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• GASTRIC CANCER •

Imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in invasiveness and metastasis of human gastric carcinoma

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Abstract

AIM: The expressive balance between matrix metalloproteinase-9 (MMP-9) and its tissue inhibitor of metalloproteinase-1 (TIMP-1) plays a critical role in maintaining the degradation and synthesis of extracellular matrix. Loss of such balance is associated with invasion and metastasis of tumors. This study aimed to determine the expression of MMP-9 and TIMP-1 in gastric carcinoma, and the association of the expressive imbalance between MMP-9 and TIMP-1 with the invasion and metastasis and prognosis of gastric carcinoma.

METHODS: We used immunohistochemistry to determine the expressions of MMP-9, TIMP-1 and proliferating cell nuclear antigen Ki-67 in the gastric specimens taken from 256 patients with primary gastric carcinoma. The patients were followed-up for up to 96 months.

RESULTS: No association between the expression of MMP-9 and TIMP-1 and patients' sex and age, tumor size and location of gastric carcinoma was observed. The incidence of the positive expression of MMP-9 in cases with tumors invasion to muscularis propria and visceral peritoneum (70.13 % and 69.09 %, respectively) was significantly higher than that in cases with tumor invasion only to lamina propria or submucosa (42.50 %, P=0.0162). The positive correlation between MMP-9 expression and the depth of tumor invasion was observed (Pearson correlation coefficient=0.2129, P=0.016). Along with the increase of the metastatic station of lymph nodes, the incidence of the MMP-9 expression was increased by degrees; a positive correlation between them was observed (Pearson correlation coefficient=0.2910, P=0.0001). There was also a significant correlation between MMP-9 expression and the TNM stage in gastric carcinoma (Pearson correlation coefficient=0.3027, P<0.0001). The incidence of MMP-9 expression in stage II and III/IV (75.00 % and 76.15 %, respectively) was significantly higher than those in stage I (46.15 %, P<0.0001). A negative correlation between TIMP-1 immunoreactivity and the depth of invasion, status of lymph node metastasis and TNM stage was observed (Pearson correlation coefficient =-0.1688, -0.3556 and -0.3004, P=0.023, <0.0001 and <0.0001, respectively). Four types of co-expression of MMP-9 and TIMP-1 were observed; i.e. MMP-9 positive but TIMP-1 negative (n=115), both positive (n=52), both negative (n=62) and MMP-9 negative but TIMP-1 positive (n=27). The frequency of serosal invasiveness was significant higher in patients with MMP-9 but without TIMP-1 expression than those with other types of the co-expression (P=0.0303). The incidence of lymph node metastasis was highest in patients with MMP-9 but without TIMP-1 expression, and lowest in those with TIMP-1 but without MMP-9 expression (P<0.0001). The survival rate in patients with MMP-9 but without TIMP-1 expression was lower than that in those with TIMP-1 but without MMP-9 expression (P=0.0014).

CONCLUSION: Our results in gastric carcinoma demonstrated a significant positive association of MMP-9 over-expression with proliferation of tumor cells, the depth of invasiveness, lymph node metastasis and TNM stage, suggesting MMP-9 can serve as a molecular marker of tumor invasion and metastasis. We also demonstrate a significant negative relationship of TIMP-1 expression with the depth of invasiveness and lymph node metastasis, which provide a new idea in the tumor biological and genetic treatment. The interaction between MMP-9 and TIMP-1 in the processes of tumor invasion and metastasis is that MMP-9 mainly promotes tumor invasion and metastasis and TIMP-1 inhibits functions of MMP-9. The imbalance between MMP-9 and TIMP-1 expression may suggest the occurrence of tumor invasion and metastasis, predict poor prognosis. For patients with imbalanced MMP-9 and TIMP-1 expression, the optimal treatment scheme needs to be selected.

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INTRODUCTION

The malignant behavior of tumor cells mainly depends on the capability of invasion and metastasis of cancer cells. After the components of the extracellular matrix (ECM) are degraded, tumor cells invade the surrounding tissue and the vascular or lymphatic vessels to form metastatic colonies at distant sites. Matrix metalloproteinase-9 (MMP-9) can degrade the main components of the ECM, type IV and V collagen and gelatin[1-6], thus, its activities are closely related to the ability of the invasiveness and metastasis of tumor cells[7,8]. Increased expression of matrix metalloproteinases (MMPs) renders the tumor cells capable of digesting essential tissue barriers especially basement membranes lining the blood vessels, thereby promoting the cells' motility. By forming a 1:1 complex with MMP-9 and inhibiting its enzymatic activity[2,9,10], tissue

inhibitor of metalloproteinase-1 (TIMP-1) plays negative role in the invasion and metastasis of tumor cells[11]. Therefore, attentions have been paid to the role of MMP-9 and TIMP-1 in the progress of tumor, and it has been reported that the expression of MMP-9 and TIMP-1 was correlated[12], but the relationship of their expressive imbalance to the invasion and metastasis in gastric carcinoma was rarely reported. In the present study, we study the expressive pattern of MMP-9 and TIMP-1 in 256 patients with primary gastric carcinoma by immunohistochemistry, as well as the relationship of their expressive imbalance to invasion and lymph node metastasis and prognosis of gastric carcinoma. We demonstrated that the expressive imbalance of MMP-9 and TIMP-1 was significantly associated with the invasion and metastasis of gastric carcinoma.

MATERIALS AND METHODS

Materials

Two hundred fifty-six patients who underwent a surgery for the primary gastric carcinoma at the First Affiliated Hospital of Fujian Medical University, between 1991 and 1999, and had sufficient clinical materials were selected for this study. These patients comprised 186 males and 70 females. The median age was 60 with a range from 23 to 84 years. All studied patients had not been accepted for radiation therapy and chemotherapy before the operation. The histological findings, lymph node metastasis and TNM stage were evaluated based on World Health Organization Classification of Tumors[13,14]. Follow-up information was available for 167 patients.

Methods

The specimens were fixed in formalin and embedded in paraffin wax, sliced serial step sections of 4 μm thickness and stained by hematoxylin-eosin.

Immunohistochemistry

Paraffin sections (4 μm thick) were immunostained with anti-mouse monoclonal antibodies for MMP-9 (GE-213, 1:10, NeoMarkers), TIMP-1 (102D1, 1:10, NeoMarkers) and Ki-67 (MB67, Ready, NeoMarkers) by the peroxidase-conjugated streptavidin complex method. Sections were deparaffinized and heated in a microwave oven for 10 min to retrieve the antigens. They were immersed in 3 % hydrogen peroxide in 100 % methanol for 10 min to block the endogenous peroxidase activity. After incubated in normal horse serum for 20 min, the tissue sections were incubated with the primary antibodies for 120 min at room temperature. The sections were incubated with biotinylated rabbit anti-mouse immunoglobulins G for 20 min and then treated with peroxidase-conjugated streptavidin for 20 min. The sections were immersed into DAB solution. The slides were counterstained with haematoxylin solution, dehydrated and mounted. Between steps, the slides were washed three times with phosphate buffered saline (PBS). As a negative control, PBS was used instead of the primary antibody.

Two independent observers without knowledge of the clinical outcomes evaluated the degree of immunohistochemical staining. All sections for which the two observers disagreed were re-evaluated until there was a complete agreement on the classification.

Immunohistochemical analyses of MMP-9, TIMP-1 and Ki-67 labeling index

Figures 1 and 2 show a positive expression of MMP-9 and TIMP-1, respectively. They were expressed within the cell membrane and/or cytoplasm. The intensity of staining in cell membrane and cytoplasm and the percentage of immunoreactive cells to total tumor cells were evaluated. The intensity of staining was graded as 0, when staining not greater than negative control, 1, for light staining, and 2, for heavy staining. Immunoreactivity was scored according to the percentage of immunoreactive cells over total tumor cells counted as 0, if <5 % cells were stained; 1 if 5-25 % cells were immunoreactive, 2 if 26-50 % cells were immunoreactive and 3 if >50 % cells were immunoreactive. The expression of MMP-9 and TIMP-1 was finally defined according to the score obtained from the grade of intensity multiplied by the score of cell immunoreactivity, i.e. negative (-, score 0-1), positive (+, score 2-3), and strong positive (++, score 4 or above).

The positive expression of Ki-67 staining was in the nuclei of the carcinoma cells. Ki-67 labeling index was defined as the ratio of immunoreactive cells over 1 000 tumor cells counted labeling.

Statistical analysis

The χ^2 analysis was used for univariable categorical analysis. The relationship of the expressive imbalance between MMP-9 and TIMP-1 to the postoperative survival was tested for prognostic significance in gastric carcinoma specific survival using Kaplan-Meier survival curves and the log-rank test. All statistical analysis was performed using the SPSS 6.0 statistical software program. A value of $P<0.05$ was considered statistically significant.

RESULTS

Relationship between MMP-9 expression and the clinical pathological parameters of gastric carcinoma

MMP-9 was mainly expressed within the cytoplasm and cytoplasmic membranes of the gastric carcinoma cells (Figure 1). Among 256 primary gastric carcinomas, the incidence of a positive expression of MMP-9 in carcinoma cells was 65.23 % (167/256), with the incidence of strong immunoreactivity of 13.67 % (35/256). No significant correlations between the expression of MMP-9 and sex, age, location and size of tumors were observed. As shown in Table 1, the incidence of the positive expression of MMP-9 in cases whose tumors invaded to muscularis propria and visceral peritoneum (70.13 % and 69.09 %, respectively) were significantly higher than those whose tumors only invaded to lamina propria or submucosa (42.50 %, $P=0.0162$). A significant correlation between MMP-9 expression and the depth of tumor invasion was observed (Pearson correlation coefficient=0.2129, $P=0.016$). Along with the increase of the metastatic station of lymph nodes, the incidence of the MMP-9 expression was increased by degrees; a positive correlation between them was observed (Pearson correlation coefficient=0.2910, $P=0.0001$). We also demonstrated a significant correlation between MMP-9 expression and the TNM stage of gastric carcinoma (Pearson correlation coefficient=0.3027, $P<0.0001$), the incidence of MMP-9 expression was significantly higher in stage II and III/IV (75.00 % and 76.15 %, respectively) than in stage I (46.15 %, $P<0.0001$, Table 1).

Relationship between TIMP-1 expression and the clinical pathological parameter in gastric carcinoma

A total of 79 (30.89 %) patients had positive immunohistochemical staining for TIMP-1 in the cytoplasm and cytoplasmic membrane of the gastric carcinoma cells, with the strong positive staining only five (1.95 %) cases (Figure 2). No statistical correlation between TIMP-1 immunoreactivity and sex, age, location and tumor size was observed. There were significant negative correlations between TIMP-1 immunoreactivity and the depth of invasion, status of lymph node metastasis and TNM stage (Pearson correlation coefficient =-0.1688, -0.3556 and -0.3004, $P=0.023$, <0.0001 and <0.0001, respectively, Table 1).

Table 1 MMP-9 (matrix metalloproteinase-9) and TIMP-1 (tissue inhibitor of metalloproteinase-1) expression and clinicopathological characteristics of gastric carcinoma

	n	Expressing levels of MMP-9				*P* value	Expressing levels of TIMP-1			*P* value
		-	+	++	Positive rate (%)		-	+	Positive rate (%)	
(1) Sex										
Male	186	61	100	25	67.20	>0.05	127	59	31.72	>0.05
Female	70	28	32	10	60.00		50	20	28.57	
(2) Age										
Mean		55.00	58.33	61.36			57.28	58.64		
±		±	±	±		>0.05	±	±		>0.05
SD		12.19	11.31	8.48			12.19	10.22		
(3) Location										
Cardia	63	14	36	13	77.78	>0.05	39	24	38.10	>0.05
Corpus	33	10	18	5	69.70		23	10	30.30	
Antrum	140	55	71	14	60.71		101	39	27.86	
Others	20	10	7	3	50.00		14	6	30.00	
(4) Tumor size(cm)										
<5	124	52	59	13	58.06	>0.05	80	44	34.38	>0.05
⩾5	132	37	73	22	71.69		97	35	26.51	
(5) Histological type										
Well-moderately differentiated	109	24	61	24	77.98	>0.05	63	46	42.20	>0.05
Poorly differentiated	98	38	53	7	61.22		77	21	21.43	
Undifferentiated	18	13	4	1	27.78		14	4	22.22	
Mucinous	31	14	14	3	54.84		23	8	25.81	
(6) Depth of invasion										
Lamina propria or submucosa	40	23	15	2	42.50	<0.05	23	17	42.50	<0.05
Muscularis propria	77	23	54	10	70.13		48	29	37.66	
Visceral peritoneum	139	43	73	23	69.06		106	33	23.74	
(7) Lymph node metastasis										
Negative	98	51	40	7	47.96	<0.01	47	51	52.04	<0.01
N1	130	34	73	23	73.85		103	27	20.77	
N2	28	4	19	5	85.71		27	1	3.57	
(8) TNM stage										
I	91	49	36	6	46.15	<0.01	46	45	49.45	<0.01
II	56	14	35	7	75.00		40	16	28.57	
III-IV	109	26	61	22	76.15		91	18	16.51	
(9)Ki-67labeling index										
Mean		669.83	720.09	751.77			716.95	684.53		
±		±	±	±		<0.01	±	±		>0.05
SD		129.48	126.64	120.81			119.67	122.34		

SD: Standard deviation.

Table 2 Association between the expression of MMP-9 (matrix metalloproteinase-9) and TIMP-1 (tissue inhibitor of metalloproteinase-1) and invasion and metastasis of gastric carcinoma

MMP-9 expression	TIPM-1 expression	*n*	Penetrating visceral peritoneum		Lymph node metastasis	
			n	Ratio(%)	*n*	Ratio(%)
+	-	115	74	64.35	97	84.35
	+	52	22	42.31[a]	23	44.23[ab]
-	-	62	32	51.61[a]	33	53.32[ab]
	+	27	12	44.44[a]	5	15.52[a]

[a]*P*<0.05 *vs* MMP-9(+)TIMP-1(-); [b]*P*<0.05 *vs* MMP-9(-)TIMP-1(+).

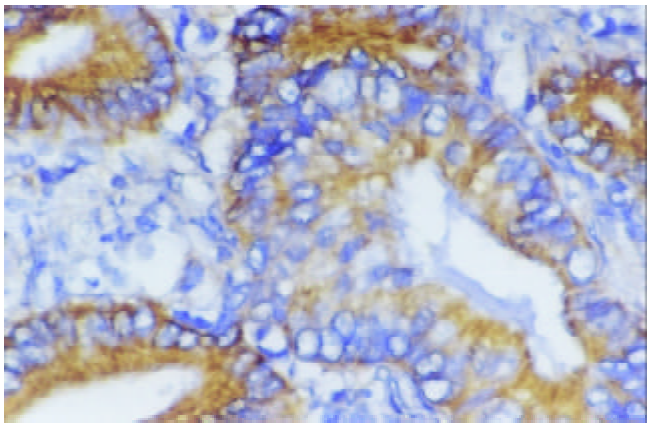

Figure 1 MMP-9 (matrix metalloproteinase-9) strongly positive staining. Membrane or cytoplasm of gastric cancer cells was stained brown. (SP method ×400).

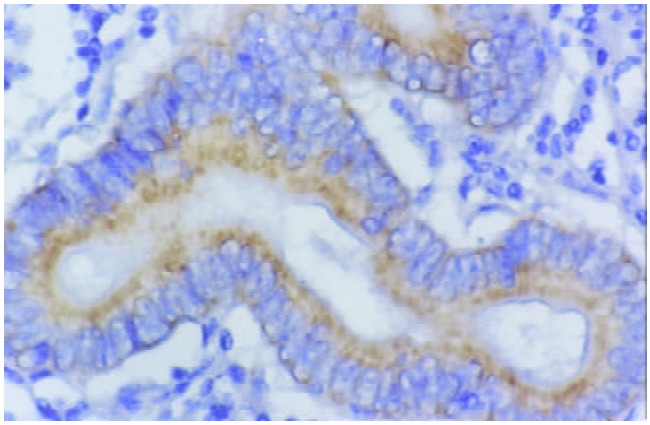

Figure 2 TIMP-1 (tissue inhibitor of metalloproteinase-1) strongly positive staining. Membrane or cytoplasm of gastric cancer cells was stained brown. (SP method. ×400).

Relationship between MMP-9 and TIMP-1 expression and Ki-67 labeling index

As shown in Table 1, the higher the expression of MMP-9 in gastric carcinoma, the higher the Ki-67 labeling indexes in tumor cells (F=6.7013, P=0.0015). There was no significant difference in Ki-67 labeling index between the positive group and negative group of TIMP-1 expression in gastric carcinoma (F=3.4474, P>0.05).

Relationship of the expressive imbalance between MMP-9 and TIMP-1 to the invasiveness and metastasis of gastric carcinoma

According to the expression of MMP-9 and TIMP-1 in gastric carcinoma tissues, four patterns of co-expression were observed: 1, MMP-9 positive but TIMP-1 negative, or MMP-9 expression greater than TIMP-1 expression, n=115 (44.92 %); 2, MMP-9 and TIMP-1 both positive, n=52 (20.13 %); 3, MMP-9 and TIMP-1 both negative, n=62(24.22 %); 4, MMP-9 negative but TIMP-1 positive, or TIMP-1 expression greater than MMP-9 expression, n=27 (10.55 %). Whereas patterns 2 and 3 of the co-expression of MMP-9 and TIMP-1 were defined as balanced, the co-expression patterns in 1 and 4 were defined as imbalanced. The frequency of the serosa invasiveness in patients with the co-expression pattern 1 was significant higher than those with other patterns (P=0.0303). Similarly, the incidence of lymph node metastasis was highest in patients with the co-expression pattern 1 and lowest in those with the pattern 4 (P<0.0001, Table 2).

Relationship of the expression of MMP-9 and TIMP-1 to the postoperative survival of patients with gastric carcinoma

Follow-up (6-97 months) information was available for 167 patients with gastric carcinoma. The postoperative survival rate appeared to decrease in patients with MMP-9 expression compared with those without MMP-9 expression, and in patients without TIMP-1 expression compared with those with TIMP-1 expression, although their difference was not statistically significant (P>0.05). However, the correlation between the expressive imbalance of MMP-9 and TIMP-1 and the postoperative survival was demonstrated. The survival rate was significantly decreased in patients with the co-expression pattern 1 compared with those with the co-expression pattern 4 (P=0.0014, Table 3, Figure 3).

Table 3 Association between the expression of MMP-9 (matrix metalloproteinase-9) and TIMP-1 (tissue inhibitor of metalloproteinase-1) and the prognosis of patients with gastric carcinoma

	n	Survival (%)			P value
		1 yr	2 yr	5 yr	
MMP-9 expression					
-	50	80.42	70.33	60.92	>0.05
+	117	68.09	55.77	44.15	
TIMP-1 expression					
-	121	67.48	56.62	41.23	>0.05
+	46	82.93	68.81	68.81	
Co-expression of MMP-9 and TIMP-1					
A	83	64.87	53.62	36.75	<0.05
B	34	76.08	60.90	60.90	
C	38	73.48	63.74	49.42	
D	12	100.00	88.89	88.89[a]	

Note: A: MMP-9(+)TIMP-1(-); B: MMP-9(+)TIMP-1(+); C: MMP-9(-)TIMP-1(-); D: MMP-9(-)TIMP-1(+), [a]P<0.05 *vs* A.

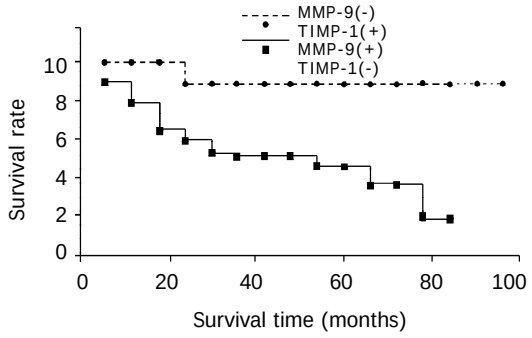

Figure 3 The association between expressive imbalance of MMP-9 (matrix metalloproteinase-9) and TIMP-1 (tissue inhibitor of metalloproteinase-1)and postoperative survival in gastric carcinoma.

DISCUSSION

Expression of MMP-9 and invasiveness, metastasis and prognosis of gastric carcinoma

Degradation of extracellular matrix (ECM) and basement membranes by the tumor cells is a critical step in the processes of tumor invasion and metastasis. MMP-9 is one member of the matrix metalloproteinase families, and characterized by substrate high-specificity and capable of degrading several components of ECM, including type IV collagen molecules which form the major component of the basement membrane.

Increased levels of MMP-9 have been implicated in the invasive potential of tumors[1-4]. There was a trend towards a higher proportion of active MMP-9 with an increasing grade of breast carcinoma, endometrial carcinoma, colorectal carcinoma, papillary thyroid carcinoma and squamous cell carcinoma of the head and neck[15-19]. In this study, we observed positive expression of MMP-9 in 65.23 % of patients with gastric carcinoma, but no association of the expression of MMP-9 with sex, age, tumor size and location. These results were similar to the results reported by Murray *et al*[12] and Hou *et al*[20]. In contrast to the Murray's and Hou's study, we also demonstrated there was a significant correlation between MMP-9 expression and proliferation of tumor cells, the depth of invasiveness, lymph node metastasis and TNM stage of gastric carcinoma. Pereda *et al* also found that high levels of matrix metalloproteinases promoted the proliferation of pituitary adenomas cells[21]. Kabashima *et al* reported that MMP-9 expression correlated with lymph node metastasis in intramucosal gastric carcinoma[22]. Torri *et al* reported that preoperative plasma MMP-9 concentration correlated closely with severity of T, N and M classification, and stage[23]. These results suggest that over-expression of MMP-9 plays an important role in the progress of gastric carcinoma, and MMP-9 protein may be served as a marker for invasiveness and metastasis of gastric carcinoma. We also noticed that MMP-9 expression increased dramatically in advanced tumors compared with early tumors, whereas there was no such difference between different stages of the advanced tumors. These results suggested that MMP-9 expression might play an important role in the early progress of gastric carcinoma. Sier et al reported that the expression and activation of MMP-9 in tumor tissues were of prognostic significance for poor overall survival of the patients with gastric carcinoma, independent of the major clinicopathological parameters[8,24]. Although there was a decreasing trend of survival in the patients with MMP-9 expression compared with those without the expression, the difference was no significant. Maatta *et al* reported the similarly results in hepatocellular carcinoma and pancreatic adenocarcinoma[25]. The relation of MMP-9 to the prognosis of gastric carcinoma still was needs to be further investigated.

Expression of TIMP-1 and invasiveness, metastasis and prognosis of gastric carcinoma

During the process of invasiveness and metastasis of tumors, the secretion and activation of metalloproteinases (MMPs) is not sufficient to degrade ECM components, as its enzymatic activity can be inhibited by a family of endogenous inhibitors, the tissue inhibitors of metalloproteinase (TIMPs). TIMP-1, a 28.5 kDa glycoprotein, is the first member of the TIMP family, and known to form a complex of 1:1 stoichiometry with activated collagenase, stromelysin and MMP-9 to inhibit their activities. Watanabe *et al* found that the transfection of the complete human TIMP-1 cDNA into highly metastatic human gastric carcinoma cell line KKLS notably decreased the formation of liver metastases when transplanted into nude mice[11]. It is suggested that TMP-1 is a negative regulators in the process of tumor metastasis. The expression of TIMP-1 in gastric carcinoma has not been widely examined so far. In our study, a negative association between TIMP-1 expression and invasiveness and metastasis and TNM stage was observed, but there was no association between TIMP-1 expression and sex, age, tumor size and location in gastric carcinoma. These results were opposite to the results reported by Mimori *et al* that the expression of TIMP-1 mRNA in the biopsy samples from human gastric carcinoma tissues (T) was higher than in the biopsy samples from the corresponding normal tissues (N), and a higher T/N ratio of TIMP-1 mRNA correlated with lately advanced stage and poor prognosis of human gastric carcinoma[26]. Other studies also showed that the increased TIMP-1 expression correlated with poor prognosis variables, including shortened survival, in patients with renal cell carcinoma and lung cancer[8,27]. Several studies have shown that TIMP-1 possesses two activities, i.e. inhibitory activity of metalloproteinases, and growth promoting function[2,28]. Our findings suggest that, TIMP-1 in the progress of human gastric carcinoma functions mainly as an inhibitor of metalloproteinases, subsequently blocking the invasiveness and metastasis of tumor cells. Our findings may offer a new idea in the biological and genetic treatment for gastric carcinoma[29-34]. In our study, the survival rate of patients with TIMP-1 expression was higher than those without TIMP-1 expression, although difference was not significant. Further studies are needed to determine whether or not TIMP-1 expression alone can serve as a marker predicting the prognosis of patients with gastric cancer.

Imbalance between expression of MMP-9 and TIMP-1 in the invasiveness, metastasis and prognosis of gastric carcinoma

Under physiological conditions the expression of MMPs and TIMPs is highly coordinated at the level of gene expression, and this balanced expression guarantees normal tissue structure and organ function, and prevents both excessive ECM deposition and increased ECM degradation. As some factors in malignant tumors contributes to the over-expression of MMPs without matched TIMP expression, and thus, this balance was broken, thereby, the ECM was degraded and the cancer metastasis was occurred. By contraries, over-expression of TIMPs can prevent the degradation of ECM and inhibit the cancer invasion and metastasis. TIMP-1 can bind to the catalytic domain of MMP-9 in a 1:1 stochiometry to form complex, so inhibiting the enzymatic activity of MMP-9 [2,9,10]. Murray *et al* revealed the correlation between the expression of MMP-9 and the expression of TIMP-1 in gastric carcinoma[12,27,35]. However, observation concerning the association of imbalance between the expression of MMP-9 and TIMP-1 with invasion and metastasis in gastric carcinoma has rarely been published. We found that the tumor invasion and metastasis was more frequent in the cases with positive expression of MMP-9. However, the extents of invasion and metastasis in gastric carcinoma significantly decreased if the TIMP-1 was also expressed in these cases at the same time. This suggests that MMP-9 mainly exert functions of promoting cancer invasion and metastasis, while TIMP-1 independently exerts the inhibiting function for cancer invasion and metastasis during the processes of the invasion and metastasis of gastric carcinoma. We also found that the incidence of visceral peritoneum invasion and lymph node metastasis was the highest in the cases with MMP-9 expression but without TIMP-1 expression, whereas, the incidence of lymph node metastasis in the cases with the TIMP-1 expression but without MMP-9 expression was the lowest, with the modest incidence in cases with balanced expression of MMP-9 and TIMP-1. These findings strongly support the hypothesis the expressive imbalance between MMP-9 and TIMP-1 is an important factor in tumor invasion and metastasis. In brief, altered balance of expression between MMP-9 and TIMP-1 plays a central role in progression of gastric carcinoma. According to our follow-up information, we found, for the first time, that although MMP-9 or TIMP-1 alone may not serve as an indicator for patient prognosis, there is a significant association of the expressive imbalance between MMP-9 and TIMP-1 with the postoperative survival of patients with gastric carcinoma. Our data suggest that patients with over-expression of MMP-9 and no expression of TIMP-1 have more aggressive tumor progression and a lower survival rate.

In conclusion, our results indicate a significant positive association between MMP-9 expression and proliferation of tumor cells, the depth of invasiveness, lymph node metastasis

and TNM stage of gastric carcinoma, suggesting MMP-9 can serve as a molecular marker of tumor invasion and metastasis. Our results also demonstrate a significant negative association of TIMP-1 expression with the depth of invasiveness and lymph node metastasis, which provides a new idea in tumor biological and genetic treatment. The interaction between MMP-9 and TIMP-1 in the processes of tumor invasion and metastasis is that MMP-9 mainly promotes tumor invasion and metastasis whereas TIMP-1 inhibits the functions of MMP-9. Imbalance between MMP-9 and TIMP-1 expression may predict the occurrence of tumor invasion and metastasis and poor prognosis. For these patients with imbalanced MMP-9 and TIMP-1 expression, the optimal treatment scheme needs to be selected.

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