SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 2: 1319-1322, 2011

Expression of MMP-3 and TIMP-3 in gastric cancer tissue and its clinical significance

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Received July 1, 2011; Accepted August 19, 2011

DOI: 10.3892/ol.2011.399

Abstract. The present study aimed to investigate the expression of matrix metalloproteinase-3 (MMP-3) and the tissue inhibitor of metalloproteinase-3 (TIMP-3) in gastric cancer tissue, as well as to analyze the correlation between their expression and the occurrence of gastric cancer. Immunohistochemistry was used to determine the expression of MMP-3 and TIMP-3 in the gastric cancer tissue from 18 patients with early-stage gastric cancer (early-stage group) and 26 patients with advanced-stage gastric cancer (advanced-stage group). Transmission electron microscopy (TEM) was used to observe the lymphocytes and tumor cells in gastric cancer tissue. The results showed that the expression of TIMP-3 was significantly higher, whereas that of MMP-3 and MMP-3/TIMP-3 was lower in gastric cancer tissue of the early-stage group than in that of the advanced-stage group ($P<0.05$). The TEM images revealed increased lymphocytes and inconspicuous tumor cells penetrating the basement membrane in gastric cancer tissue of the early-stage group, and decreased lymphocytes and obvious tumor cells penetrating the basement membrane in the advanced-stage group. In conclusion, MMP-3 and TIMP-3 may be used as indices for the invasion and metastasis of gastric cancer and possess marked clinical significance in the prognostic judgment of gastric cancer.

Introduction

Invasion and metastasis are the two key characteristics of malignant tumors, and the direct cause of death in patients with tumors. Tumor prognosis is closely correlated to the invasive and metastatic potential of tumor cells (1). Invasion and metastasis of tumor cells form a complex, multi-step, multi-link cascade and reactive process including local invasion, tumor cell infiltration into blood vessels and tumor cell adhesion. The tumor cells damage the basement membrane and degrade the extracellular matrix during this process to achieve tumor invasion and metastasis (2). Matrix metalloproteinase-3 (MMP-3) and the tissue inhibitor of metalloproteinase-3 (TIMP-3) are the two crucial *in vivo* enzymes present during extracellular matrix synthesis and regulation of the degradation metabolism balance that are closely correlated to tumor cell invasion and metastasis (3).

In the present study, immunohistochemistry was used to determine the expression of MMP-3 and TIMP-3 in the gastric cancer tissue of 44 patients with gastric cancer in order to investigate the role of MMP-3 and TIMP-3 in the occurrence and development of gastric cancer. Transmission electron microscopy (TEM) was used to observe lymphocytes and tumor cells in gastric cancer tissue to further investigate the relationship between MMP-3, TIMP-3 and clinical stages.

Materials and methods

Clinical information. A total of 44 gastric cancer patients who had received surgery at the Yancheng First People's Hospital, China, between January 2009 and December 2010 were selected, all of whom were pathologically diagnosed as gastric cancer following surgery. None of the patients had received any treatment for gastric cancer prior to surgery. According to the invasion depth of gastric cancer, the patients were staged as follows: 18 cases in the early and medium stages (early-stage group, cancer tissue limited to the mucosa and submucosa, with or without lymph node metastasis) comprising 13 males and 5 females aged between 32 and 62 years, mean age 50.2±7.3 years; and 26 cases in the advanced stage (advanced-stage group, cancer tissue invading into the muscular layer) comprising 16 males and 10 females aged between 42 and 64 years, mean age 52.9±6.9 years. There was no statistically significant difference in age and gender distribution between the two groups ($P>0.05$).

Experimental methods

Immunohistochemistry method. MMP-3 and TIMP-3 streptavidin-biotin complex immunohistochemistry kits were purchased from American Santa Cruz Company. Immuno-

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Key words: gastric cancer, matrix metalloproteinase-3, tissue inhibitor of metalloproteinase-3

Table I. The average gray values of MMP-3 and TIMP-3 in gastric cancer tissue during early and advanced stages.

Groups	n	MMP-3	TIMP-3	MMP-3/TIMP-3
Early-stage	18	63.32±18.23	122.20±16.07	0.53±0.16
Advanced-stage	26	99.87±22.24	69.41±17.87	1.53±0.53
t		5.757	10.032	7.769
P-value		0.001	0.001	0.001

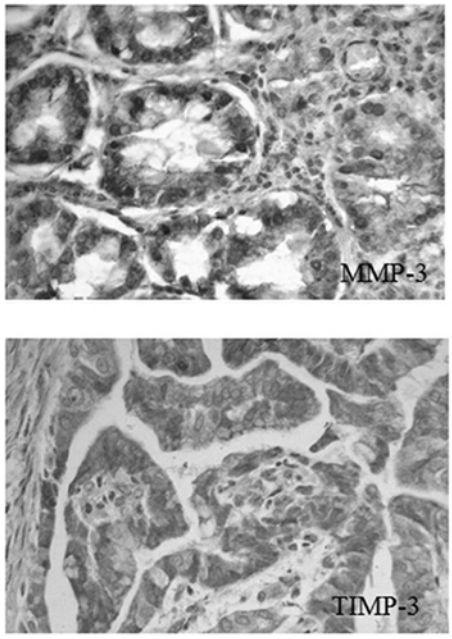

Figure 1. The expression of MMP-3 and TIMP-3 in gastric cancer tissue.

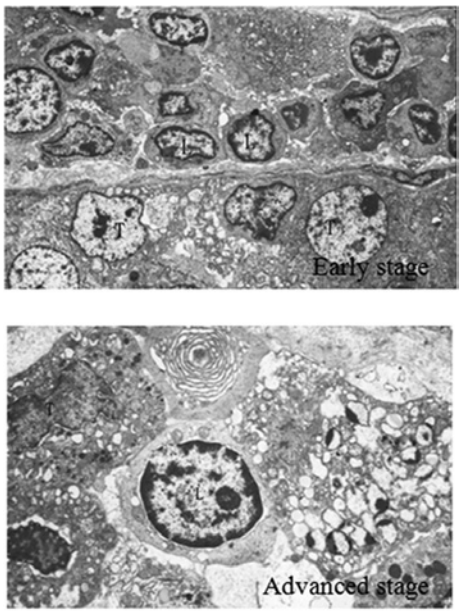

Figure 2. The expression of gastric cancer tissue (L, lymphocyte; T, tumor cell).

histochemistry was applied using the S-P method and the laboratory operations were performed according to the kit instructions. The gastric cancer tissues were fixed, embedded and conventionally sliced with PBS buffer solution formalin solution, then dewaxed to water and treated as follows: 3% H_2O_2 was used to block endogenous peroxidase and was repaired using a microwave in 0.01 mol/l citrate buffer (pH 6.0) for 30 min and sealed with normal goat serum A. The primary and secondary antibodies and horseradish acid were successively added to indicate streptomycin working solution (MMP-3 and TIMP-3 antibodies were diluted and incubated at 1:100 and stored overnight at 4˚C). The solutions were then colored with DAB, re-stained with hematoxylin and sealed with neutral gum. PBS was used as a blank control instead of the primary and secondary antibodies. MMP-3 and TIMP-3 immunohistochemical staining slices were observed, and 10 fields of vision were randomly selected under high magnification (x40) for each slice, with 20 randomly tested positive cancer cells for each field. A total of 200 positive cells were tested for each sample. The average gray values of the positive area were tested, where the strength of immunohistochemistry was positively proportional with the gray values, higher positive material corresponding to deeper dyeing and greater gray value, otherwise smaller.

Transmission electron microscope (TEM) method. The gastric cancer tissue samples were cut into 1-mm^3 slices and placed into 3% glutaraldehyde phosphate buffer (pH 7.4) and fixed for 2-4 h. The samples were then re-fixed with 1% osmic acid and gradually dehydrated with alcohol. They were then embedded with epoxy resin 812, sliced with LKB IV ultramicrotome and double-stained with uranyl acetate-lead citrate. The samples were then observed using a JEM-1220 transmission electron microscope.

Statistical analysis. SPSS 13.0 software was used to process data; the metrological data were expressed as the mean ± standard deviation ($\bar{x}$+s). The independent samples t-test was used to compare the MMP-3 and TIMP-3 expression in early and advanced gastric cancer tissue, as well as the MMP-3:TIMP-3 ratio. The above-mentioned hypothesis test was a two-sided test, with a test level (α) of 0.05. $P<0.05$ was considered to be statistically significant.

Results

Expression of MMP-3 and TIMP-3. MMP-3 was mainly expressed in the cytoplasm of cancer cells, in brownish-yellow

granules. The granules with a positive reaction of TIMP-3 were mainly expressed in the cytoplasm of cancer cells, as shown in Fig. 1.

Comparison of MMP-3 and TIMP-3. Table I shows a significantly decreased MMP-3 expression and increased TIMP-3 expression in gastric cancer tissue during the early stage, compared with that in the advanced stage. The MMP-3/TIMP-3 ratio was therefore significantly decreased ($P<0.05$).

Lymphocytes and tumor cells in gastric cancer tissue. During the early stage of gastric cancer, a substantial lymphocyte invasion was observed in the peripheral tissues. The lymphocytes were arranged in clusters along the basement membrane close to each other, with moderately integrated basement membranes. Cancer cells were observed in the nests, with an irregular nucleus, and visible vacuolar degeneration. During the advanced stage of gastric cancer, the basement membrane was markedly damaged, with less lymphocyte infiltration compared to that during the earlier stage. However, the number of tumor cells penetrating the basement membrane increased, as shown in Fig. 2.

Discussion

Gastric cancer is one of the most common malignant tumors, representing the second and fourth most common malignancies in males and females, respectively, worldwide (4). In China, gastric cancer is the most common malignant tumor, with an average annual mortality rate of 25.53 per 10 million deaths. Thus, gastric cancer is a clear threat to human health. Consequently, the identification of new prevention and treatment measures to improve the diagnosis and treatment levels of gastric cancer in China is crucial.

The extracellular matrix (ECM) and basement membrane of the tissue are natural barriers preventing tumor invasion and metastasis. Degraded ECM and loss of integrity of the basement membrane lead to tumor invasion and metastasis (5). *In vivo*, matrix metalloproteinases (MMPs) are the key proteolytic enzymes by which the ECM and basement membrane are degraded, thereby promoting cancer cell invasion into the surrounding tissue (6). MMP-3 is generated by connective interstitial cells, fibroblasts, capillary endothelial cells, macrophages and tumor cells (7), with extensive substrates, and is capable of degrading the basement membrane, proteoglycans, laminin, fibronectin, and II, III, IV, V and VI collagen (8). The unique function of MMP-3 is to activate other types of MMP, such as MMP-2 and MMP-9 (9). The activity of MMP-3 is specifically inhibited by TIMP-3 (10). TIMP-3 is a new member of the TIMP family, mainly generated by the mesoblastema. TIMP-3 possesses additional regulation sites for MMPs and is capable of promoting the proliferation and transformation of non-transformed cells. Moreover, it is capable of developing covalent binding with MMP enzyme precursors or active enzymes, so as to inhibit the activation and activity of MMP enzyme precursors (11).

Studies on tissues of nasopharyngeal carcinoma (12), cervical cancer (13), breast cancer (14), lung cancer (15) and colon cancer (16) have shown that MMP-3 and TIMP-3 expression is correlated to the invasion and metastasis of cancer cells. This study has shown that TIMP-3 expression in the gastric tissues of the early-stage group was significantly higher than that of the advanced-stage group, whereas MMP-3 expression and the MMP-3/TIMP-3 ratio were significantly lower than the advanced-stage group. The TEM images demonstrated increased lymphocytes and inconspicuous tumor cells penetrating the basement membrane in the gastric cancer tissue of the early-stage group, and decreased lymphocytes and obvious tumor cells penetrating the basement membrane in the advanced-stage group. This observation indicates that MMP-3 is capable of degrading ECM and causing marked damage to the basement membrane: The cancer cells are therefore capable of infiltrating and growing to distant locations along the basement membrane matrix defects and matrix space. On the other hand, the lymphocytes surrounding the cancer area increase during the advanced stage, with decreased defense capacity. Therefore, a large number of cancer cells are capable of permeating the basement membrane and causing invasion and metastasis.

In conclusion, MMP-3 and TIMP-3 are closely correlated to the occurrence and development of gastric cancer. However, studies regarding the relationship between the imbalance of MMP-3 and TMIP-3 expression and the occurrence and development of gastric cancer are in the initial stage. Further studies regarding this relationship are therefore required.

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