ANNALS OF CANCER RESEARCH AND THERAPY

VOLUME 12, NOS. 1 & 2, COPYRIGHT © 2004 BY

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Biomedical Publisher to the World

Box 966, Westbury, NY 11590-0966 USA

**MATRIX METALLOPROTEINASE-7 IS A USEFUL MARKER FOR
PREDICTING AGGRESSIVE BEHAVIOR OF SUBMUCOSAL GASTRIC
CANCER**

Jiro Shimazaki¹⁾, Masayuki Itabashi²⁾, Motonobu Katano¹⁾ and Takafumi Tabuchi¹⁾

Department of Fourth Surgery, Tokyo Medical University Kasumigaura Hospital¹⁾

Department of Pathology, Ibaraki Prefectural Central Hospital and Cancer Center²⁾

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Key Words: Carcinoma, clinicopathologic findings, extracellular matrix, gastric cancer, immunoreactivity, invasion, lymphatic invasion, malignancy, metastasis, MMPs, regression rates, TIMPs, tumor invasion, vascular vessel invasion.

Abstract

To elucidate the process of metastasis in submucosal gastric cancer (SM-GC), we examined the immunohistochemical expression of matrix metalloproteinases (MMPs: MMP-2, MMP-7, and MMP-9) and tissue inhibitors of metalloproteinases (TIMPs: TIMP-1, and TIMP-2) in 66 cases of SM-GC, and compared them with clinicopathological findings of the cases. The positive rates for MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 were 42.4%, 33.3%, 9.1%, 0%, and 48.5%, respectively. There was a significant correlation between MMP-7 positive rate and the degree of tumor invasion. Moreover, only MMP-7 positive rate was significantly higher in cases with lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis than in cases without them. MMP-2 positive rate was significantly higher in cases with massive submucosal invasion

¹ Corresponding author: Dr. Jiro Shimazaki, Department of Fourth Surgery, Tokyo Medical University Kasumigaura Hospital, 3-20-1 chyuou, Ami-machi, Inasiki-gun, Ibaraki 300-0395, Japan. Telephone: +81-29-887-1161 (ext. 7076; Fax: +81-29-840-2089; E-mail: shimau@plum.plala.or.jp.

(sm₂) and intestinal-type carcinoma. There were no significant correlations between clinicopathological findings and positive rates for MMP-9, TIMP-1, and TIMP-2. In logistic regression, MMP-7 immunoreactivity was found to be an independent risk factor of lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis. These data suggest that MMP-7 is an important factor implicated in local invasion and metastasis, and would be a useful marker for predicting aggressive behavior of SM-GC.

Introduction

Cancer cell metastasis comprises a multi-step process, and degradation of the extracellular matrix, such as the connective tissue and the basement membrane, by cancer cells is an important step in this process (Hart and Saini, 1992, Kohn and Liotta, 1995). At present, matrix metalloproteinases (MMPs), which are involved in the degradation of the extracellular matrix, and tissue inhibitors of metalloproteinases (TIMPs), which are potential inhibitors of MMPs, are investigated in several tumor cell types. In gastric cancer, MMPs and TIMPs are investigated with respect to cell differentiation, invasion, metastasis, malignant potential, and prognosis. Apart from the present study, only a few reports concerned with early gastric cancer are available, particularly with regard to submucosal gastric cancer (SM-GC). Establishing a correlation between the clinicopathological findings and the expression of MMPs, and TIMPs is useful for understanding the biological features of SM-GC. Moreover, the degree of tumor invasion, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis are important factors that determine the SM-GC treatment. Evaluation of these factors by observing MMPs and TIMPs will serve as an aid for the diagnosis and treatment of SM-GC. This study aims to elucidate the correlation between the clinicopathological findings and the immunoreactions of MMPs and TIMPs in SM-GC. This is performed by using a primary antibody to MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2.

Material and Methods

1. Material

The 66 cases of SM-GC, which were surgically resected at the Ibaraki Prefectural Central Hospital and Cancer Center during January 1997 and June 2000, were studied (Table 1). Clinicopathological findings were evaluated based on the Japanese Classification of Gastric Carcinoma (Japanese Research Society for Gastric cancer, 1999).

In histological type, papillary adenocarcinoma and tubular adenocarcinoma were defined as an intestinal-type carcinoma, and poorly differentiated adenocarcinoma and signet ring cell carcinoma were defined as a diffuse-type carcinoma.

2. Methods

Immunohistochemistry of MMPs and TIMPs: A single paraffin block which includes whole the layers of tumor tissue was selected from each case. To retrieve the antigen, deparaffinized sections for MMPs were heated in microwave oven (500W) for 20 minutes and those for TIMPs were treated with 0.04% trypsin. The endogenous peroxidase activity was suppressed by a solution of 0.3% H₂O₂. The sections were incubated with primary antibody to MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 (Fuji Chemical Industry, Table 2) for 14 hours at 4°C, and the labeled streptoavidin biotin complex method (LSAB) was adopted using a LSAB kit (DAKO). The sections were developed in 0.05M Tris-HCl buffer containing 0.6 ng/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.006% H₂O₂, and counterstaining was performed with hematoxylin.

Definition as a positive immunoreaction: MMPs and TIMPs immunoreactions were considered positive if MMPs and TIMPs immunoreactivity were found in more than 20% of the tumor cells. This cut off value was adopted because of the statistical significant difference that was found.

Statistical significance of differences were determined by Fisher's exact probability test and logistic regression as multivariate analysis. The level of significance was $p < 0.05$.

Results

1. Immunohistochemical staining of MMPs and TIMPs

Immunohistochemically, all MMPs and TIMPs stained diffusely and granularly to intracytoplasm of the tumor cells (Figure 1a-e). In part, MMP-2 stained on tumor cell membranes. MMP-7, MMP-9 and TIMP-1 showed no special distribution patterns of immunoreactive carcinoma cells in tumor cell nests, but staining of MMP-2 and TIMP-2 had a tendency to be stronger in the tumor center than deep point of the tumor. Inflammatory cells and fibroblasts of the tumor were strongly positive for MMP-2, MMP-9, TIMP-1, and TIMP-2. Moreover, endothelial cells were positive for MMP-2 and

MMP-9. MMP-7 was localized predominantly to the tumor cells than inflammatory cells and fibroblasts.

TABLE I

Clinicopathological findings of SM-GC cases.

Clinicopathological findings	SM-GC (n=66)
Degree of tumor invasion	
sm1	23 (34.8%)
sm2	43 (65.2%)
Histological type	
intestinal-type carcinoma	42 (63.6%)
diffuse-type carcinoma	24 (36.4%)
Amount of tumor stroma	
medullary-type	30 (45.5%)
intermediate-, and scirrhous-type	36 (54.5%)
Pattern of tumor infiltration (INF)	
α	24 (36.4%)
β and γ	42 (63.6%)
Lymphatic invasion (ly)	
absent	40 (60.6%)
present	26 (39.4%)
Vascular vessel invasion (v)	
absent	59 (89.4%)
present	7 (10.6%)
Lymph nodes metastasis (n)	
absent	47 (71.2%)
present	19 (28.8%)
Clinical stage	
I A	47 (71.2%)
I B	19 (28.8%)

TABLE II

List of primary antibodies used.

	Clone No.	working dilutions
MMP-2	75-7F7	1ug/ml
MMP-7	141-7B2	5ug/ml
MMP-9	56-2A4	5ug/ml
TIMP-1	147-6D11	5ug/ml
TIMP-2	67-4H11	2.5ug/ml

Fuji Chemical Industry

TABLE III
Clinicopathological findings and MMP-7 positive rate in SM-GC cases.

Clinicopathological findings	66cases	MMP-7 positive (%)	MMP-7 negative (%)	p-value
Degree of tumor invasion				
sm1	23	3 (13.0%)	20 (87.0%)	p<0.05
sm2	43	19 (44.2%)	24 (55.8%)	
Histological type				
intestinal-type carcinoma	42	11 (26.2%)	31 (73.8%)	N.S.
diffuse-type carcinoma	24	11 (26.2%)	13 (54.2%)	
Amount of tumor stroma				
medullary-type	30	9 (30.0%)	21 (70.0%)	N.S.
intermediate-, and scirrhous-type	36	13 (36.1%)	23 (63.9%)	
Pattern of tumor infiltration (INF)				
α	24	5 (20.8%)	19 (79.2%)	N.S.
β and γ	42	17 (40.5%)	25 (59.5%)	
Lymphatic invasion (ly)				
absent	40	2 (5.0%)	38 (95.0%)	p<0.01
present	26	20 (76.9%)	6 (23.1%)	
Vascular vessel invasion (v)				
absent	59	16 (27.1%)	43 (72.9%)	p<0.01
present	7	6 (85.7%)	1 (14.3%)	
Lymph nodes metastasis (n)				
absent	47	8 (17.0%)	39 (83.0%)	p<0.01
present	19	14 (73.7%)	5 (26.3%)	
Clinical stage				
IA	47	8 (17.0%)	39 (83.0%)	p<0.01
IB	19	14 (73.7%)	5 (26.3%)	

Fisher's exact probability test

TABLE IV
Clinicopathological findings and MMP-2 positive rate in SM-GC cases

Clinicopathological findings	66cases	MMP-2 positive (%)	MMP-2 negative (%)	p-value
Degree of tumor invasion				
sm1	23	5 (21.7%)	18 (78.3%)	p<0.05
sm2	43	23 (53.5%)	20 (46.5%)	
Histological type				
intestinal-type carcinoma	42	22 (53.4%)	20 (47.6%)	p<0.05
diffuse-type carcinoma	24	6 (25.0)	18 (75.0%)	
Amount of tumor stroma				
medullary-type	30	16 (53.3%)	14 (46.7%)	N.S.
intermediate- and scirrhous-type	36	12 (33.3%)	24 (66.7%)	
Pattern of tumor infiltration (INF)				
α	24	11 (45.8%)	13 (54.2%)	N.S.
β and γ	42	17 (40.5%)	25 (59.5%)	
Lymphatic invasion (ly)				
absent	40	17 (42.5%)	23 (57.5%)	N.S.
present	26	11 (42.3%)	15 (57.7%)	
Vascular vessel invasion (v)				
absent	59	24 (40.7%)	35 (59.3%)	N.S.
present	7	4 (57.1%)	3 (42.9%)	
Lymph nodes metastasis (n)				
absent	47	22 (46.8%)	25 (53.2%)	N.S.
present	19	6 (31.6%)	13 (68.4%)	
Clinical stage				
I A	47	22 (46.8%)	25 (53.2%)	N.S.
I B	19	6 (31.6%)	13 (68.4%)	

Fisher's exact probability test

TABLE V
Multivariate analysis of MMPs and TIMPs in SM-GC cases

	Lymphatic invasion			Vascular vessel invasion			Lymph nodes metastasis		
	p-value	Relative risk (odds ratio)	95% confidence interval	p-value	Relative risk (odds ratio)	95% confidence interval	p-value	Relative risk (odds ratio)	95% confidence interval
Degree of tumor invasion (sm1, sm2)	0.3236	2.918	0.346~24.463	0.9733	290163.458	9.334E-316~.	0.3646	2.28	0.384~13.546
Histological type (intestinal, diffuse)	0.2187	0.195	0.014~2.638	0.248	0.198	0.013~3.088	0.3496	0.411	0.064~2.650
Amount of tumor stroma (med, int and sci)	0.4549	2.362	0.248~22.527	0.5364	0.472	0.044~5.105	0.6558	1.473	0.268~8.081
Pattern of tumor infiltration (INF: α , β and γ)	0.1146	6.912	0.626~76.291	0.4971	2.647	0.159~43.923	0.3044	2.578	0.423~15.712
MMP-2 (positive, negative)	0.6778	0.621	0.066~5.879	0.8617	1.202	0.151~9.555	0.1248	0.279	0.055~1.425
MMP-7 (positive, negative)	0.0004	267.462	12.089~5917.272	0.0344	14.872	1.219~181.510	0.0013	13.125	2.726~63.187
MMP-9 (positive, negative)	0.473	0.201	0.003~16.057	0.9874	6.509-E6	0.000~.	0.6612	0.489	0.020~11.995
TIMP-2 (positive, negative)	0.0594	0.101	0.009~1.095	0.9494	0.931	0.102~8.532	0.4962	0.593	0.131~2.675

Multivariate analysis of MMPs and TIMPs immunoreactions in 66 cases cases submucosal gastric cancer (SM-GC) considering additional clinicopathological findings in gastric cancer (Degree of tumor invasion, histological type, amount of tumor stroma, pattern of tumor infiltration). Level of significance, relative risk estimated by odds ratio and 95% confidence interval are given in the table for those parameters. MMP-7 is revealed to be independent risk factor of lymphatic invasion, vascular vessel invasion and lymphnodes metastasis (significant p value, relative risk>1).

Figure 1 a

Figure 1: Immunohistochemical staining of MMPs and TIMPs in SM-GC. Immunostaining was performed with primary antibody to MMP-2 (a), MMP-7 (b), MMP-9 (c), TIMP-1 (d), and TIMP-2 (e). (x200).

Figure 1. b

Figure 1.c

Figure 1. d

Figure 1. e

2. *Correlation between the clinicopathological findings and the MMPs, TIMPs expression in SM-GC cases.*

The positive rates for MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 were 42.4%, 33.3%, 9.1%, 0%, and 48.5%, respectively. MMP-7 positive rate was significantly higher in cases with massive submucosal invasion (sm₂), clinical stage IB group, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis (44.2%, 73.7%, 76.9%, 85.7%, and 73.7%, respectively: Table 3). There was no significant correlation between MMP-7 expression and histological type, amount of tumor stroma, or pattern of tumor infiltration (INF). MMP-2 positive rate was significantly higher in cases with sm₂ and intestinal-type carcinoma (53.5% and 53.4%, respectively: Table 4). There were no significant correlations between clinicopathological findings and MMP-9, TIMP-1, and TIMP-2 expression.

In logistic regression considering 8 factors which contained MMP-2, MMP-7, MMP-9, TIMP-2, and four clinicopathological findings, such as histological type, degree of tumor invasion, amount of tumor stroma, and INF, MMP-7 expression was found to be an independent risk factor of lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis (p-value were 0.0004, 0.0344, and 0.0013, respectively) (Table 5).

Discussion

Approximately 20 different types of MMP, which is a family of closely related proteins that degrade the extracellular matrix, have been identified. MMP-7 (matrilysin) is the smallest type of MMP, and exhibits high activities against a wide range of substrates, such as type-IV collagen, laminin, proteoglycans, fibronectin, gelatin, and elastin (Sires, Griffin *et al.*, 1993; Imai, Yokohama *et al.*, 1995). Honda, Mori *et al.* (1996) and Yamashita, Azumano *et al.* (1998) demonstrated by means of reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry that in gastric cancer tissue, the activity of MMP-7 is higher in the tumor tissue rather than in the non-tumor tissue, and the over-expression of MMP-7 significantly correlated with lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis. Early and advanced gastric cancers were studied previously, but these studies did not focus on

SM-GC. In this immunohistochemical study of 66 SM-GC cases, the MMP-7 positive rate was significantly higher in cases with sm₂, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis, and it was independent risk factor of lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis in SM-GC. These data suggest that MMP-7 expression is an important factor implicated in local invasion and metastasis, and would be a useful marker for predicting aggressive behavior of SM-GC.

Evaluation of the degree of tumor invasion, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis during SM-GC pre-treatment would enable us to perform a minimum treatment, such as endoscopic resection or gastrectomy without dissection of the lymph nodes. In this study, we demonstrated that MMP-7 was an important factor implicated in the local invasion and metastasis of SM-GC. This result suggests that it is possible to evaluate the degree of tumor invasion, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis by observing MMP-7 expression in biopsy specimens of SM-GC. However, there are some contradictions because the biopsy specimens are minimum samples of the surface of the tumor tissue. These are often fragmentary and necrotic, so it is unclear whether they can correctly evaluate MMP-7 expression. Further, there are some cases with sm₂, lymphatic invasion, vascular vessel invasion, and lymph nodes metastasis, although these were negative for MMP-7 expression. It is thought as a reason of this result that another proteolytic enzyme, such as a MT-MMP, affects invasiveness of gastric cancer (Bando, Yonemura *et al.*, 1998). Only results of this study are not enough to determine the SM-GC treatment by observing MMP-7 expression in biopsy specimens. In order to resolve these problems, quantitative evaluation using a more specific primary antibody to MMP-7, and investigation using many proteolytic enzymes and biopsy specimens of SM-GC, are required.

MMP-2 and MMP-9 belong to the group of type IV collagenase in the MMP family, and degrade type IV collagen, which is the main component of the basement membrane (Emonard, Remacle *et al.*, 1992; Cao, Sato *et al.*, 1995; Ballin, Gomez *et al.*, 1988). In previous studies on MMP-2 in gastric cancer using immunohistochemistry, Allgayer, Babic *et al.* (1998) and Grigioni, D'Frizzo *et al.* (1994) demonstrated that MMP-2 immunoreaction significantly correlated with diffuse-type carcinoma and this

affected the overall survival of the gastric cancer patients. In contrast, David, Nesland *et al.* (1994) concluded that there were no significant correlations between the MMP-2 expression and the clinicopathological findings. Using gelatin zymography and immunohistochemistry, Nomura, Fujimoto *et al.* (1996) reported that MMP-2 activation correlated with local invasion and lymphatic permeation in gastric cancer. In this study, the MMP-2 positive rate was significantly higher in cases with sm₂ and intestinal-type carcinoma. Regarding the role of MMP-9 in gastric cancer, Murray, Duncan *et al.* (1998) and Migita, Sato *et al.* (1999) demonstrated that MMP-9 expression was higher in cases with intestinal-type carcinoma, although no correlations were found between MMP-9 expression and the clinicopathological findings in this study. Differences in the primary antibody and the methods of immunohistochemical staining may account for the discrepancies observed in MMP-2 and MMP-9. Furthermore, we observed the MMP and TIMP immunoreactions only in tumor cells, although stromal cells that surround the tumor cells had strong immunoreactivities for MMP-2 and MMP-9. It is unclear which immunoreactivities affect the invasion and metastasis of tumor cells. Detailed molecular biological studies and quantitative evaluation of MMP-2 and MMP-9 immunoreactivities, which distinguish between tumor cells and stromal cells, would be required to elucidate the relationship of MMP-2 and MMP-9 to cancer metastasis.

Four different types of TIMPs have been identified, which are specific inhibitors of the MMPs. The TIMPs play a central role in restraining the activation of individual MMPs. Based on these functions of TIMPs, it is hypothesized that TIMPs restrain the invasion and metastasis of tumor cells through MMP functions. On the other hand, a recent molecular biological study reported that TIMP-1 and TIMP-2 played a role not only in MMP inhibition but also in cell growth-promoting activities (Hayakawa, Yamashita *et al.*, 1992; 1994; Gomez, Alonso *et al.*, 1997). Furthermore, Nagase (1997) reported that TIMP-2 affected the proMMP-2 activation process. These complex functions of TIMPs provide an explanation for the absence of significant correlations between TIMP-1 and, TIMP-2 immunoreactions and clinicopathological findings of SM-GC cases in this study.

References

- Allgayer, H., Babic, R., Beyer, B.C.M., Grutzer, K.U., Tarabichi, A., Schildberg, F.W. and Heiss, M.M. (1998). Prognostic relevance of MMP-2 (72-kD collagenase IV) in gastric cancer. *Oncology* 55:152-160.
- Allgayer, H., Babic, R., Grutzer, K.U., Beyer, B.C.M., Tarabichi, A., Schildberg, F.W. and Heiss, M.M. (1998). Tumor-associated proteases and inhibitors in gastric cancer: analysis of prognostic impact and individual risk protease patterns. *Clin.Exp.Metastasis* 16:62-73.
- Ballin, M., Gomez, D.E., Sinha, C.C. and Thorgeirsson, U.P. (1988). Ras oncogene mediated induction of a 92kDa mettalloproteinase; strong correlation with the malignant phenotype. *Biochem.Biophys.Res.Commun.* 154:832-838.
- Bando, E., Yonemura, Y., Endou, Y., Sasaki, T., Taniguchi, K., Fujita, H., Fushida, S., Fujimura, T., Nishimura, G., Miwa, K. and Seiki, M. (1998). Immunohistochemical study of MT-MMP tissue status in gastric carcinoma and correlation with survival analyzed by univariate and multivariate analysis. *Oncol.Rep.* 5:1483-1488.
- Cao, J., Sato, H., Takino, T. and Seiki, M. (1995). The C-terminal region of membrane type metrix metalloproteinase is a functional transmembrane domain required for pro-gelatinases A activation. *J.Biol.Chem.* 270:801-805.
- David, L., Nesland, J.M., Holm, R. and Sobrinho-Simoes, M. (1994). Expression of laminin, collagen IV, fibronectin, and type IV collagenase in gastric carcinoma. *Cancer* 73:518-527.
- Emonard, H.P., Remacle, A.G., Noel, A.C., Grimaud, J.A., Stetler-Stevenson, W.G. and Foidart, J.M. (1992). Tumor cell surface-assoiciated binding site for the M_r 72,000 type IV collagenase. *Cancer Res.* 52:5845-5848.
- Grigioni, W.F., D'Errico, A., Fiorentino, M., Mancini, M., Stetler-Stevenson, W.G., Sobel, M.E., Liotta, L.A., Onisto, M. and Garbisa, S. (1994). *Mod.Pathol.* 7:220-225.
- Gomez, D.E., Alonso, D.F., Yoshiji, H. and Thorgeirsson, U.P. (1997). Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *EJCB.* 74:111-122.
- Hart, I.R. and Saini, A. (1992). Biology of tumor metastasis. *Lancet* 339:1453-1457.
- Hayakawa, T., Yamashita, K., Tanzawa, K., Uchijima, E. and Iwata, K. (1992). Growth-promoting of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. *FEBS.Lett.* 298:29-32.
- Hayakawa, T., Yamashita, K., Ohuchi, E. and Shinagawa, A. (1994). Cell growth-promoting activity of tissue inhibitor of metalloprotenases-2 (TIMP-2). *J.Cell.Sci.* 107:2373-2379.
- Honda, M., Mori, M., Ueo, H., Sugimachi, K. and Akiyoshi, T. (1996). Matrix metalloproteinase-7 expression in gastric cancer. *Gut.* 39:444-448.

- Imai, K., Yokohama, Y., Nakanishi, I., Ohuchi, E., Fujii, Y., Nakai, N. and Okada, Y. (1995). Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. *J.Biol.Chem.* 270:6691-6697.
- Japanese Classification of Gastric Carcinoma. Japanese Research Society for gastric cancer. (1999). Kanehara & Co., Ltd., Tokyo, Japan.
- Kohn, E.C. and Liotta, L.A. (1995). Molecular insights into cancer invasion: Strategies for prevention and intervention. *Cancer Res.* 55:1856-1862.
- Murray, G.I., Duncan, M.E., Arbuckle, E., Melvin, W.T. and Fothergill, J.E. (1998). Matrix metalloproteinases and their inhibitors in gastric cancer. *Gut.* 43:791-797.
- Migita, T., Sato, E., Saito, K., Mizoi, T., Shiba, K., Matsuno, S., Nagura, H. and Ohtani, H. (1999). Differing expression of MMPs-1 and -9 and urokinase receptor between diffuse- and intestinal-type gastric carcinoma. *Int.J.Cancer* 84:74-79.
- Nomura, H., Fujimoto, N., Seiki, M., Mai, M. and Okada, Y. (1996). Enhanced production of matrix metalloproteinases and activation of matrix metalloproteinase-2 (gelatinase A) in human gastric carcinomas. *Int.J.Cancer* 69:9-16.
- Nagase, H. (1997). Activation mechanisms of matrix metalloproteinases. *Biol.Chem.* 378:151-160.
- Sires, U.I., Griffin, G.L., Broekelmann, T.J., Mecham, R.P., Murphy, G., Chung, A.E., Welgus, H.G. and Senior, R.M. (1993). Degradation of entactin by matrix metalloproteinases. *J.Biol.Chem.* 268:2069-2074.
- Yanashita, K., Azumano, I., Mai, M. and Okada, Y. (1998). Expression and tissue localization of matrix metalloproteinase 7 (matrilysin) in human gastric carcinomas. Implications for vessel invasion and metastasis. *Int.J.Cancer* 79:187-194.

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