Expression of Matrix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Gastric Carcinoma

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Abstract **Objective** To study the expression of matrix metalloproteinase-9(MMP-9),its native tissue inhibitor (TIMP-1) in gastric carcinoma, and the relationship between their expressions and the clinicopathologic features of gastric carcinoma. **Methods** 40 specimens resected from patients with primary gastric carcinoma were investigated with immunohistochemical SP method using monoclonal antibodies to MMP-9 and TIMP-1. **Results** Among 40 cases, 29 cases were MMP-9 positive(72.5%);19 cases were TIMP-1 positive(47.5%). The overexpression of MMP-9 was correlated to higher lymph node metastasis($p<0.01$ and $p<0.05$, respectively)and poorer differetiation; The enhanced expression of TIMP-1 was associated with lymph node involvement and distant metastasis ($p<0.05$). **Conclusions** The results suggest that the overexpression of MMP-9 and TIMP-1 can indicate the malignant biologic behavior of gastric carcinoma. In addition, TIMP-1 may prove to be a useful marker for determining the biologic aggressiveness of gastric carcinoma.

Key words gastric carcinoma; matrix metalloproteinases-9; tissue inhibitors of metalloproteinases-1; biologic behavior

Degradation of extracellular matrix (ECM) is required for malignant tumor invasion and metastasis[1]. Matrix metalloproteinase-9(MMP-9), one of the important family members of Matrix metalloproteinases (MMPs)which degrade the various ECM components, is thought to play a key role in these processes[2]. The activity of MMP-9 is normally inhibited by native tissue inhibitor of metalloproteinase-1 (TIMP-1). The potency of tumor cells to invade into the ECM is regulated by the balance of MMP-9 and TIMP-1.

In this study, we investigated the expressions of MMP-9 and TIMP-1 in gastric carcinoma by immunohistochemical staining and analysed the relationship between their expressions and the clinicopathologic features of gastric cancer.

MATERIALS AND METHODS

Gastric cancer patients

Forty curative resected gastric carcinoma and ten normal specimens were selected and fixed in a 10% formaldehyde solution and embedded in paraffin from April 1998 to April 1999 at Surgical Department of The first Hospital affiliated to Suzhou university. There were 28 men and 12 women, the mean age was 56.8 years with a range of 35 to 77 years. 28 patients were verified histologically lymph node positive and 13 were found distant metastasis. According to Laurren's pathologic classification, 22 were intestinal type and the rest were diffusive type.

Immunohistochemical staining

Paraffin embedded tissues were sliced in consecutive 5-um sections and evaluated histologically with HE and immunohistochemical staining with the ultrasensitiveTM streptavidin-peroxidase method (S-P method) using monoclonal antibodies to MMP-9(mouse anti human,GE-213) and TIMP-1(mouse anti human,102D1)which were purchased from MaiXin-Bio Inc. (FuZhou, china)

Statistical analysis

Correlations between the clinicopathologic characteristics of patients and the expressions MMP-9, TIMP-1 were statistically evaluated by using Chi-square test. The difference were considered to be significant $P<0.05$.

RESULTS

Staining position

MMP-9 and TIMP-1 were immunolocalized predominantly in the cytoplasm of the cancer nests and it's infiltrating margin in the stroma.

Additionally, MMP-9 staining was also found in macrophages in tumor matrix, and in normal gastric tissue, there was no MMP-9 staining. TIMP-1 was stained scatteredly in the normal gastric carcinoma.

Relations between MMP-9, TIMP-1 expression and clinicopathologic factors

Expression of MMP-9 was analyzed for all patients in relation to tumor size, histologic type, lymph node invasion and distant metastasis (Table 1). These were significant correlations between the expression of MMP-9 and histologic type, lymph node invasion ($P<0.05$ and $p<0.01$, respectively). As to tumor size, distant metastasis, the expression of MMP-9 had no significant difference. TIMP-1 expression was enhanced significantly in patients with lymph node invation ($P<0.05$) and in patients with distant metastasis ($P<0.05$). there were no significant correlations between the expression of TIMP-1 and tumor size, histologic type. The positive rate of MMP-1 in all patients was higher than that of TIMP-1 (72.5% Vs 42.7%).

DISCUSSION

Degradation of ECM and basement membrane is required for tumor invasion and metastasis. MMPs which can degrade the various ECM components are important enzymes in regulating the balance of ECM. Among MMPs families, MMP-2 (the 72-KD Type Ⅳ collagenase/gelatinase A)and MMP-9(the 92-KD Type Ⅳ collagenase/gelatinase B), which selectively degrade Type Ⅳ collagen, a major component of ECM and basement membrane, are reported to be markedly associated with the invasion and metastasis of tumor cells[3]. Many reports have been published about the associations of MMP-9 and/or MMP-2 with the metastasis of a large variety of cancers, such as breast and colon carcinomas[4, 5].

In this study, MMP-9 was negative in normal gastric tissue, while in cancer tissue, MMP-9 was expressed at different extent. The strongest staining position was at the tumor infiltrating margin in stroma which indicated that MMP-9 plays a role in the tumor invasion and progression. MMP-9 expressed more highly in the diffuse type gastric carcinoma than that in intestinal type's, thus suggested that poorer differentiated cancer cells have higher competence to induce expression of MMP-9 and higher tendency to invasion and metastasis. MMP-9 expressed highly in the lymph node positive group than in the negative's, this showed it's important role in cancer lymphtic metastasis. Seir[6] and co-workers found a negative association of the tissue level of MMP-9 mRNA expression with the overall survival of patients with gastric carcinoma. Zeng[7] et al showed that the expression level of MMP-9 could act as a independent indicator to predict the free tumor survival and overall survival of the colorectal cancer. Many study found the MMP-9 mRNA was located in tumor stroma cell, for example, macrophages, rather than cancer cell itself, however, other reports including ours showed, in the level of protein, the MMP-9 was located largely at the plasm of cancer cells, Based on the above findings, we speculate the following opinions: The first, It is the host cells, rather than tumor cells that produce MMP-9 in the tumor progression. In other words, many host cells including the human monocyte-derived macrophages that were stimulated by tumor cells induced the release of MMP-9, then through some way, it was transported to tumor cells to play a role. The second, immunocytes maybe play a key role in the course of interaction of tumor and matrix which has dual facets: one is antitumor, the other is to promote the progress of cancer by some complex factors. We didn't find associations of expression of MMP-9 with staging and distant metastasis. We suggest that is due to the different detecting method, unlike the zymogram analysis, that the immunohistochemistry we used couldn't differentiate the active type of MMP-9 from the inactive type's. In addition, the mechanism of tumor invasion and metastasis is complex, which couldn't be clarified in a simple way.

As one of five members of the TIMP family, TIMP-1 can inhibite the activity of MMP-9. In this study, TIMP-1 expressed scatteredly in normal gastric mucosa, while in cancer tissue, its expression enhanced. There was a positive correlation of TIMP-1 to lymph node invasion and distant metastasis in gastric carcinoma, which seem to be contradictory to the conventional confirmation that TIMP might be inhibite the tumor metastasis, just as many reports verified, the enhanced TIMP-1 could decrease the malignant phenotype of cancer cell lines in vitro. However, although the expression of TIMP-1 in our study was upregulated, its level contrast to that of MMP-9 was imbalanced, MMP-9 was over TIMP-1 in both the strength of staining and the ratio of

Table 1 The Correlation between Clinicopathologic Characteristics and MMP-9, TIMP-1 Expression.

Variable	Total	MMP-9 Positive(%)	P Value	TIMP-1 Positive(%)	P Value
Tumor size					
<6cm	24	18(75)		13(54)	
>6cm	16	11(69)	>0.05	6(38)	>0.05
Histologic type					
Intestinal	22	12(59)		8(36)	
Diffusive	18	17(89)	<0.05	11(61)	>0.05
Lymph node invation					
Positive	28	24(86)		17(61)	
Negative	12	5(42)	<0.01	2(17)	<0.05
Distant metastasis					
Positive	13	9(69)		10(77)	
Negative	27	20(74)	>0.05	9(33)	<0.05

positive cell to negative's (72.5% Vs 47.5%). Based on this findings, we guess the enhanced TIMP-1 reflects the body's needs to regulate the overexpression of MMP-9 as well as the tumor cells' demand to it's survival microenviroment. to survive and progress, tumor cells must be not only degrade the surrounding stroma, but also sustain a balanced MMP-9/TIMP-1 system to rebuild microenviroment and remodel the microvessels. Aimori et al[8] found associations of high TIMP-1 level with the lymph node, distant metastasis and poor prognosis in patients with gastric carcinoma. Other report[9] showed the colorectal cancer patients with enhanced TIMP-1 had higher Duke's staging and poorer 5-year-survival.

In conclusion, we suggest that the overexpression of MMP-9 and TIMP-1 may be a useful indicator to predicting the malignant biologic behavior of gastric carcinoma. As the function of TIMP-1 gene is to be disclosed deeply, TIMP-1 may prove to be a useful marker for determining the biologic aggressiveness of cancer.

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