J Gastroenterol 2007; 42:120–128
DOI 10.1007/s00535-006-1975-y

Journal of Gastroenterology

© Springer 2007

Clinical significance of tissue levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in gastric cancer

Ki-Nam Shim, Sung-Ae Jung, Yang-Hee Joo, and Kwon Yoo

Department of Internal Medicine, Ewha Medical Research Institute, Ewha Womans University College of Medicine, Seoul, Korea

Background. Matrix metalloproteinases (MMPs) are one of the major classes of proteolytic enzymes involved in tumor invasion and metastasis, being inhibited by naturally occurring tissue inhibitors of metalloproteinases (TIMPs). In this study, we examined the expression of MMP-2, MMP-9, membrane-type 1 (MT1)-MMP, TIMP-1, and TIMP-2 in biopsy tissues of gastric cancer, and the correlation between their expression and clinicopathological parameters. ***Methods.*** Biopsy specimens from 66 patients with gastric carcinoma were available for this study. To determine the expression of MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2, semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out on tumor and normal tissues, respectively, sampled during diagnostic gastroscopic examination. Immunohistochemical staining of representative samples using monoclonal antibody directed against MT1-MMP was done, and the clinicopathological variables were reviewed retrospectively. ***Results.*** The expression level of MMPs and TIMPs was evaluated using the tumor : normal (T/N) ratios of MMPs and TIMPs. The T/N ratio of MT1-MMP mRNA showed a significant correlation with lymph node metastasis and tumor stage ($P < 0.05$). The other RT-PCR data of MMP-2, MMP-9, TIMP-1, and TIMP-2 did not show any significant correlation with clinicopathological parameters. Immunohistochemistry for MT1-MMP showed a positive immunoreaction in gastric adenocarcinoma and negative staining in normal mucosa. ***Conclusions.*** The correlation between the increased expression of MT1-MMP and clinicopathological variables reflects a role in predicting the aggressive behavior of gastric cancer. Because an RT-PCR assay can be performed on biopsy specimens obtained before surgery, an evaluation of MT1-MMP expression in biopsy specimens by RT-PCR may provide useful preoperative information on tumor aggressiveness.

Key words: matrix metalloproteinase, tissue inhibitor of metalloproteinase, semi-quantitative RT-PCR, gastric carcinoma

Introduction

Gastric cancer usually shows extensive local tumor invasion and early spread to metastatic sites. Metastasis of gastric cancer cells depends on some only partly understood factors: angiogenesis, cellular attachment, proteolysis, migration through the barrier into secondary sites, and, of course, colonization and proliferation in distant organs.[1] One important step in tumor invasion is the penetration of the basement membrane.[2] The basement membrane is a strong barrier to the movement of tumor cells. The invasion of the basement membrane proceeds through a series of discrete steps.[3] The matrix degradation in the basement membrane is closely related to activities of various subtypes of matrix metalloproteinases (MMPs) and the corresponding tissue inhibitors of matrix metalloproteinase (TIMPs). There are now more than 20 related enzymes, which are classified as secreted or soluble-type MMPs and membrane-type (MT)-MMPs. Among the MMPs, MMP-2 and MMP-9 have been the focus of attention in connection with cancer metastasis because of their ability to degrade type IV collagen, a major constituent of the vascular basement membrane.[4–6] MT1-MMP was the first member of the MT-MMP family to be discovered, since it is tethered to the plasma membrane.[7,8] MT1-MMP is distinguished from the soluble or secreted MMPs, such as MMP-2 and MMP-9, by the presence of a hydrophobic transmembrane domain at the C-terminus. The expression of MT1-MMP has been

Received: November 7, 2005 / Accepted: November 1, 2006
Reprint requests to: K.-N. Shim
Present Address: 911-1 Mokdong Yangcheon-gu, Seoul, 158-710, Korea

thought to initiate multiple protein cascades on the cell surface.[9–11]

MMP-2 (gelatinase A; 72-kDa gelatinase; type IV collagenase) is an important enzyme of the MMP family, which is able to degrade collagen IV, a basic component of constitutive basement membranes.[12] Like other members of the MMP family, MMP-2 is secreted in a latent form, which requires cleavage of the N-terminal 80 amino acids to become active.[13] The activation and enzymatic activity of MMP-2 is regulated by TIMP-2.[13] MMP-2 has been considered essential for metastasizing tumor cells. In this context, evaluation of MMP-2 expression in lung, breast, and colon cancer appears to be a useful prognostic indicator.[14–16]

TIMP-1-transfected cells or carcinoma cells with abundant expression of TIMP-1 mRNA inhibit MMP activity and ability to invade the basement membranes in various human carcinoma cell lines.[17–19] Recent studies have reported an alternative function of TIMP-1, that is, as a growth factor; it is highly homologous with erythroid-potentiating activity, which is an autocrine growth factor for the erythroid leukemia cell line K562.[20,21] Moreover, TIMP-1 also shares homology with a fibroblast elongation factor that is secreted from colon carcinoma cells and which stimulates tumor cell proliferation.[22] TIMP-1 RNA levels are higher in primary colorectal carcinomas with distant metastasis than in those without metastasis,[23] and the expression of TIMPs increases with the advance of the neoplastic process.[24]

The expression and involvement of several MMPs and TIMPs in human gastric carcinoma have been determined in several studies. However, the studies showed relatively conflicting results about their contribution to the clinicopathological findings and prognosis of the patients with gastric cancer. In the present study, we examined the expression of MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2 mRNA in human gastric carcinoma tissues by a reverse transcriptase-polymerase chain reaction (RT-PCR) assay, which enabled us to analyze small sample amounts, such as biopsy specimens before surgery, and the correlation between their expression and clinicopathological parameters.

Patients and methods

Patients

Biopsy specimens from 66 patients (41 men and 25 women) with gastric carcinoma, from whom clinical and histopathological data concerning patients and carcinomas were available, were obtained during a diagnostic gastroscopic examination between August 2003 and July 2004. All patients underwent gastrectomy with curative intent. Mean age was 61.3 ± 13.5 years, ranging from 28 to 84 years. Four biopsy specimens of representative samples of the carcinoma and macroscopically normal mucosa from each patient were frozen and stored at −70°C until extraction. The study was approved by the Human Research Review Committee, and informed consent was obtained from all patients.

Methods

RNA extraction

Total RNA was extracted from biopsy tissues using an easy-BLUE (Intron Biotechnology, Sungnam, Korea) total RNA extraction kit. Prepared fresh tissues were added to 800 µml easy-BLUE reagent and homogenized using a homogenizer or equivalent and vigorously vortexed at room temperature for 10 s. Then, 200 µl of chloroform was added and a vortex was applied. After centrifuging the solution at 12 000 g (4°C) for 10 min, 400 µl of the supernatant was transferred to an empty 1.5-ml tube. Then, 400 µl isopropanol (2-propanol) was added, and we mixed it well by inverting the tube 2–3 times. It was left for 10 min at room temperature. After centrifuging the solution at 12 000 g (4°C) for 10 min, we removed the supernatant to obtain the RNA pellet. Then, 1 ml of 75% EtOH was added and the solution was mixed well by inverting the tube 2–3 times. The mixture was centrifuged for 5 min at 12 000 g (4°C). The supernatant was discarded, and the remaining RNA pellet was dried. RNA was dissolved using 20–50 µl of diethylpyrocarbonate-treated distilled water for storage at −70°C. The amount and purity of the extracted RNA was determined by spectrophotometry.

cDNA synthesis

cDNA was synthesized with 5 µg of total RNA and oligo dT primer. In a sterile RNase–free microcentrifuge tube, 0.5 µg of oligo dT primer and 5 µg of RNA sample were added. The tube was heated at 70°C for 5 min, and cooled immediately on ice. Moloney murine leukemia virus reverse transcriptase (M-MLV RT; Promega, Madison, WI, USA) 200 units, rRNasin ribonuclease inhibitor (Promega) 25 units, 5× RT buffer, and dNTP were added to the tube. The tube was gently mixed, incubated for 60 min at 42°C, and heated for 5 min at 95°C. The cDNA was stored at −20°C.

Oligonucleotide primers

The primers used were 5′-AAG ATG ACC CAG ATC ATG TTT GAG-3′ and 5′-AGG AGG AGC AAT GAT CTT GAT CTT-3′ for β-actin, 5′-ACC TGG ATG CCG TCG TGG AC-3′ and 5′-TGT GGC AGC ACCAGG GCA GC-3′ for MMP-2,[25] 5′-CCA TTT CGA CGA TGA CGA GTT G-3′ and 5′-CTT GTC GCT GTC AAA GTT CGA G-3′ for MMP-9,[26] 5′-ATC

TGT GAC GGG AAC TTT GAC-3′ and 5′-ACC TTC AGC TTC TGG TTG TTG-3′ for MT1-MMP,[25] 5′-CTT CTG GCA TCC TGT TGT TGC T-3′ and 5′-GGC TGT TCC AGG GAG CCA CGA-3′ for TIMP-1,[26] and 5′-TGC AGC TGC TCC CCG GTG CAC-3′ and 5′-TTA TGG GTC CTC GAT GTC GAG-3′ for TIMP-2.[25] All primers were synthesized by TaKaRa Korea Biomedical (Seoul, Korea).

PCR amplification

The amplification reaction was carried out in a 20-μl PCR mixture containing 4 μl of the synthesized cDNA solution, 4 μl of 5× polymerase reaction buffer, 200 μM of dNTPs, 0.5 μM of each primer (sense and antisense) and 1 unit of *Taq* polymerase (Promega). The PCR mixture was amplified using a GeneAmp PCR System 9600 (PerkinElmer, Wellesley, MA, USA). Amplified products (10 μl) were identified by electrophoresis of the PCR products on 1% agarose gel containing ethidium bromide and ultraviolet illumination. The housekeeping gene β-actin was used as a control and for semiquantitative analysis of the MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2. A negative control, with H_2O instead of cDNA, was also used. The levels of gene transcripts were quantified as the ratio of the intensity of the target gene to the intensity of β-actin.

Immunohistochemical analysis

The paraffin-embedded sections were deparaffinized in xylene and rehydrated in a graded series of ethanol solutions. After the endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide for 30 min, the primary polyclonal rabbit anti-human MMP-14/MT1-MMP (NeoMarkers, Fremont, CA, USA) was applied to the sections at dilutions of 1 : 100, and the sections were next incubated in a moist chamber for 30 min at room temperature. After a 5-min phosphate-buffered saline (PBS) rinse, the streptavidin peroxidase label reagent was applied for 20 min at room temperature. A final PBS rinse was followed by a 5-min incubation with 3′3-diaminobenzidine tetrahydrochloride (DAB; Zymed, San Francisco, CA, USA). Finally, the slides were counterstained with Mayer's hematoxylin. A tumor was regarded as positive if any tumor cells showed immunostaining, whereas it was classified as negative if there was a complete absence of immunostaining in tumor cells.

Statistical analysis

The results were expressed as means ± the standard deviation. The association between the clinicopathological variables and the expression of MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2 was analyzed using Student's *t* test and analysis of variance. The data were considered significant if the *P* value was <0.05. Statistical analyses were performed using SPSS (v. 11.0, SPSS, Chicago, IL, USA).

Results

The patients comprised 41 men and 25 women with a mean age of 61.3 ± 13.5 years. The gross findings were 24 cases (36.4%) of early gastric cancer (EGC) and 42 cases (63.6%) of advanced gastric cancer (AGC); histologically, 9 cases were well differentiated (13.6%), 25 cases were moderately differentiated (37.9%), and 32 cases were the poorly differentiated/signet ring cell type (48.5%). There were 24 cases (36.4%) with lymphatic invasion and 42 cases (63.6%) of groups without invasion. The blood vessels were not affected in 51 cases (77.3%) and were affected in 15 cases (22.7%). Of the 66 cancer cases, 24 (36.4%) were classified as T1, 10 (15.1%) were T2, 26 (39.4%) were T3, and 6 (9.1%) were T4; 30 cases (45.5%) were N0, 22 (33.3%) were N1, 4 (6.1%) were N2, and 10 (15.2%) were N3; 57 cases (86.4%) were M0 and 9 (13.6%) of M1. If we consider the TNM stage on the whole, there were 27 (40.9%), 8 (12.1%), 17 (25.8%), and 14 cases (21.2%) of stage I, II, III, IV tumors, respectively (Table 1).

Table 1. Patients' characteristics and clinicopathological parameters

Variables	No. (%)
Gross findings	
EGC	24 (36.4)
AGC	42 (63.6)
Differentiation	
Well	9 (13.6)
Moderate	25 (37.9)
Poor/signet ring cell	32 (48.5)
Lymphatic invasion	
–/+	42 (63.6)/24 (36.4)
Venous invasion	
–/+	51 (77.3)/15 (22.7)
Depth of invasion	
T1	24 (36.4)
T2	10 (15.1)
T3	26 (39.4)
T4	6 (9.1)
Nodal status	
N0	30 (45.5)
N1	22 (33.3)
N2	4 (6.1)
N3	10 (15.1)
Distant metastasis	
M0	57 (86.4)
M1	9 (13.6)
Stage	
I	27 (40.9)
II	8 (12.1)
III	17 (25.8)
IV	14 (21.2)

EGC, early gastric cancer; AGC, advanced gastric cancer

Expression of MMPs and TIMPs could be detected in all biopsy specimens obtained from normal tissue, and their levels varied among cases. We thus considered it to be appropriate to compare the expression level in tumor samples with that in normal samples for each case. The expression of MMPs and TIMPs was evaluated using the tumor:normal (T/N) ratio for MMPs and TIMPs. The mRNA expression of the proteolytic enzymes MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2 (Fig. 1) did not differ significantly between well-differentiated and moderately/poorly differentiated histological types (Fig. 2).

Similarly, the T/N ratio of MMP-2, MMP-9, MT1-MMP, TIMP-1, and TIMP-2 mRNA expression did not differ significantly in relation to the presence of lymphatic invasion (Fig. 3), nor in relation to the presence of vascular invasion (Fig. 4).

However, the T/N ratio of MT1-MMP mRNA expression, but not those of MMP-2, MMP-9, TIMP-1, or TIMP-2, was significantly higher when there was tumor invasion to the lymph nodes ($P < 0.05$) (Fig. 5).

Moreover, the T/N ratio of MT1-MMP mRNA expression significantly increased with the TNM stage of the tumor ($P < 0.05$), but the expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 did not differ significantly among the stages of gastric cancer (Fig. 6).

Immunohistochemistry of representative samples using a monoclonal antibody directed against MT1-MMP

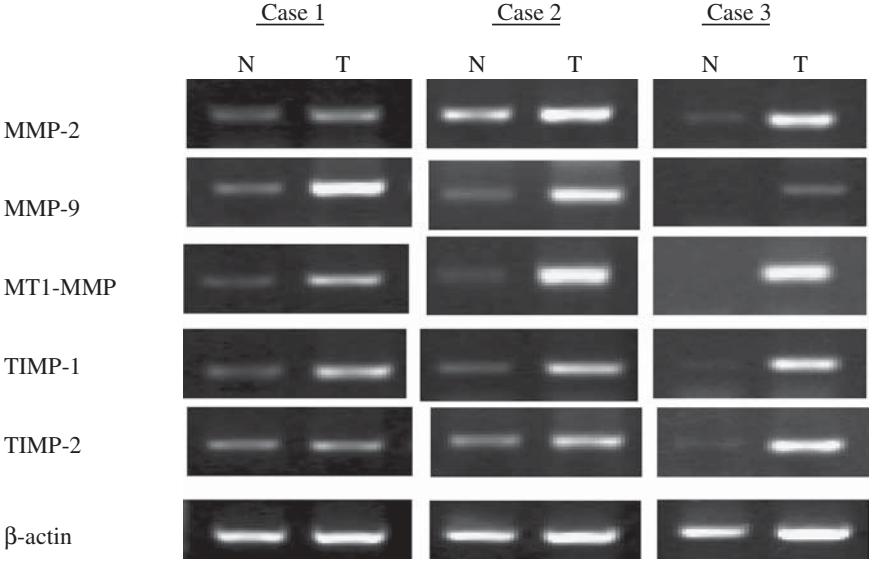

Fig. 1. Reverse transcriptase-polymerase chain reaction analysis of mRNA expression for proteolytic enzymes in normal and tumor tissues. *N*, normal tissue; *T*, tumor tissue; *MMP*, matrix metalloproteinase; *MT1-MMP*, membrane-type 1 matrix metalloproteinase; *TIMP*, tissue inhibitor of matrix metalloproteinase

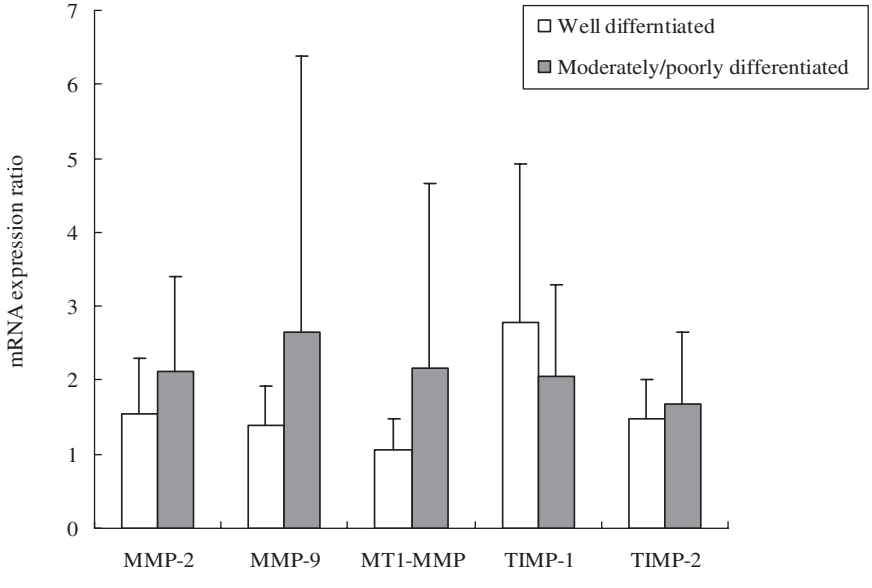

Fig. 2. Relationship between the differentiation of tumor and mRNA expression of proteolytic enzymes. *White bars*, well differentiated; *shaded bars*, moderately/poorly differentiated. $P > 0.05$

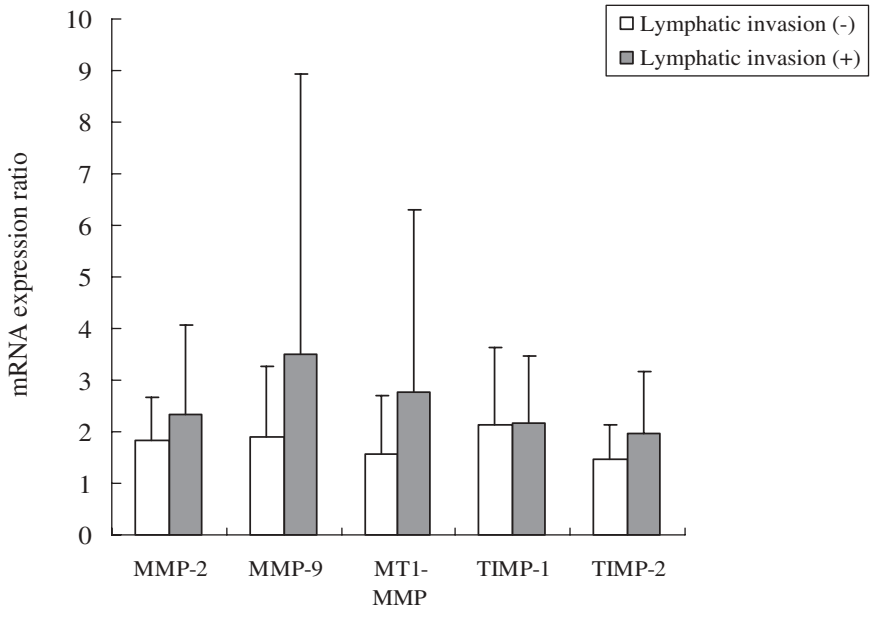

Fig. 3. Relationship between lymphatic invasion and mRNA expression of proteolytic enzymes. *White bars*, lymphatic invasion (–); *shaded bars*, lymphatic invasion (+). $P > 0.05$

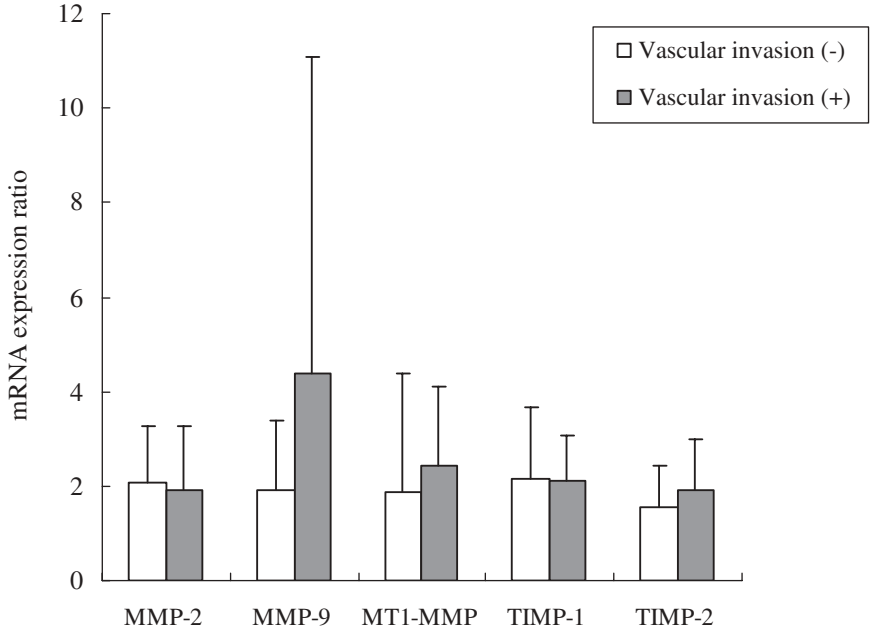

Fig. 4. Relationship between vascular invasion and mRNA expression of proteolytic enzymes. *White bars*, vascular invasion (–); *shaded bars*, vascular invasion (+). $P > 0.05$

showed a positive immunoreaction in gastric adenocarcinoma and negative staining in normal mucosa (Fig. 7).

Discussion

Invasion and metastasis are the most insidious and life-threatening aspects of cancer. A critical proteolytic event occurring early in the metastatic cascade appears to be the degradation of the basement membranes. Recent studies have suggested that the major role of MMP-2 and MMP-9 in the digestion of basement membrane type IV collagen is an important mechanism for vessel invasion and metastasis.[4–6] Although soluble MMPs have been shown to be produced as zymogen (proMMP-2) in cells, the fate of MMPs and TIMPs after their secretion from the producing cells or the spatial regulation of their molecular activation is not fully understood. However, it has been shown that the activation of MMPs is strictly controlled by plasma membrane-associated events.[9–11] Briefly, among the TIMPs, TIMP-

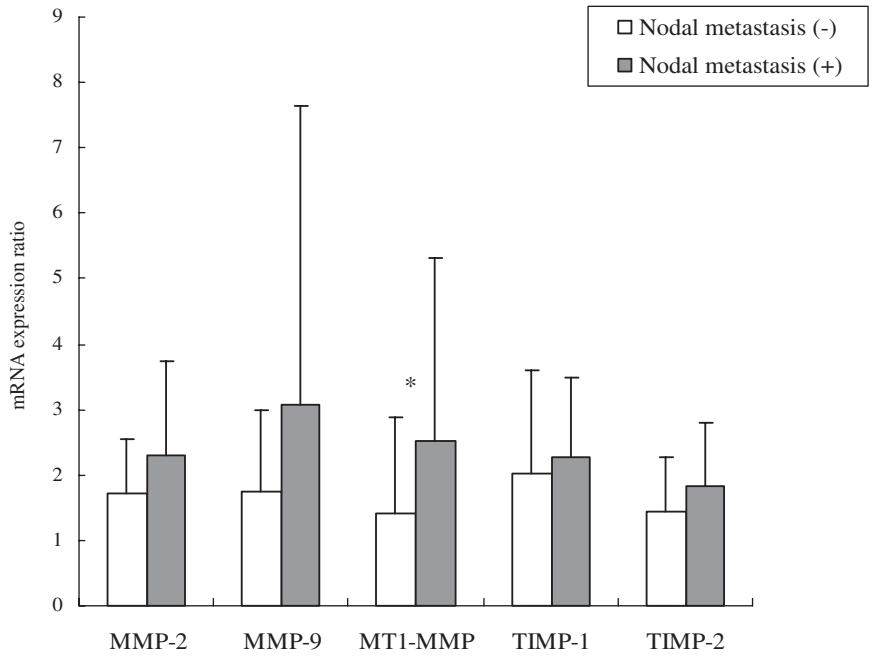

Fig. 5. Relationship between nodal metastasis and mRNA expression of proteolytic enzymes. *White bars*, nodal metastasis (–); *shaded bars*, nodal metastasis (+). *$P < 0.05$

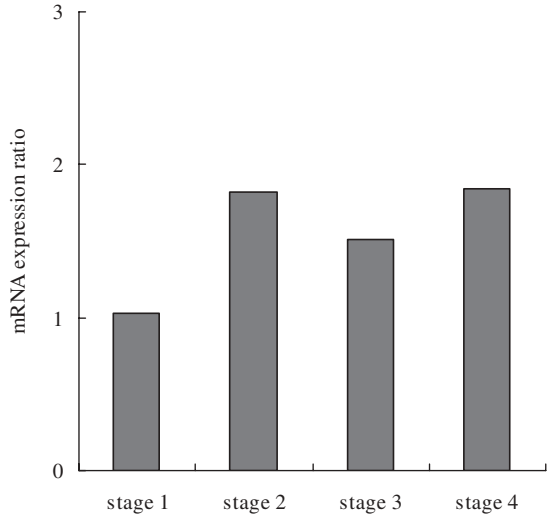

Fig. 6. Relationship between tumor stage and MT1-MMP mRNA expression of proteolytic enzymes. $P < 0.05$

2 preferentially binds first to the MT1-MMP localized on the cell surface. The TIMP-2/MT1-MMP complex subsequently functions as a receptor, allowing proMMP-2 to form a ternary complex. ProMMP-2 in the complex can then be activated by the adjacent TIMP-2-free MT1-MMP, and the active MMP-2 in turn activates MMP-9.[9,10,26–29] Koyama[30] showed that the cell surface expression of MMP-2, MMP-9, MT1-MMP, and TIMP-2 increases during invasion or metastasis of gastric carcinoma.

Because of its ability to degrade the basement membrane, MMP-2 has been postulated to be a potential marker of tumor progression and prognosis. In several studies, MMP-2 staining by immunohistochemistry correlated significantly with depth of tumor infiltration (T stage), lymph node metastasis (N stage), and distant metastasis (M stage).[4,5,31] Bando et al.[32] reported that MMP-2 immunoreactivity was associated with advanced-stage gastric cancer and that it contributed to tumor progression, invasion, and metastasis. On the other hand, Allgayer et al.[33] have demonstrated a correlation between distant metastasis (M stage) and MMP-2 status using immunohistochemistry, but not between lymph node metastasis and MMP-2 immunoreactivity in gastric cancer. The latter feature was also noticed by Kabashima et al.,[34] who also demonstrated no correlation between MMP-2 expression and lymph node metastasis. Some in vitro and in vivo experiments showed that MMP levels were related to the invading and metastatic potential of colorectal cancer. Sier et al.[5] demonstrated that higher tissue levels of total MMPs and the pro-forms of MMP-2 and MMP-9, as well as the active form of MMP-2 indicated a poor prognosis in patients with gastric carcinoma.

It has been reported that the matrix-degrading activity of MMP-9 is nearly 25 times that of MMP-2, and that MMP-9 is more important for the metastatic potential of carcinoma than MMP-2.[35,36] Moreover, MMP-9 expression by immunohistochemistry has been found to be significantly correlated with lymphatic permeation and lymph node metastasis in intramucosal gastric cancers.[37] Another study, however, showed no significant

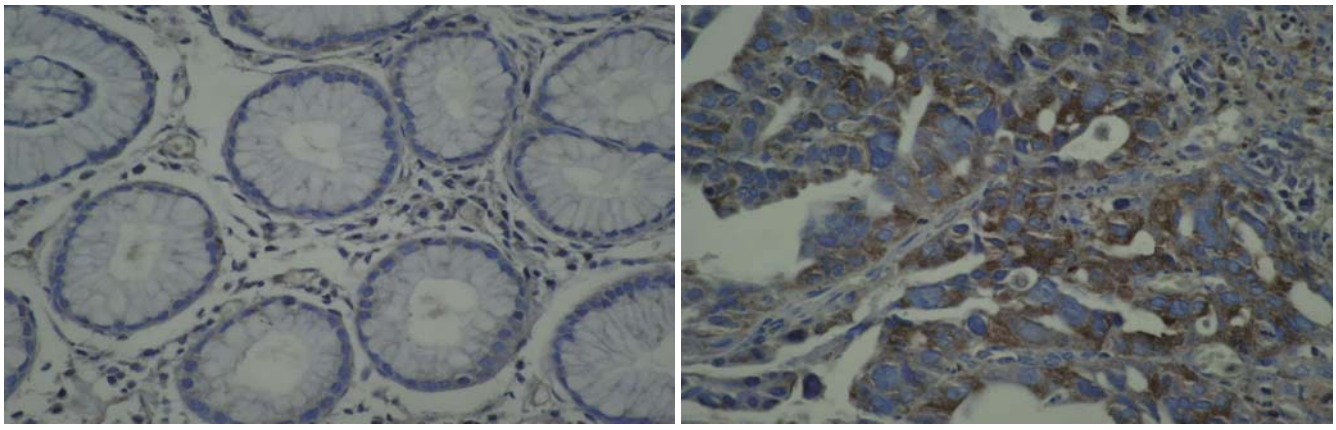

Fig. 7A,B. Immunostaining for MT1-MMP. Strong immunoreactivity was present in tumor cells (**A**), and was not present in normal gastric epithelium (**B**) (×100)

correlation between MMP-9 mRNA expression and the prognosis of patients with hepatocellular carcinoma. In this study, the levels of MMP-2 and MMP-9 mRNA expression were not correlated with the clinicopathologic parameters of gastric cancer.

MT1-MMP, the first member of a more recently established group of MMPs containing a membrane-spanning sequence, has been shown to have an important role in MMP-2 activation in cell membranes, and its overexpression seems to have a significant effect on tumor growth. Expression of MT1-MMP mRNA tends to be associated with a lower degree of differentiation in hepatocellular carcinoma and has a strong statistical association with a poor outcome of patients.[37] A similar tendency was also observed in relation to pancreatic adenocarcinomas, but the association did not reach statistical significance.[37] Moreover, Caenazzo et al.[38] showed that augmented MT1-MMP mRNA expression was associated with a poor prognosis in gastric cancer. In this study, increased expression of MT1-MMP was significantly correlated with nodal metastasis and the tumor stage of gastric cancer. To date, at least four mechanisms have been identified by which MT1-MMP can enhance tumor progression: it can activate MMP-2 on tumor cell membranes;[7,8] it is a very effective degradative enzyme by itself, having substrates such as fibronectin, tenascin, nidogen, aggregan, and perlecan;[39] it is a very potent regulator of neovascularization,[40] a phenomenon that is critical for malignant growth; and it has been shown to process directly laminin-5 r2 chains, causing a strong migration effect of tumor cells over laminin-5 surfaces.[41]

In general, MMPs facilitate tumor invasion. On the other hand, TIMPs play an important role in inhibiting MMPs. TIMPs have been reported to be negative regulators of MMPs in human and mouse tumor models, in vivo and in vitro.[42] However, in another study using clinical samples, the expression of TIMP mRNA was higher in carcinoma tissues. In studies of various types of carcinoma, such as stomach, colorectal, head and neck, and pancreatic carcinomas, both MMPs and TIMPs were found to correlate with increased metastatic and invasive potential of tumor cells.[23,43] TIMP-1 RNA levels were higher in primary colorectal carcinomas with distant metastasis than in those without distant metastasis, and the expression of TIMPs increased with the advance of the neoplastic process.[23,25] A discrepancy still exists, however, between the function of TIMP-1 as an inhibitor of tumor cell invasion in vitro and the higher expression of TIMP-1 in human carcinoma cells reported in previous studies. There are several possible explanations for this discrepancy. First, higher expression of MMPs was observed in tissues with invasive carcinoma cells, which induced macrophages with cytokines and thus elevated the expression of TIMPs.[44,45] Second, TIMP-1 has two distinct activities, that is, a metalloproteinase inhibitory activity and a growth factor activity.[21,44,45]

Although both normal and neoplastic cells produce MMPs and other proteinases, only malignant cells are invasive.[46] Therefore, it is more likely that the control of MMP activity by specific inhibitors is one cause of the different functions of these enzymes in normal and neoplastic tissues. Ko et al.[47] has demonstrated that TIMP-2 is inversely correlated with nodal metastasis and that TIMP-2 expression is stronger in EGC than in AGC, indicating that TIMP-2 may play an important role in protection against MMPs. Another study, however, showed that the expression of TIMP-2 was not associated with variable clinicopathological parameters, and that the status of TIMP-2 expression was variable in many types of cancer tissues. In our study, the levels

of TIMP-1 and TIMP-2 mRNA expression were not correlated with the clinicopathologic parameters of gastric cancer.

In summary, our data support previous suggestions of the importance of MT1-MMP in malignant tumor growth, and increased MT1-MMP mRNA expression by tumor cells in gastric cancer reflect a role in predicting the aggressive behavior of gastric cancer. As an RT-PCR assay can be performed on biopsy specimens obtained before surgery, evaluation of MT1-MMP expression in biopsy specimens by RT-PCR may provide useful preoperative information on tumor aggressiveness.

Acknowledgment. The present study was supported in part by year 2003 research funding from Ewha Womans University for new faculty members.

References

1. Liotta LA, Kohn E. Cancer invasion and metastases. JAMA 1990;263:1123–6.
2. Schwartz GK. Invasion and metastases in gastric cancer: in vitro and in vivo models with clinical correlations. Semin Oncol 1996;23:316–24.
3. Kohn EC, Liotta LA. Molecular insights into cancer invasion: strategies for prevention and intervention. Cancer Res 1995; 55:1856–62.
4. Monig SP, Baldus SE, Hennecken JK, Spiecker DB, Grass G, Schneider PM, et al. Expression of MMP-2 is associated with progression and lymph node metastasis of gastric carcinoma. Histopathology 2001;39:597–602.
5. Sier CF, Kubben FJ, Ganesh S, Heerding MM, Griffioen G, Hanemaaijer R, et al. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer 1996;74:413–7.
6. Kabashima A, Maehara Y, Kakeji Y, Baba H, Koga T, Sugimachi K. Clinicopathological features and overexpression of matrix metalloproteinases in intramucosal gastric carcinoma with lymph node metastasis. Clin Cancer Res 2000;6:3581–4.
7. Cao J, Sato H, Takino T, Seiki M. The C-terminal region of membrane type matrix metalloproteinase is a functional transmembrane domain required for pro-gelatinase A activation. J Biol Chem 1995;270:801–5.
8. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanisms of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloproteinase. J Biol Chem 1995;270:5331–8.
9. Curran S, Murray GI. Molecular aspects of their roles in tumor invasion and metastasis. Eur J Cancer 2000;36:1621–30.
10. Seiki M, Yana I. Role of pericellular proteolysis by membrane type-1 matrix metalloproteinase in cancer invasion and angiogenesis. Cancer Sci 2003;94:569–74.
11. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, et al. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994;370:61–5.
12. Chen WT. Membrane proteases: roles in tissue remodeling and tumor invasion. Curr Opin Cell Biol 1992;4:802–9.
13. Stetler-Stevenson WG, Krutzsch HC, Liotta LA. Tissue inhibitors of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. J Biol Chem 1989;264:17374–8.
14. Passlick B, Sienel W, Seen-Hibler R, Wockel W, Thetter O, Mutschler W, et al. Overexpression of matrix metalloproteinase 2 predicts unfavorable outcome in early-stage non-small cell lung cancer. Clin Cancer Res 2000;6:3944–8.
15. Pyke C, Ralfkiaer E, Tryggvason K, Dano K. Messenger RNA for two type IV collagenase is located in stromal cells in human colon cancer. Am J Pathol 1993;142:359–65.
16. Tryggvason K, Hoyhtya M, Pyke C. Type IV collagenases in invasive tumors. Breast Cancer Res Treat 1993;24:209–18.
17. Sato H, Kida Y, Mai M, Endo Y, Sasaki T, Tanaka J, et al. Expression of genes encoding type IV collagen-degrading metalloproteinases and tissue inhibitors of metalloproteinases in various human tumor cells. Oncogene 1992;7:77–83.
18. Azuma M, Tamatani T, Fukui K, Yoshida H, Kamogashira T, Ogino K, et al. Role of plasminogen activators, metalloproteinases and the tissue inhibitor of metalloproteinase-1 in the metastatic process of human salivary-gland adenocarcinoma cells. Int J Cancer 1993;54:669–76.
19. Belloc C, Lu H, Soria C, Fridman R, Legrand Y, Menashi S. The effect of platelets on invasiveness and protease production of human mammary tumor cells. Int J Cancer 1995;60:413–7.
20. Docherty AJ, Lyons A, Smith BJ, Wright EM, Stephens PE, Harris TJ, et al. Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. Nature 1985;318:66–9.
21. Avalos BR, Kaufman SE, Tomonaga M, Williams RE, Golde DW, Gasson JC. K562 cells produce and respond to human erythroid-potentiating activity. Blood 1988;71:1720–5.
22. Agrez MV, Meldrum CJ, Sim AT, Aebersold RH, Clark IM, Cawston TE, et al. A fibroblast elongation factor purified from colon carcinoma cells shares sequence identify with TIMP-1. Biochem Biophys Res Commun 1995;206:590–600.
23. Zeng ZS, Cohen AM, Zhang ZF, Stetler-Stevenson W, Guillem JG. Elevated tissue inhibitor of metalloproteinase 1 RNA in colorectal cancer stroma correlates with lymph node and distant metastases. Clin Cancer Res 1995;1:899–906.
24. Urbanski SJ, Edwards DR, Hershfield N, Huchcroft SA, Shaffer E, Sutherland L, et al. Expression pattern of metalloproteinases and their inhibitors changes with the progression of human sporadic colorectal neoplasia. Diagn Mol Pathol 1993; 2:81–9.
25. Chung HW, Lee JY, Moon HS, Hur SE, Park MH, Wen Y, et al. Matrix metalloproteinase-2, membranous type 1 matrix metalloproteinase, and tissue inhibitor of metalloproteinase-2 expression in ectopic and eutopic endometrium. Fertil Steril 2002;78: 787–95.
26. Chou CS, Tai CJ, MacCalman CD, Leung PC. Dose-dependent effects of gonadotropin releasing hormone on matrix metalloproteinase (MMP)-2, and MMP-9 and tissue specific inhibitor of metalloproteinases-1 messenger ribonucleic acid levels in human decidual stromal cells in vitro. J Clin Endocrinol Metab 2003; 88:680–8.
27. Shofuda K, Moriyama K, Nishihashi A, Higashi S, Mizushima H, Yasumitsu H, et al. Role of tissue inhibitor of metalloproteinases-2 (TIMP-2) in regulation of pro-gelatinase A activation catalyzed by membrane-type matrix metalloproteinase-1 (MT1-MMP) in human cancer cells. J Biochem 1998;124:462–70.
28. Wang Z, Juttermann R, Soloway PD. TIMP-2 is required for efficient activation of proMMP-2 in vivo. J Biol Chem 2000; 275:26411–5.
29. Itoh Y, Takamura A, Ito N, Maru Y, Sato H, Suenaga N, et al. Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. EMBO J 2001;20:4782–93.
30. Koyama S. Enhanced cell surface expression of matrix metalloproteinases and their inhibitors, and tumor-induced host-response in progression of human gastric carcinoma. Dig Dis Sci 2004; 49:1621–30.
31. Nomura H, Sato H, Seiki M, Mai M, Okada Y. Expression of membrane type matrix metalloproteinase in human gastric carcinomas. Cancer Res 1995;55:3263–6.

32. Bando E, Yonemura Y, Endou Y, Sasaki T, Taniguchi K, Fujita H, et al. Immunohistochemical study of MT-MMP tissue status in gastric carcinoma and correlation with survival analyzed by univariate and multivariate analysis. Oncol Rep 1998;5:1483–8.
33. Allgayer H, Babic R, Grutzner KU, Beyer BC, Tarabichi A, Schildberg FW, et al. Tumor-associated proteases and inhibitors in gastric cancer: analysis of prognostic impact and individual risk protease patterns. Clin Exp Metastasis 1998;16:62–73.
34. Kabashima A, Maehara Y, Kakeji Y, Baba H, Koga T, Sugimachi K. Clinicopathological features and overexpression of matrix metalloproteinases in intramucosal gastric carcinoma with lymph node metastasis. Clin Cancer Res 2000;6:3581–4.
35. Yasumitsu H, Miyazaki K, Umenishi F, Koshikawa N, Umeda M. Comparison of extracellular matrix-degrading activities between 64-kDa and 90-kDa gelatinases purified in inhibitor-free forms from human schwannoma cells. J Biochem 1992;111:74–80.
36. Yamagata S, Tanaka R, Ito Y, Shimizu S. Gelatinases of murine metastatic tumor cells. Biochem Biophys Res Commun 1989;158: 228–34.
37. Maatta M, Soini Y, Liakka A, Autio-Harmainen H. Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumor progression and clinical prognosis. Clin Cancer Res 2000;6:2726–34.
38. Caenazzo C, Onisto M, Sartor L, Scalerta R, Giraldo A, Nitti D, et al. Augmented membrane type 1 matrix metalloproteinase (MT1-MMP):MMP-2 messenger RNA ratio in gastric carcinomas with poor prognosis. Clin Cancer Res 1998;4:2179–86.
39. d'Ortho MP, Will H, Atkinson S, Butler G, Messent A, Gavrilovic J, et al. Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. Eur J Biochem 1997;250:751–7.
40. Hiraoka N, Allen E, Apel IJ, Gyetko MR, Weiss SJ. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell 1998;95:365–77.
41. Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J Cell Biol 2000;148:615–24.
42. Khokha R, Waterhouse P, Yagel S, Lala PK, Overall CM, Norton G, et al. Antisense RNA-induced reduction in murine TIMP levels confers oncogenicity on Swiss 3T3 cells. Science 1989;243:947–50.
43. Nomura H, Fujimoto N, Seiki M, Mai M, Okada Y. Enhanced production of matrix metalloproteinases and activation of matrix metalloproteinase 2 (gelatinase A) in human gastric carcinomas. Int J Cancer 1996;69:9–16.
44. Partridge CA, Jeffrey JJ, Malik AB. A 96-kDa gelatinase induced by TNF-alpha contributes to increased microvascular endothelial permeability. Am J Physiol 1993;265:L438–47.
45. Richards CD, Agro A. Interaction between oncostatin M, interleukin 1 and prostaglandin E2 in induction of IL-6 expression in human fibroblasts. Cytokine 1994;6:40–7.
46. Alexander CM, Werb Z. Targeted disruption of the tissue inhibitor of metalloproteinases gene increases the invasive behavior of primitive mesenchymal cells derived from embryonic stem cells in vitro. J Cell Biol 1992;118:727–39.
47. Ko BK, Cho HR, Choi DW, Nam CW, Park CJ, Kim GY, et al. Reduced expression of tissue inhibitor of metalloproteinase in nodal metastasis of stomach cancer. J Korean Med Sci 1998; 13:286–90.