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BRIEF ARTICLE

Levels of matrix metalloproteinase-1 and tissue inhibitors of metalloproteinase-1 in gastric cancer

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Abstract

AIM: To evaluate the levels of preoperative serum matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in gastric cancer.

METHODS: One hundred gastric cancer patients who underwent gastrectomy were enrolled in this study. The serum concentrations of MMP-1 and TIMP-1 in these patients and in fifty healthy controls were determined using an enzyme-linked immunosorbent assay.

RESULTS: Higher serum MMP-1 and TIMP-1 levels were observed in patients than in controls ($P < 0.001$). Serum MMP-1 and TIMP-1 levels were positively associated with morphological appearance, tumor size, depth of wall invasion, lymph node metastasis, liver metastasis, perineural invasion, and pathological stage. They were not significantly associated with age, gender, tumor location, or histological type.

CONCLUSION: Increased MMP-1 and TIMP-1 were associated with gastric cancer. Although these markers are not good markers for diagnosis, these markers show in advanced gastric cancer.

Key words: Gastric cancer; Matrix metalloproteinase-1; Tissue matrix metalloproteinase-1

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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral endopeptidases that play a significant role in the degradation of all matrix partitions, which

are crucial for malignant tumor growth, invasion, and metastasis[1,2]. MMPs are inhibited by tissue inhibitors of metalloproteinase (TIMPs), which are secreted proteins. TIMPs bind to enzymatically active MMPs at a 1:1 molar stoichiometry, thus inhibiting proteolysis[3]. The role of TIMPs in the imbalance of the extracellular matrix is significant and may inhibit or stimulate tumorigenesis[4].

MMP-1 is also known as collagenase (EC 3.4.23.7)[5]. Saffarian *et al*[6] showed that activated MMP-1 acts by processing on the collagen fibril. The biological implications of MMP-1 acting as a molecular retainer, tied to the cell surface, prompted recent mechanisms for its status in tissue remodeling and cell-matrix interaction to be proposed. MMP-1 in the stromal tumor microenvironment can change the behavior of cancer cells to promote cell migration and invasion[7].

TIMP-1 is a 28.5 kDa glycoprotein that has been studied in many human malignancies, including gastric cancer[8]. TIMP-1 mRNA expression is increased in gastric, esophageal, and pancreatic cancer[9-11]. TIMP-1 is present in human peripheral blood and body fluids[12]. MMP-1 and TIMP-1 levels have been studied in plasma or serum of patients with cumulative malignancies[13,14].

Our study was carried out to analyze serum MMP-1 and TIMP-1 levels in gastric cancer patients and to investigate their clinicopathological correlations.

MATERIALS AND METHODS

A total of 100 patients who underwent gastrectomy with gastric cancer between December 2007 and April 2010 were enrolled. Their median age was 58.5 years (range, 34-78 years), and the ratio of men/women was 47/53. There were 50 healthy volunteer controls without family history of cancer, whose average age was 56 years (range, 48-65 years) (22 men, 28 women). Peripheral venous blood of patients and controls was taken before gastrectomy and stored at 4℃. Blood from controls was taken on the day of a physical examination. The blood samples were centrifuged 1000 rpm, in 15 min, at 20℃ to separate the serum, which was stored at -70℃ until analysis. The mean storage time of all samples was 2 mo (45-80 d).

Resected tumor specimens were studied pathologically according to the criteria of the UICC's pTNM classification[15]. Information recorded included age, gender, tumor location, tumor size, wall invasion, resection margin, histological type, lymph node metastasis, vascular invasion, lymphatic invasion, and perineural invasion. The histological features were classified into two types: (1) intestinal or differentiated type, consisting of papillary and/or tubular adenocarcinomas; and (2) diffuse or undifferentiated type, consisting of poorly differentiated, signet-ring cells, and/or mucinous adenocarcinomas.

Enzyme-linked immunosorbent assay (ELISA) for serum MMP-1 and TIMP-1 was performed using an ELISA kit (R&D System, USA) following the manufacturer's instructions.

As appropriate, the Mann-Whitney *U* test or Fisher's exact test was used for group comparisons. Correlations between parameters were tested by Spearman's correlation coefficient. A $P < 0.05$ was considered statistically significant.

Table 1 Serum matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 levels in patients and controls

Variables	Controls (n = 50)	Patients (n = 100)	P
Age (yr)	56 (48-65)	58 (47-64)	
Gender female (%)	37	40	
MMP-1 (ng/mL)	256 (109-342)	785 (457-900)	< 0.0001
TIMP-1 (ng/mL)	220 (198-267)	725 (417-1134)	< 0.0001

MMP-1: Matrix metalloproteinase-1; TIMP-1: Tissue inhibitor of metalloproteinase-1.

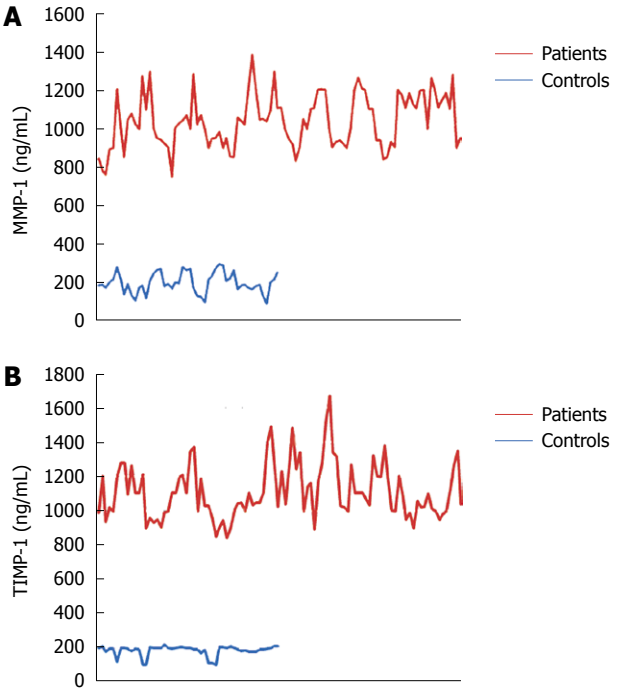

Figure 1 Serum matrix metalloproteinase-1 (A) and tissue inhibitor of metalloproteinase-1 (B) levels of controls and patients. MMP-1: Matrix metalloproteinase-1; TIMP-1: Tissue inhibitor of metalloproteinase-1.

RESULTS

Serum MMP-1 and TIMP-1 levels in gastric cancer patients and controls are shown in Table 1 and Figure 1A and B. The serum levels of MMP-1 and TIMP-1 in gastric cancer patients were significantly higher than in the control group ($P < 0.0001$). Clinicopathological variables are shown in Table 2. Serum MMP-1 and TIMP-1 levels were positively associated with the depth of wall invasion ($P < 0.01$), lymph node metastasis ($P < 0.001$), and lymphatic invasion ($P < 0.001$). The serum levels of MMP-1 and TIMP-1 were closely associated with distant metastasis ($P < 0.001$). In particular, higher MMP-1 and TIMP-1 levels were significantly associated with positive lymphovascular invasion ($P < 0.001$), tumor size ≥ 4 cm ($P < 0.001$), positive lymph node metastasis ($P < 0.001$), T stage

Table 2 Clinicopathological variables of serum matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in patients

Variables	MMP-1	TIMP-1	*P*
Lymphovascular invasion			
Negative	543 (500-678)	489 (450-573)	
Positive	801 (768-845)	642 (567-703)	< 0.001
Tumor size (cm)			
< 4	478 (460-501)	429 (425-479)	
⩾ 4	675 (509-725)	671 (532-690)	< 0.001
Lymph node metastasis			
Negative	563 (503-650)	642 (598-709)	
Positive	742 (657-799)	756 (570-876)	< 0.001
T stage			
T0-2	521 (498-599)	598 (564-783)	
T3-4	674 (578-783)	749 (570-794)	< 0.001
TNM stage			
Ⅰ	469 (458-502)	476 (423-512)	
Ⅱ	534 (467-563)	521 (478-589)	
Ⅲ	714 (546-857)	753 (512-699)	< 0.001
Ⅳ	765 (699-900)	975 (812-1134)	< 0.001

MMP-1: Matrix metalloproteinase-1; TIMP-1: Tissue inhibitor of metalloproteinase-1.

(T3-T4) ($P < 0.001$), or TNM stage (Ⅲ and Ⅳ) ($P < 0.001$). MMP-1 and TIMP-1 levels were not significantly associated with negative lymphovascular invasion, tumor size < 4 cm, negative lymph node metastasis, T stage (T0-T2), and TNM stage (Ⅰ and Ⅱ). Overall, they were associated with pathological stage ($P < 0.001$). Serum MMP-1 and TIMP-1 levels were not associated with age ($P = 0.237$), gender ($P = 0.281$), tumor location ($P < 0.142$), histological type ($P = 0.103$), vascular invasion ($P = 0.247$), or peritoneal seeding ($P = 0.271$).

Higher serum MMP-1 and TIMP-1 levels were correlated with gastric cancer ($P < 0.001$, $r = 0.77$). Figure 1A shows that MMP-1 levels in patients with gastric cancer were significantly higher than in control groups. Figure 1B shows that TIMP-1 levels in patients with gastric cancer were significantly higher than in control groups.

DISCUSSION

In our study, we investigated MMP-1 and TIMP-1 levels in gastric cancer patients and compared them with a control group. We also investigated their associations with clinicopathological features.

Matrix metalloproteinases are involved in many normal biological processes (e.g. embryonic development, blastocyst implantation, organ morphogenesis, nerve growth, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, wound healing, angiogenesis, and apoptosis) and pathological processes (e.g. arthritis, cancer, cardiovascular disease, nephritis, neurological disease, breakdown of the blood brain barrier, periodontal disease, skin ulceration, corneal ulceration, liver fibrosis, emphysema, and fibrotic lung disease). Although the main function of matrix metalloproteinases is elevation of ECM during tissue resorption and progression of many diseases, it is obvious that matrix metalloproteinases also alter the biological functions of ECM molecules by definite proteolysis. MMP-1 and TIMP-1 are thought out to be involved in dissemination of cancer cells by dissolving the ECM, but they are also important in creating an environment that supports the initiation and growth of primary and metastatic tumors. These effects may be associated with proteolytic release of growth factors and/or modification of cellular environments[16].

The most important finding in our study was the association between high MMP-1 and TIMP-1 levels in gastric cancer patients. In addition, high MMP-1 and TIMP-1 levels were significantly associated with certain clinicopathological variables. High MMP-1 expression has been associated with hematogenous metastasis[17,18], rising depth of invasion, and metastasis in colorectal cancer[18,19]. Our study also suggested that MMP-1 levels are associated with depth of invasion and metastasis.

Patients with colorectal cancer, ovary, lung, and liver diseases have increased TIMP-1 levels compared to control groups[14,20-22]. Wang *et al*[23] suggested that serum TIMP-1 levels were higher in gastric cancer patients than control groups and were associated with clinicopathological variables. However, they suggested that serum TIMP-1 levels were associated with depth of wall invasion, distant metastasis, peritoneal seeding, lymphatic invasion, lymph node metastasis, and perineural invasion. However, we did not find that serum TIMP-1 levels were associated with peritoneal seeding and perineural invasion.

MMP-1 is associated with the primal pace of invasion and angiogenesis in gastric cancer, which may make it a useful marker for prognosis. TIMP-1 is more simply released into the blood[24]; therefore, the sensitivity of the assay is higher than that for MMP-1.

High blood levels of MMP-1 and TIMP-1 are associated with poor prognosis of malignancies. Thus, they might useful as markers for malignant potential (i.e. tumor growth and/or differentiation) for cancer. Notably, serum TIMP-1 levels have been established as an independent factor in gastric cancer[23].

Some metalloproteinases have been shown to degrade over time when measured in stored blood samples. However, we do not think that such protein decay is a significant factor when proteins are stored for 2 mo. This assumption is supported by the work of Papazoglou *et al*[25], Kardeşler *et al*[26] and Karapanagiotidis *et al*[27].

MMP-1 and TMP-1 can be considered as 'traditional' and conventional serum biomarkers; many studies have measured both of these proteins as serum biomarkers[28].

This study demonstrated that high serum MMP-1 and TIMP-1 levels in gastric cancer patients are significantly associated with disease progression. Their levels are important markers of tumor progression or advanced tumor stages.

COMMENTS

Background

The incidence of gastric cancer is rising worldwide. Collagenases may play a role

in degradation of the cell matrix, possibly leading to growth of malignant tumors, lymph node metastasis, increased depth of invasion and other metastases.

Research frontiers

Matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) change the environment of cancer cells to promote cell migration and invasion. Changes caused by these endopeptidases have a role in the progression of the gastric cancer.

Innovations and breakthroughs

High blood levels of MMP-1 and TIMP-1 are associated with poor prognosis of malignancies, making them potentially useful biomarkers for the malignant potential (i.e. tumor growth and/or differentiation) of cancer. These effects may be associated with proteolytic release of growth factors and/or modification of tumor cells.

Applications

The date generated in this paper might be used to explain the development of gastric cancer, to prevent metastasis, and to aid early diagnosis.

Terminology

MMP-1 and TIMP-1 zinc-dependent neutral endopeptidases. The role of MMP-1 and TIMP-1 in the imbalance of the extracellular matrix is significant and may inhibit or stimulate tumorigenesis. These effects have been demonstrated, and these molecules may represent useful markers of tumorigenesis.

Peer review

It is a nice study, with interesting results.

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