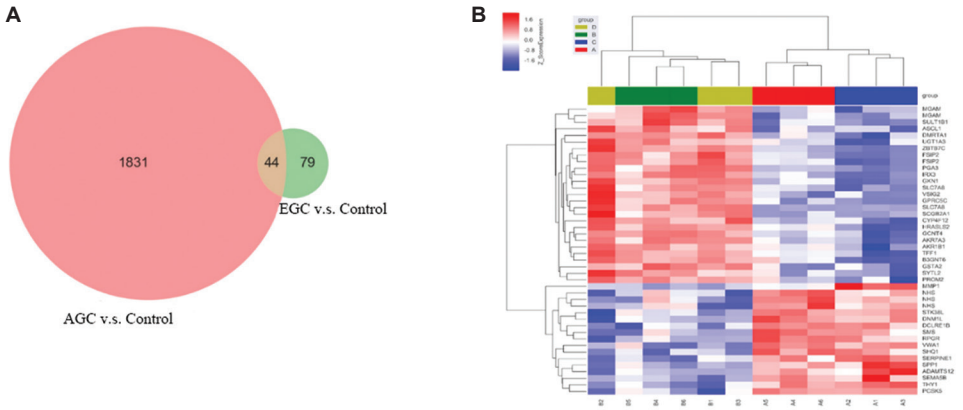


Figure 1. Results related to whole transcriptome sequencing of early gastric cancer/paracancerous tissues and advanced gastric cancer/paracancerous tissues: (A) Differentially expressed coding genes in early gastric cancer (EGC) and advanced gastric cancer (AGC); (B) Heatmap of 44 differentially expressed genes shared by both EGC and AGC.

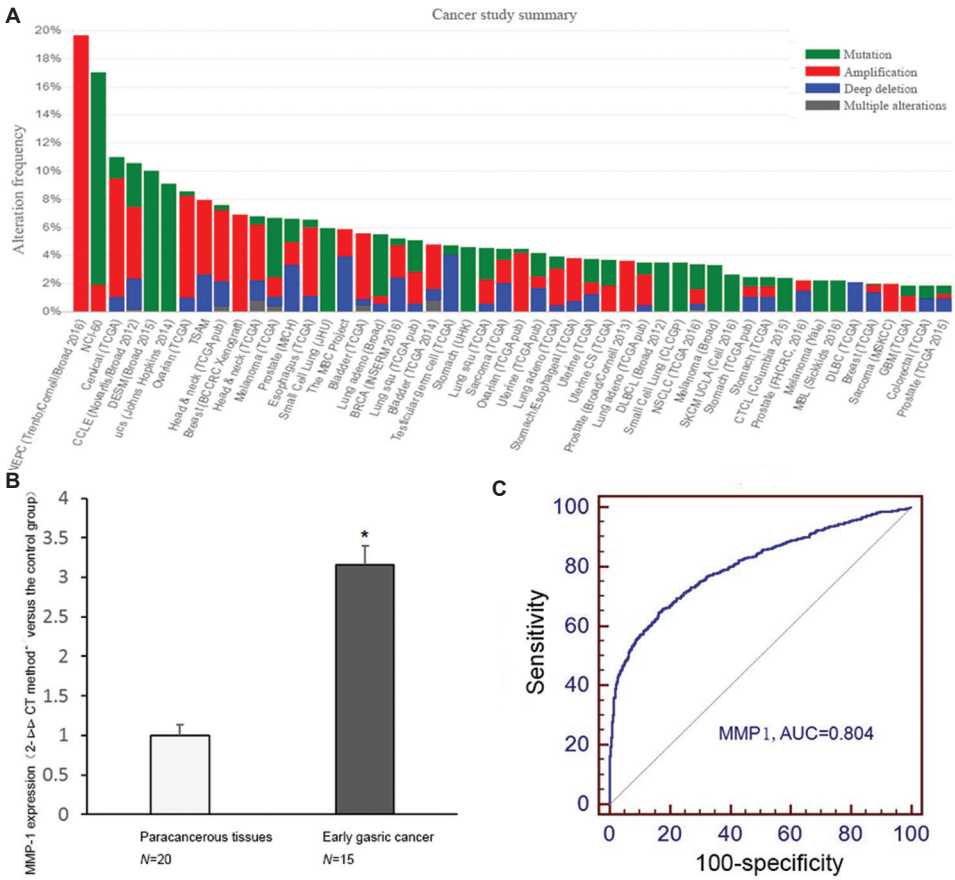


Figure 2. MMP-1 as a potential diagnostic marker for early gastric cancer: (A) A search of TCGA database revealed that high expression of or mutations in MMP-1 was present in a variety of malignancies, and MMP-1 polymorphisms were found to be associated with gastric cancer in gastric cancer-related studies; (B) MMP-1 expression in early cancer and paracancerous tissues was detected by fluorescence quantitative PCR. *$P < 0.05$; (C) Receiver operating characteristic curve (ROC) analysis was performed using SPSS software.
Abbreviations: AUC: Area under the curve; PCR: Polymerase chain reaction; TGCA: The Cancer Genome Atlas; MMP-1: Matrix metalloproteinase-1.

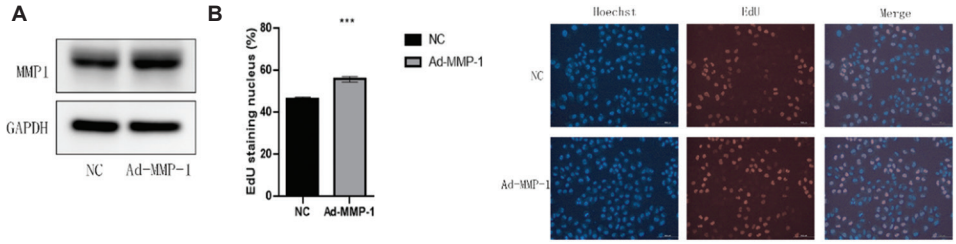


Figure 3. Effect of MMP-1 overexpression on the proliferative ability of gastric cancer cells: (A) Western blot validation of the upregulation of MMP-1 expression; (B) Percentage of EdU-positive cells.
Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$
Abbreviations: Ad-MMP-1: MMP-1-overexpression plasmid; NC: Negative control; MMP-1: Matrix metalloproteinase-1.

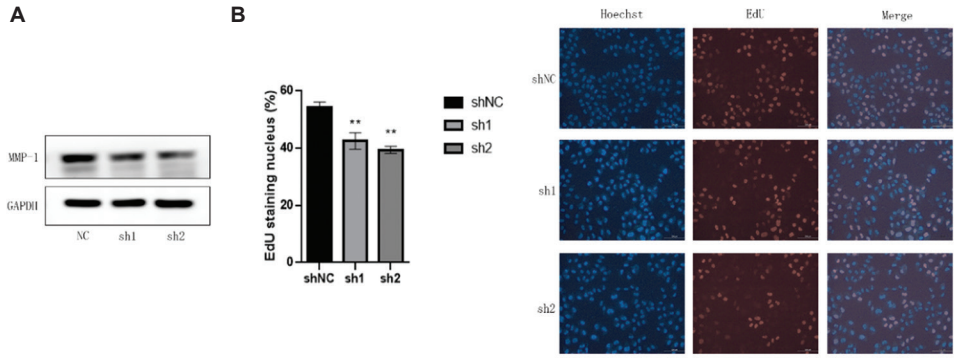


Figure 4. Effect of MMP-1 disruption on the proliferative ability of gastric cancer cells: (A) Western blot validation of MMP-1 disruption and (B) statistics of the percentage of EdU-positive cells.
Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$
Abbreviation: NC: Negative control; MMP-1: Matrix metalloproteinase-1.

3.4. Effect of MMP-1 on the epithelial-mesenchymal transition (EMT)-related protein expression in gastric cancer cells

Analysis of the MMP-1 effect on EMT-related protein expression in gastric cancer cells revealed significantly increased vimentin expression, whereas a lower expression of E-cadherin was observed with the overexpression of MMP-1. Similarly, following the downregulation of MMP-1, a significant decrease in vimentin expression and an increase in E-cadherin expression were observed in N87 cells (Figure 6).

4. Discussion

Matrix metalloproteinases are secreted by many cells and are involved in various physiological processes, such as angiogenesis[10] and embryogenesis.[11] They also play a role in many pathological conditions, including myocardial infarction,[12] fibrotic diseases,[13] osteoarthritis,[14,15] and cancer.[16,17] At least 11 MMPs function within cells, including MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-14, MMP-23, and MMP-26.[18,19] MMPs can disrupt the histologically observed barrier in tumor cells. These enzymes are essential for tumor infiltration and metastasis. Moreover, MMPs such as MMP-1, MMP-2, and MMP-9[20-22] are involved in tumor EMT by interacting with each other.[9,23]

Differentially expressed genes were identified using BioGRID, STRING, GO, and KEGG pathway analyses, revealing that MMP-1 expression was considerably upregulated in gastric cancer tissues and cells, as well as in clinical serum samples, based on tissue protein assays.[23-27] Similar findings were reported for esophageal carcinoma, demonstrating that MMP-1 functions as an oncogene and the high expression of MMP-1 in esophageal cancer was closely associated with lymph node metastasis, microvascular density, and advanced TNM (tumor, node, and metastasis) staging.[28-30] A combination of 19 serum proteins was reported to act as a diagnostic marker to distinguish patients with TNM Stages I-II, with MMP-1 being the best in terms of performance. These outcomes suggest that MMP-1 could be a potential biomarker for the early diagnosis and prevention of gastric cancer.[31] In colorectal cancer, *in vivo* and *ex vivo* experiments confirmed that the expression of MMP-1 was higher than

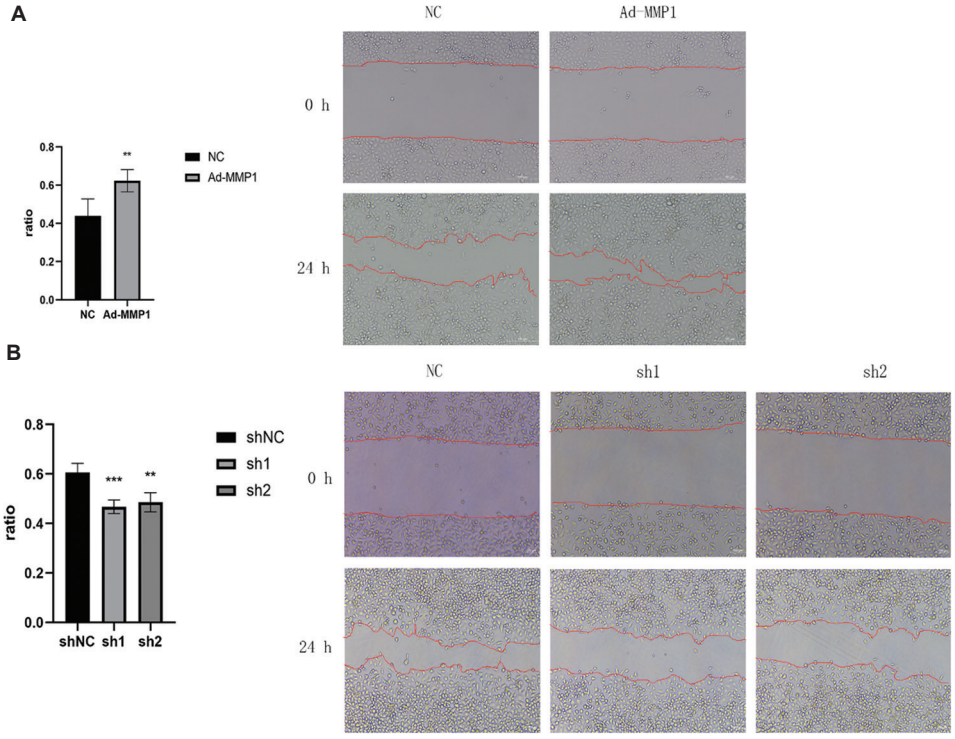


Figure 5. Effect of MMP-1 on the migration of gastric cancer cells detected by scratch healing assay: (A) Effect of upregulation of MMP-1 expression on the migration of gastric cancer cells and (B) effect of downregulation of MMP-1 expression on the migration of gastric cancer cells.
Abbreviations: Ad-MMP-1: MMP-1-overexpression plasmid; NC: Negative control.

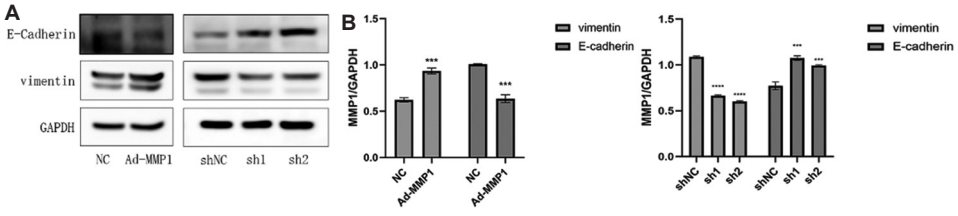


Figure 6. Effect of MMP-1 on the expression of epithelial-mesenchymal transition-related proteins in gastric cancer cells, as detected by western blotting: (A) Western blot detection of related protein expression and (B) grayscale value comparisons of differences in related protein expression levels.
Abbreviations: Ad-MMP-1: MMP-1-overexpression plasmid; NC: Negative control.

that of matched paraneoplastic non-cancerous tissues, and its high expression was significantly associated with lymphatic metastasis as well as TNM staging.[32] An association between polymorphisms in the MMP-1 promoter and the risk of gastric cancer, particularly in conjunction with smoking behavior and *Helicobacter pylori* infection, has been reported in Taiwan, potentially useful in the early detection and prevention of gastric cancer.[4] This conclusion was drawn from a survey involving 121 gastric cancer patients and 363 matched healthy volunteers, supported by literature evidence.[33] Effective screening methods for early detection can facilitate the timely treatment of patients and improve their chances of survival.[34-37]

The low survival rate of numerous stomach cancer patients is due to the fact that the tumor is often detected in the middle or late stages.[38,39] Timely and comprehensive treatment is crucial for a better prognosis.[40-43] The diagnosis of gastrointestinal tumors still relies on invasive upper gastrointestinal endoscopy.[44,45] However, noninvasive tests and highly specific biomarkers can provide valuable data for gastric cancer screening and diagnosis. For instance, peripheral blood,[46,47] urine,[48,49] and body fluids such as saliva,[50,51] gastric lavage,[52] and gastric juices[53,54] can serve as good sources.[55] Population-based surveillance programs have been effective in reducing gastric cancer-related morbidity and mortality in several countries.[56,57] Therefore, early diagnosis strategies are crucial for providing timely treatment

to improve patient prognosis and survival. In addition, medical imaging and mathematical modeling techniques have been used to diagnose gastric cancer at an early stage.[58-62] Bioinformatics methods have shown that ITGAX, CCL14, ADHFE1, and HOXB13 are expressed at significantly higher levels in gastric cancer tumor tissues than in adjacent normal tissues.[63] Furthermore, immunohistochemistry was employed to study the low expression of MLL2 in tissue microarrays of 529 cases of human gastric cancer and its correlation with early-stage cancer.[64] These genes may serve as gastric cancer marker genes, providing ideas for future experimental studies, and similar stories abound.

Metastasis is a leading contributor to cancer morbidity and mortality, resulting in approximately 90% of cancer-related deaths. The biochemical events and parameters involved in the metastatic process and tumor microenvironment have been targeted or can be potential targets for metastasis prevention and inhibition.[65-67] Epithelial-mesenchymal transition is a process that is required for the progression of many types of malignant tumors.[68-72] Matrix metalloproteinases are involved in various essential functions, including cell regeneration, programmed cell death, and angiogenesis, as well as normal developmental and pathological processes such as EMT.[73] MMP-1 is a critical factor in the development of several tumors, and several articles have reported that MMP-1 is involved in EMT-associated processes, including hepatocellular carcinoma,[9] head-and-neck cancer,[74] cervical cancer,[75] and colorectal cancer.[76] Furthermore, increased expression levels of MMP-1 have been observed in the tumor cells and tumor-associated stroma of breast cancer individuals with EMT markers present in the peripheral blood compared to those without EMT markers. In addition, tumor grade is independently correlated with MMP-1 expression in cancer cells,[77,78] suggesting that MMP-1 plays an essential role in tumor EMT and staging.

5. Conclusion

This study demonstrated that MMP-1 holds promise as a specific diagnostic marker for EGC. In addition, it illustrated the effects of MMP-1 modulation on the proliferation and migration of gastric cancer cells, a finding that has not been reported previously. Therefore, these findings provide important insights for setting future research directions.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Qing Li, Ke Yi
Data analysis: Xiaoli Zhu
Formal analysis: Ke Yi
Investigation: Ke Yi
Methodology: Yan Hu
Writing – original draft: Ke Yi
Writing – review & editing: Xiaoli Zhu, Qing Li

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets analyzed in the current study are available from the corresponding author on reasonable request.

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