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Reduced Expression of Tissue Inhibitor of Metalloproteinase in Nodal Metastasis of Stomach Cancer

The matrix metalloproteinases (MMPs) have been associated with tumor cell invasion and metastasis of human cancers by mediating the degradation of extracellular matrix components. Therefore, these enzymes and their inhibitor (TIMP-2) constitute promising targets in the development of anticancer therapies. In order to investigate the correlation between expressions of TIMP-2, MMPs and clinical outcome, immunohistochemical staining of MMP-2, MMP-9, and TIMP-2 were performed on paraffin-embedded tissue sections of 15 early gastric cancers (EGC) and 15 advanced gastric carcinomas (AGC) without nodal metastasis and 15 AGC with nodal metastasis (AGCn+). MMP-2 and MMP-9 were expressed in neoplastic cell plasma membrane in 83.3% and 88% of cases of AGC, respectively with inter-tumoral variability of staining intensity. MMP-2 and MMP-9 staining were not correlated with presence of nodal metastasis or degree of invasion depth at the time of diagnosis ($p>0.05$). The immunoreactivity of TIMP-2 was detected in the peri-tumoral stroma. Residual benign stomach tissue showed no or weak immunoreactivity for TIMP-2 staining. Among AGC, neoplasms with diffuse and strong TIMP-2 staining have less frequent metastasis (28.6%) than cases with focal and weak (68.8%) ($p<0.05$). Early gastric cancer revealed diffuse and strong TIMP-2 expressions. We conclude that clinical outcome such as depth of invasion or metastasis is more closely related to the expression of TIMP-2 than the corresponding MMPs.

Key Words : *Immunohistochemisty; Metalloproteinases; Tissue inhibitor of metalloproteinase-2; Stomach neoplasms*

Byung Kyun Ko, Hong Rae Cho, Dae Wha Choi, Chang Woo Nam, Chan Jin Park, Gyu Yeol Kim, Sung Sook Kim*, Yeong Ju Woo*, Jooryung Huh†, Min Young Kim‡

Department of General Surgery and Pathology*, University of Ulsan Hospital
Department of Pathology, University of Ulsan, Asan Medical Center†
Department of Cancer Biology‡, Hanhyo Research Center

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Address for correspondence
Sung Sook Kim, M.D.
Department of Pathology, University of Ulsan Hospital, 290-3, Jeonha-dong, Dong-gu, Ulsan 682-060, Korea
Tel : (0522) 32-1301, Fax : (0522) 52-3024
E-mail : sskseh@chollian.net

INTRODUCTION

Metastasis of an initially localized tumor to vital organs is the dominant cause of related deaths. The mechanism controlling the metastatic progression of a localized tumor is a very complex process, involving many biochemical and cellular events. One such biochemical event may be the secretion of proteolytic enzymes, capable of degrading the extracellular matrix (ECM) by invading tumor cells (1-3). Penetration of the basement membrane surrounding the tumor cells that can secrete and locally initiate a proteolytic cascade is the first step in tumor invasion (4-6).

Degradation of basement membrane, which is mainly composed of type IV collagen, laminin, and fibronectin, is mediated by a set of secreted MMPs, also termed matrixins (7). These naturally occurring, Zn^{++} dependent endopeptidases are involved in the stromal turnover of connective tissue matrix, as well as in certain disease processes (8, 9). Increased levels of these proteinases have been implicated with the invasive potential of tumors (10, 11). Proteolysis by MMPs is regulated by a family of naturally occurring endogenous proteinase inhibitors known as TIMPs. There are at least two molecular species of TIMPs: TIMP-1 (MW, 28000) and TIMP-2 (MW, 21000), as well as a large TIMP-like inhibitor (MW, 76000).

Although both normal and neoplastic cells produce MMPs and other proteinases, only malignant cells are invasive (12). Therefore, it is more likely that control of MMP activity by specific inhibitors (TIMPs) is a cause for the differential functioning of these enzymes in normal and neoplastic tissues (13). Several studies about the MMPs expression in stomach cancer (14-16), have been reported, but few papers about the TIMP expression (17) has been reported yet.

We tested the different functionings of MMPs and TIMP-2 by measuring their expressions in stomach cancer tissue with immunohistochemistry.

MATERIALS AND METHODS

Sample collection

Thirty advanced gastric carcinomas and adjacent non-malignant tissues were available for this study. Samples were processed routinely as done in Department of Pathology for diagnosis of tumor type and staging. Among thirty advanced cancer, fifteen cases revealed nodal metastasis (AGCn+) and fifteen cases didn't (AGCn−). Another fifteen cases of early gastric cancer tissue (EGC) were available. Among fifteen early gastric cancer, five cases were mucosal type and ten cases were submucosal type. All fifteen early gastric cancers didn't reveal nodal metastasis.

Immunohistochemistry

Immunohistochemical staining was done using the LSAB kit from Dako Company as described previously (5). Primary monoclonal antibodies for MMP-2, MMP-9 and TIMP-2 were obtained commercially (MMP-2, MMP-9 from Calbiochem) and as a generous gift (TIMP-2 from Hanhyo RC). The slides were developed for 10 minutes with the enzyme substrate, 3 amino-9-ethyl carbazole (AEC). The slides then were counterstained with hematoxylin, dehydrated, and mounted. The grade of staining was evaluated by two observers when the slides were scored. The staining was graded as 0, when staining not greater than negative control, 1+ for light or focal staining, 2+ for diffuse or heavy staining. Differences between observers were resolved by consensus.

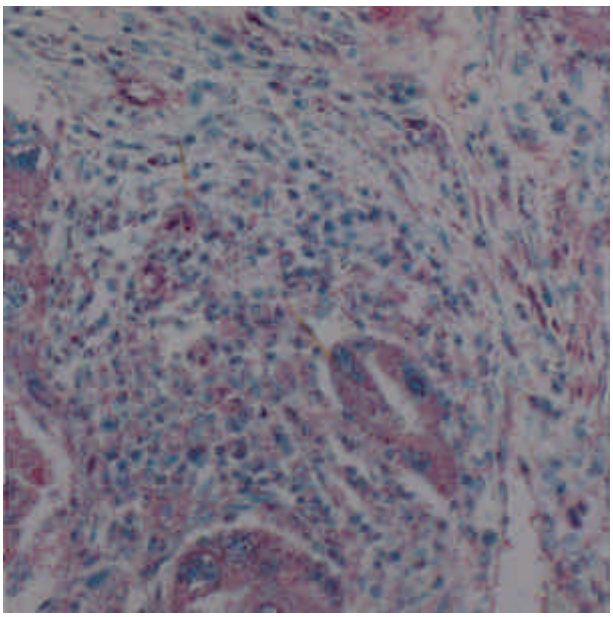

Fig. 1. Advanced stomach cancer revealed MMP-2 expression (Immunostain, ×200).

Statistical analysis

All relationships between variables were assessed using the chi-square test with Yates' correlation or the Fischer's exact test.

RESULTS

MMP-2, MMP-9 expression

Normal gastric tissue showed no reactivity for MMP-2, and MMP-9. But the MMP-2 and MMP-9 were expressed in cytoplasm and cytoplasmic membranes of the tumor cells of 83.3% and 88%, respectively (Fig. 1). Some stromal fibroblasts and endothelial cells were focally positive. There is no grade 2+ in all cases. The differences of immunoreactivity between MMP-2 and MMP-9 were not remarkable, except for less reaction in MMP-9. The intensity was relatively weak but the positive cells were diffuse. Most advanced carcinomas and some EGC showed immunoreactivity (Table 1). Advanced carcinomas revealed stronger reaction than EGC. Among EGC, mucosal type did not show immunoreactivity for metalloproteinases. According to nodal metastasis, 14 out of 15 with nodal metastasis, and 11/15 without metastasis were positive for MMP-2 (Table 1), and 12/15 with nodal metastasis and 10/15 without nodal metastasis were positive for MMP-9. There is no statistical correlation between expression of MMPs and nodal status ($p>0.05$).

TIMP-2 expression

Normal gastric tissue showed no or weak immunoreactivity for TIMP-2 (Fig. 2). TIMP-2 was expressed

Table 1. Expression of MMP-2, MMP-9 and TIMP-2 in stomach cancer tissue

	TIMP-2			MMP-2		MMP-9	
	−	+	++	−	+	−	+
EGC (15)	0	6	9	2	13	4	11
AGC without nodal metastasis (15)	0	5	10	4	11	5	10
AGC with nodal metastasis (15)	0	11	4	1	14	3	12

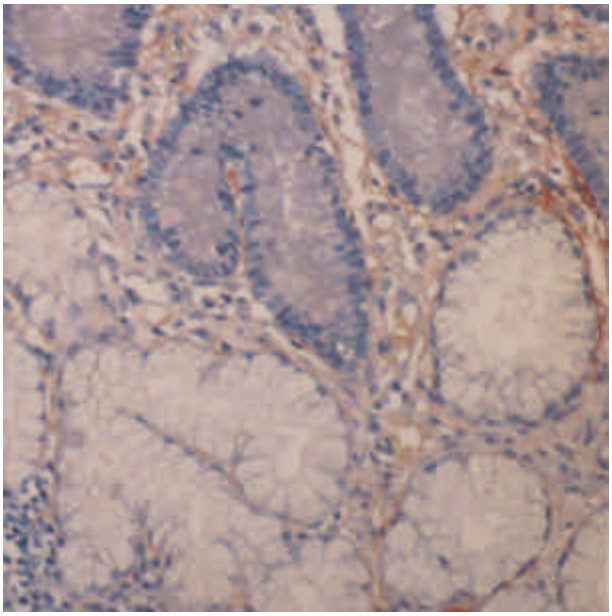

Fig. 2. Normal gastric mucosa showed no or weak immunoreactivity for TIMP-2 (Immunostain, ×200).

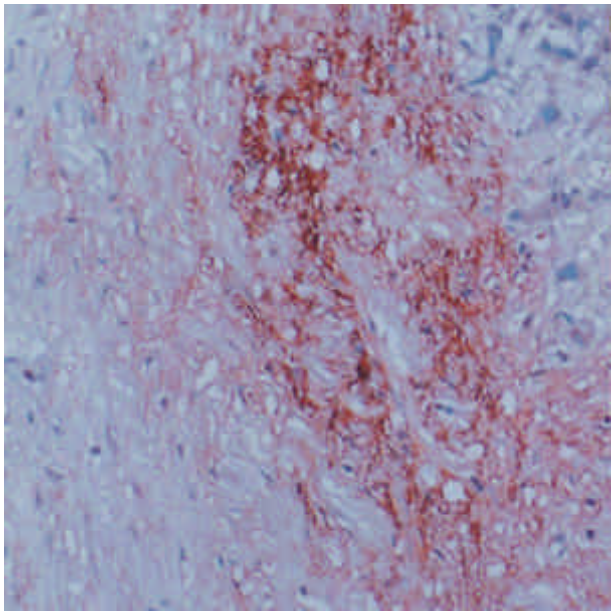

Fig. 3. Early gastric cancer showed strong TIMP-2 expression in stroma (Immunostain, ×200).

mainly by stromal fibroblasts and vascular endothelial cells. Tumor cells did not express TIMP-2. The immunoreactivity for TIMP-2 was positive in all cases of AGC and EGC. However, the staining patterns were different by degree of invasion and nodal metastasis. In case of AGC, the immunoreactivity TIMP-2 was relatively weak to moderate and focal, especially AGC with nodal metastasis cases, whereas strong and diffuse in EGC (Fig. 3). Neoplasms with strong and diffuse TIMP-2 staining (Fig. 4A) have metastasis significantly less frequently (28.6% metastasis) than cases with focal staining (68.8% metastasis) ($p<0.05$). Also some AGC with nodal metastasis (Fig. 4B) revealed weak and focal immunoreactivity for TIMP-2. EGC showed strong reaction, but mucosal type showed less reactivity. Dysplastic lesion adjacent cancer showed no expression (Table 2).

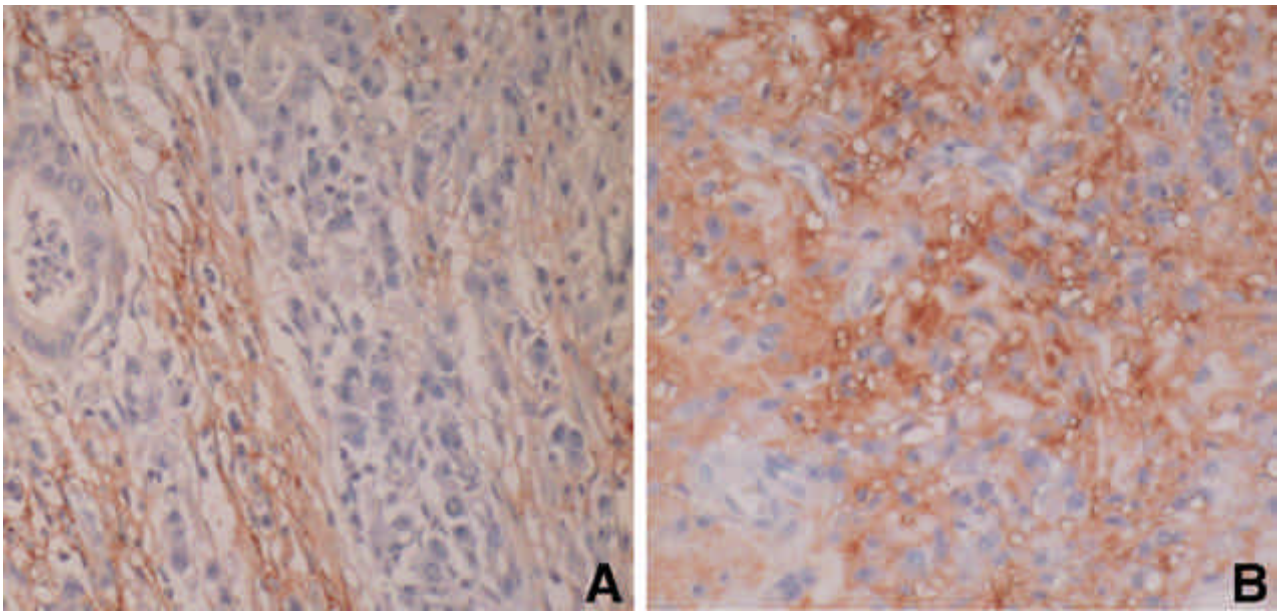

Fig. 4. Advanced gastric cancer with nodal metastasis revealed weaker TIMP-2 (A), whereas cancer without nodal metastasis showed stronger reactivity for TIMP-2 (B) (Immunostain, ×200).

Table 2. Expression of MMP-2, MMP-9 and TIMP-2 in types of EGC

Type	TIMP-2			MMP-2		MMP-9	
	−	+	++	−	+	−	+
Dysplastic lesion (5)*	5	0	0	5	0	5	0
Mucosal type (5)	0	5	0	2	3	4	1
Submucosa (10)	0	1	9	0	10	0	10

* Dysplstic lesion is observed in adjacent obvious cancer tissue.

DISCUSSION

Tumor invasion and metastasis are the major causes of morbidity and death for cancer patients. The exact mechanisms responsible for the formation of metastases are not fully understood. The critical event of tumor invasion that signals the initiation of the metastatic cascade is thought to be interaction of the tumor cell with the basement membrane. There are at least three critical steps involved in this process (2, 3). The first step is attachment to the ECM, which may be mediated by pre-existing or newly formed contact sites. The second one is creation of a proteolytic defect in the ECM. The final phase is migration through the proteolytic and modified matrix. Many biological processes involving ECM turnover have been linked with expression of matrix metalloproteinases. The metalloproteinases multigene family comprises three subclasses of zinc-binding enzymes, namely, collagenases, stromelysins and gelatinases that together have the ability to degrade all protein components of the ECM (5-7). Metalloproteinases play a major role in normal tissue remodeling as well as in invasion by malignant cells (18). The role of MMPs in ECM degradation can be regulated at many stages, including gene activation and transcription, messenger ribonucleic acid (mRNA) stability, translation, and secretion of latent proenzymes, binding of proenzymes to cell membranes and/or ECM components, proenzyme activation, inactivation by endogenous inhibitors and degradation or removal of active or inactive enzyme species. Immunohistochemical staining study in gastric neoplasia has shown that gastric carcinoma contains enhanced amount of matrix metalloproteinases (16, 22). In the present study, we show that in a majority of gastric carcinomas the MMP-2 and MMP-9 levels are significantly higher than in the corresponding gastric mucosa, irrespective of the activity state of the enzymes. These findings strongly suggest that the basement membrane underlying stomach epithelium probably undergoes rapid break-down due to the matrix-degrading enzymes secreted by the neoplastic cells. The recent immunohistochemical staining data in which MMP-2 was found to be higher in advanced vs early gastric tumor (16) is not in agreement with our results that there was no statistical correlation between expression of MMPs and nodal metastasis.

Tissue inhibitors of metalloproteinases, TIMP-1 and TIMP-2, are specific inhibitors of MMPs (19). Proteolysis by MMPs is regulated by a family of these naturally occurring endogenous proteinase inhibitors. There are at least two molecular species of TIMPs: TIMP-1 (MW 28,000) and TIMP-2 (MW 21,000), as well as a large TIMP-like inhibitor (MW 76,000). Many cell lines secrete MMP-2 and MMP-9 as proenzyme-inhibitor complexes with TIMPs. Although both normal and neoplastic cells produce MMPs and other proteinases, only malignant cells are invasive. Therefore, it is more likely that control of MMP activity by specific inhibitors is one of causes for the different function of these enzymes in normal and neoplastic tissues. For example, uncontrolled secretion or constitutive activation of secreted MMPs with a concomitant decrease in TIMPs production might be responsible for the invasive property of some stomach tumor cells (20). These findings are similar to out results. Stromal cells of stomach cancer tissue secrete TIMP-2, especially in early gastric cancer. Compared with the findings showing some basal expression in the control tissue, TIMP-2 appears to be expressed more specially in the gastric carcinoma tissue. Our present study has proven the importance of the role of TIMP-2 in demonstrating inverse correlation of TIMP-2 expression in nodal metastasis. Furthermore, the fact that TIMP-2 expression in EGC is more strong than AGC, indicates that TIMP-2 might play an important role in protection against MMPs. However, among EGC, the noninvasive or mucosal types did not show TIMP-2 expression. These findings could show that TIMP-2 secretion is the response to MMPs. Enhanced TIMP-2 expression, therefore, may denote a stromal response to tumor invasion, indicative of protective behavior in special subset of stomach carcinomas unlike breast carcinoma (20). These findings could support the Hong et al's report that TIMP-2, produced by stromal cells, may play an important role in inhibiting the proteolytic activity of matrix metalloproteinases that originated from cancer cells, in gastric carcinoma, especially in nodal metastasis (21).

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