Prognostic Value of Protease-activated Receptor-1 (PAR-1) and Matrix Metalloproteinase-1 (MMP-1) in Gastric Cancer

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Abstract. *Background: Protease-activated receptors (PARs) are proposed to be involved in the invasive and metastatic processes of various types of cancer. Matrix metalloproteinase-1 (MMP-1) plays a role in cancer invasion and tissue remodelling. It has been reported that MMP-1 can alter the behavior of cancer cells through PAR-1 to promote cell migration and invasion. We considered whether the expression of PAR-1 and MMP-1 has relevance to progression in gastric cancer. Materials and Methods: An immunohistochemical study was carried out on 129 samples of gastric cancer using anti-PAR-1 and anti-MMP-1 mouse monoclonal antibodies. Associations between immunostaining and clinicopathological factors were analyzed statistically. Results: There were 58 carcinomas positive for PAR-1 expression. The expression of PAR-1 was associated with the depth of wall invasion and peritoneal dissemination. There were 42 carcinomas positive for both PAR-1 and MMP-1 expression which was associated with the histological stage, depth of wall invasion, lymph node metastasis and peritoneal dissemination. These patients had a significantly poorer prognosis than those with expression-negative tumors. Multivariate analysis indicated that PAR-1 expression and combined PAR-1 and MMP-1 expression were independent prognostic factors. Conclusion: The results led us to believe that the expression of PAR-1 and MMP-1 is associated with the progression of gastric cancer and an independent prognostic predictor.*

Cells sense variations in their environment through interactions between receptors and their cognate ligands. Typically, extracellular ligands and cell surface receptors are different molecular entities. Protease-activated receptors (PARs) do not simply form a ligand–receptor complex for activation. The activation of PARs is a two-step process. First, the tethered ligand is unmasked by proteolytic cleavage, then an intramolecular rearrangement allows the ligand and the receptor moieties to interact (1). Four different PARs have been identified: PAR-1, -2, -3 and -4. PAR-1 has been shown to respond to a highly select group of serine proteases that include thrombin, plasmin, coagulation factor Xa and activated protein C (2-5). These ligands recognize and cleave the *N*-terminal exodomain of PAR-1 between Arg^{41} and Ser^{42}. Proteolytic cleavage exposes a new *N*-terminus that binds to the body of the receptor to induce transmembrane signaling to internally located G-proteins (6). The activated G-proteins in turn trigger a cascade of downstream events, leading to engagement of integrins, cell adhesion, migration and mitogenesis.

Investigation of the role of PAR-1 in tumorigenesis and metastasis was initiated recently. PAR-1 expression has been detected in human colon adenocarcinoma, a pancreatic carcinoma cell line, and a laryngeal carcinoma cell line (7-9). In breast cancer, PAR-1 expression levels were directly correlated with the degree of invasiveness in both primary breast tissue specimens and established cancer cell lines. High levels of PAR-1 mRNA were found in infiltrating ductal carcinoma while they were absent from normal and pre-malignant hyperplasia (10, 11). Matrix metalloproteinase-1 (MMP-1) can alter the behavior of breast cancer cells through PAR-1 to promote cell migration and invasion (12). PAR-1 was subsequently found to mediate the migration of some tumor cells to ward factors secreted by host fibroblasts. These studies culminated in the observation of a direct correlation between PAR-1 expression and the degree of invasiveness of breast tumor cells (10).

Matrix metalloproteinases (MMPs) are well known proteinases that are considered to play significant roles in tumor invasion and metastasis. In many types of solid cancer, MMPs have been shown to be overexpressed either by tumor cells or more commonly by stromal fibroblasts (13, 14). Furthermore, in certain types of cancer including

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Key Words: Gastric cancer, protease activated receptor-1 (PAR-1), matrix metalloproteinase-1 (MMP-1), immunohistochemistry, prognostic factor.

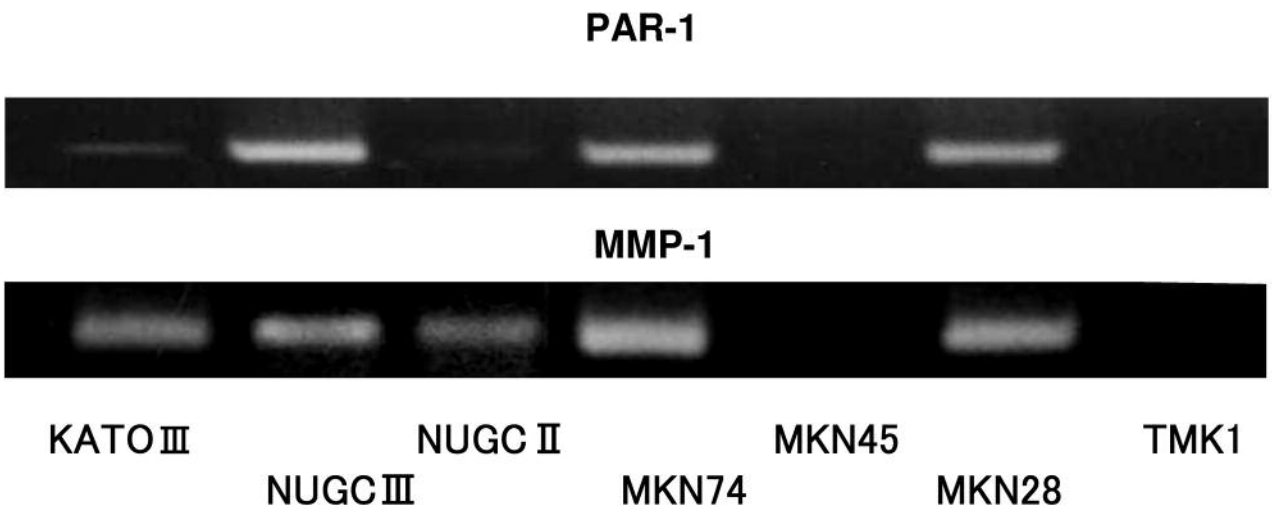

Figure 1. *The expression of PAR-1 and MMP-1 mRNA in 7 gastric cancer cell lines as evaluated by RT-PCR. PAR-1 mRNA expression was confirmed in MKN28, MKN74 and NUGCIII cell lines. MMP-1 mRNA expression was confirmed in MKN28, MKN74, NUGCII, NUGCIII, and KATOIII cell lines.*

gastric cancer, the extent of MMP expression has been shown to correlate with tumor grade and stage (15, 16). In gastric cancer, MMP-1 plays an important role in the progression of peritoneal dissemination (17).

Therefore, in this study, we investigated the clinical significance of the immunohistochemical detection of PAR-1 and MMP-1 to clarify whether MMP-1 can alter the behavior of cancer cells through PAR-1 to promote cell invasion and metastasis.

Materials and Methods

Patients. In this study, 129 patients with a diagnosis of primary gastric cancer received their initial treatment at the first Department of Surgery, University of Fukui, Japan. All patients underwent surgery without preoperative radio- or chemotherapy. Ninety-four patients underwent curative resections, while the others underwent non-curative resections. The records and histological specimens of the 129 patients were available for review.

Cell culture. The human gastric cancer cell lines MKN28, MKN45, MKN74, TMK1, KATOIII, NUGCII and NUGCIII were cultured at 37ïC in 95% air and 5% CO_2 in RPMI 1640 medium containing 10% fetal bovine serum (FBS; Gibco, Calabasas, CA, USA), 10 units/ml penicillin G and 10 mg/ml streptomycin (Gibco).

Reverse transcription-PCR and quantitative RT-PCR analysis. Total RNA was extracted from gastric cancer cells with ISOGEN reagent (NipponGene, Tokyo, Japan). Single-stranded cDNA prepared from 3 μg of total RNA using MMLV (Moloney murine leukemia virus) reverse transcriptase (Gibco) with an oligo $(dT)_{14}$ primer was used as the template for PCR and qPCR. The following primer pairs were used: GAPDH: 5'-GGGAGCCAAAAGGGTCATC ATCT-3' and 5'-GACGCCTGCTTCACCACCTTCTTG-3'; PAR-1: 5'-TGTGAACTGATCATGTTTATG-3' and 5'-TTCGTAA GATAAGAGATATGT-3'; MMP-1: 5'-CTCATGAACTCGG CCATTCT-3' and 5'-CCGGGTAGAAGGGATTTGTG-3'.

Clinical findings. The Japanese Classification of Gastric Carcinoma, 2nd English edition, (18) was used for pathological diagnosis and for the classification of variables. Mortality statistics for all patients were obtained from their follow-up records.

Immunohistochemical staining. Mouse monoclonal antibody against PAR-1 was purchased from Immunotech (Immunotech, Marseille, France), and mouse monoclonal antibody against MMP-1 was purchased from Daiichi Fine Chemicals (Daiichi Fine Chemicals, Japan). Sections prepared from MKN28 human gastric cancer cell cultures were used as positive controls and appropriate antibody dilutions were determined by titration in the presence of positive control. The final dilution of PAR-1 was 1:100 and that of MMP-1 antibodies was 1:100.

The tumor tissues from resected specimens were routinely fixed in 10% paraformaldehyde and embedded in paraffin. The sections were dewaxed using xylene and rehydrated in graded alcohols. To reduce nonspecific background staining, endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes at room temperature. After being washed in Tris-buffered saline (TBS), the sections were incubated overnight with the primary antibodies (in TBS containing 1% bovine serum albumin) at 4ïC in a humidified chamber. The sections were then washed three times with TBS, and incubated for 60 minutes at room temperature with labeled-dextran polymer (Envision; Dako, Carpinteria, CA, USA). After further washing in TBS, the sections were developed with activated 3'-diaminobenzidine-tetrahydrochloride (DAB) for 5 min and the reaction was stopped in TBS. The sections were lightly counterstained with methyl green. PAR-1-and MMP-1-positive cells exhibited the deposition of brown DAB precipitate.

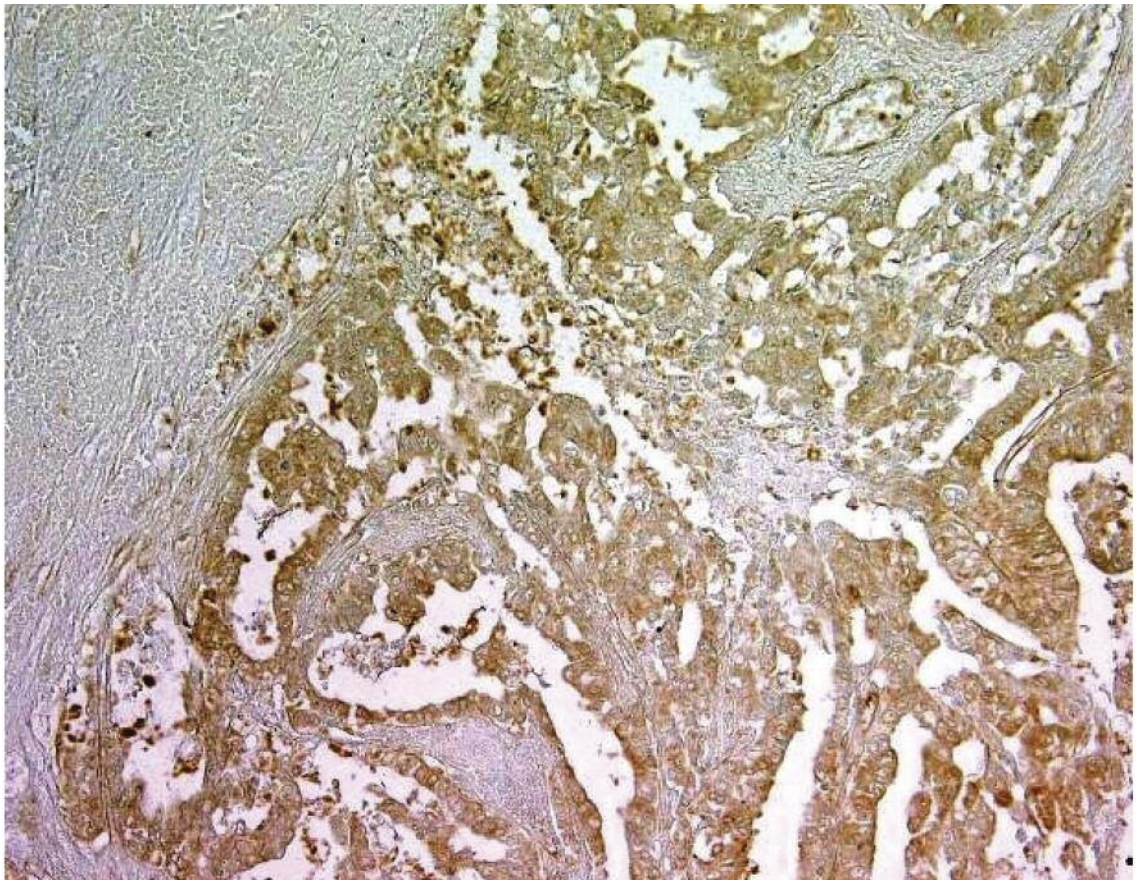

Figure 2. *Immunohistochemical staining of PAR-1 in human gastric cancer. The PAR-1 expression was intensely strong on the cell membrane of primary cancer tissues. Tumor cells in the invasive front were more predominantly stained for PAR-1 than in the tumor center. Staining was judged as +++. Original magnification x100.*

Histological evaluation and scoring. The combined histological results were assessed independently by two of the authors (DF and YH) who classified and scored the sample. Positive reactions were classified using the following criteria: (–) <10% cells stained negative, (+) 10% to <20%, (++) 20% to <40%, and (+++) 40% or more. In accordance with Migita *et al.* (19) we found that no significant reactivity was observed for PAR-1 and MMP-1 in non-neoplastic gastric mucosa. Hence tissues in which more than 10% of the tumor cells were stained were classified as PAR-1 and MMP-1 protein-positive.

Statistical analysis. The survival curve of patients was determined according to the Kaplan-Meier method; the outcomes of different groups of patients were compared by log rank test. Statistically significant differences in clinicopathological findings were identified using the Chi-square test. Univariate and multivariate analysis were performed using the Cox proportional hazard regression model. Patients who died of diseases other than gastric cancer were treated as censored cases. StatView-5.0 software (SAS Institute, Cary, NC, USA) was used to perform all statistical analyses. A value of $p<0.05$ was considered statistically significant.

Results

Expression of PAR-1 and MMP-1 mRNA in gastric cancer cell lines. The expression of PAR-1 and MMP-1 mRNA in seven gastric cancer cell lines was evaluated by reverse transcription-PCR (Figure 1). The definitive expression of PAR-1 mRNA was confirmed in MKN28, MKN74 and NUGC III cell lines, but no bands corresponding to the RT-PCR product of PAR-1 mRNA were observed for NUGCII, MKN45 or TMK1 cell lines; KATOIII showed only a faint band. The definitive expression of MMP-1 mRNA was confirmed in MKN28, MKN74, NUGCII, NUGCIII and KATOIII cell lines. MKN28 was selected as positive control because it expressed both PAR-1 and MMP-1 mRNA.

Expression of PAR-1 and MMP-1 in gastric cancer. Of the 129 specimens of gastric cancer, tumor tissue of 58 (45%) showed positive immunoreactivity for the PAR-1. The PAR-1 expression was intensely strong on the cell membrane of

Table I. *Association between of PAR-1 expression and clinicopathological findings.*

Variable	Negative	Positive	*P*-value*
Histopathological stage			
IA	9	3	0.0854
IB	19	10	
II	13	7	
IIIA	8	7	
IIIB	6	4	
IV	16	27	
Histological differentiation			
Differentiated (pap, tub1, tub2)	42	31	0.5154
Undifferentiated (por1, por2, sig, muc)	29	27	
Macroscopic type			
Infiltrating	25	23	0.6035
Localized	46	35	
Depth of wall invasion			
T1	13	3	0.0028
T2	40	29	
T3	13	9	
T4	5	17	
Lymph node metastasis			
n0	30	16	0.0729
n1	19	12	
n2	15	16	
n3	7	14	
Peritoneal dissemination			
Negative	66	47	0.041
Positive	5	11	
Liver metastasis			
Negative	67	53	0.5077
Positive	4	5	
Curability			
Curative resection	56	38	0.0897
Non-curative resection	15	20	

*Chi-square test. pap: papillary adenocarcinoma, tub1: well differentiated tubular adenocarcinoma, tub2: moderately differentiated tubular adenocarcinoma, por1: solid type poorly differentiated adenocarcinoma, por2: non-solid type poorly differentiated adenocarcinoma, sig: signet-ring cell carcinoma, muc: mucinous adenocarcinoma.

Table II. *Associated between MMP-1 expression and clinicopathological findings.*

Variable	Negative	Positive	*P*-value*
Histopathological stage			
IA	7	5	0.0022
IB	20	9	
II	14	6	
IIIA	3	12	
IIIB	6	4	
IV	14	29	
Histological differentiation			
Differentiated (pap, tub1, tub2)	37	36	0.7809
Undifferentiated (por1, por2, sig, muc)	27	29	
Macroscopic type			
Infiltrating	22	26	0.5087
Localized	42	39	
Depth of wall invasion			
T1	11	5	0.0416
T2	38	31	
T3	9	13	
T4	6	16	
Lymph node metastasis			
n0	29	17	0.0143
n1	18	13	
n2	9	22	
n3	8	13	
Peritoneal dissemination			
Negative	57	56	0.6163
Positive	7	9	
Liver metastasis			
Negative	62	58	0.0884
Positive	2	7	
Curability			
Curative resection	51	43	0.0839
Non-curative resection	13	22	

*Chi-square test.

primary cancer tissues (Figure 2). No association was found between PAR-1 immunostaining and histological stage, histological type, macroscopic type, lymph node metastasis or liver metastasis (Table I). Nor was any significant association found between PAR-1 immunostaining and operative curability (Table I). However, significant association between PAR-1 immunostaining and both depth of wall invasion ($p=0.0028$) and peritoneal dissemination ($p=0.041$) were found (Table I). The patient survival analysis for gastric cancer overexpressing PAR-1 showed a higher risk of death (Figure 3).

The expression of MMP-1 was analyzed in the same samples of gastric cancer. Sixty-five of 129 primary tumors (50.3%) showed positive immunoreactivity for MMP-1; immunoreactivity was detected in the tumor and stromal cells (Figure 4). Associations between MMP-1 immunostaining and histological stage ($p=0.0022$), depth of wall invasion ($p=0.0416$) and lymph node metastasis ($p=0.0231$) (Table II) were found. Statistical analysis did not reveal any further significant associations between MMP-1 expression and the other clinicopathological factors (Table II). Significant association was found between PAR-1 immunostaining and MMP-1 immunostaining ($p<0.001$) (Table III). Moreovers, there were 42 (32.6%) carcinomas positive for both PAR-1 and MMP-1 expression. The patients with tumors expressing both PAR-1 and MMP-1 concomitantly was associated with higher histological stage ($p=0.008$), greater depth of infiltration ($p<0.001$), lymph node metastasis ($p=0.0074$) and peritoneal dissemination ($p=0.0307$) (Table IV). The patients with both PAR-1 and MMP-1expression-positive tumors had

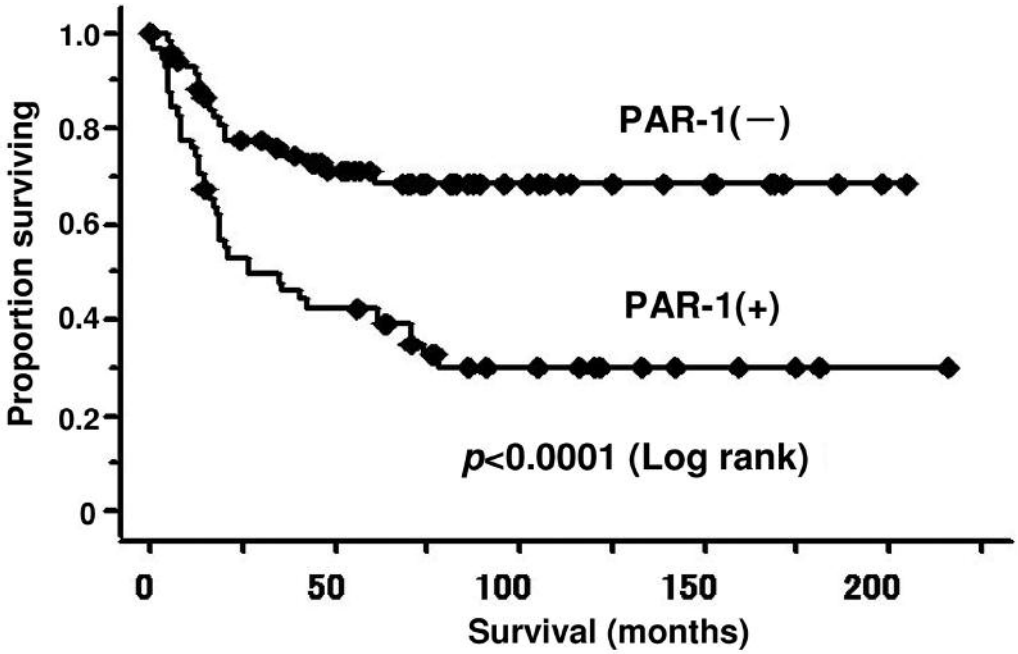

Figure 3. *Overall survival curves of patients with gastric cancer subdivided according to expression of PAR-1.*

a significant poorer prognosis than those with one side or double expression-negative tumors (Figure 5).

Univariate and multivariate analysis of survival. We analyzed the value of the expression of the different proteins on survival. Each analysis was performed independently because the known biological interactions among the different molecules could interfere with the results.

Univariate analysis identified PAR-1 expression, MMP-1 expression, both PAR-1 and MMP-1 expression, macroscopic type, depth of wall invasion, lymph node metastasis, peritoneal dissemination, liver metastasis and vascular invasion as predictors of worse survival (Table V); multivariate analysis indicated that PAR-1 expression, concomittant PAR-1 and MMP-1 expression, depth of wall invasion, peritoneal dissemination and liver metastasis were independent prognostic factors (Table VI).

Discussion

In Japan, gastric cancer is one of the most common malignancies. In spite of the improvement in surgical treatment and chemotherapy, gastric cancer of an advanced stage is still subject to a poor prognosis, although cases of early stage are successfully controlled. We previously had reported several progression-related factors, including PAR-2, in gastric cancer (20). But the mechanism of gastric cancer cell invasion and metastasis has not yet been clarified.

Although PAR-1 expression has correlated with tumor invasion and metastasis in several types of cancer, this is the first study that presents associations between the immunohistochemical status for PAR-1 and MMP-1 and clinicopathological factors and patients' prognosis in gastric cancer. Patients with tumors expressing both PAR-1 and MMP-1 concomitantly was associated with higher histological stage greater depth of infiltration, lymph node metastasis and peritoneal dissemination. These patients had a significantly poorer prognosis than those with either PAR-1 or MMP-1 immunostaining-positive tumors or with PAR-1 and MMP-1 immunostaining-negative tumors. MMP-1 positive cells were detected in the tumor and stromal cells. These findings may indicate that PAR-1 and MMP-1 play an important role in the gastric cancer cell, especially regarding invasion and metastasis.

Peritoneal dissemination requires that cancer cells that have invaded the serosal surface be scattered over the abdominal cavity, with attachment to mesothelial cells or the extracellular matrix under the mesothelium, and invasive growth in the extracellular matrix. Hence, malignant cells solicit the help of other cells, such as stromal fibroblasts, mast cells, monocytes and vascular endothelial cells, to facilitate their invasion into the surrounding tissue (21). The interface between the invading malignant cells and the hosting stromal cells, referred to as the tumor microenvironment (TME) (22), possesses a vast array of well-orchestrated cell signaling molecules which function to

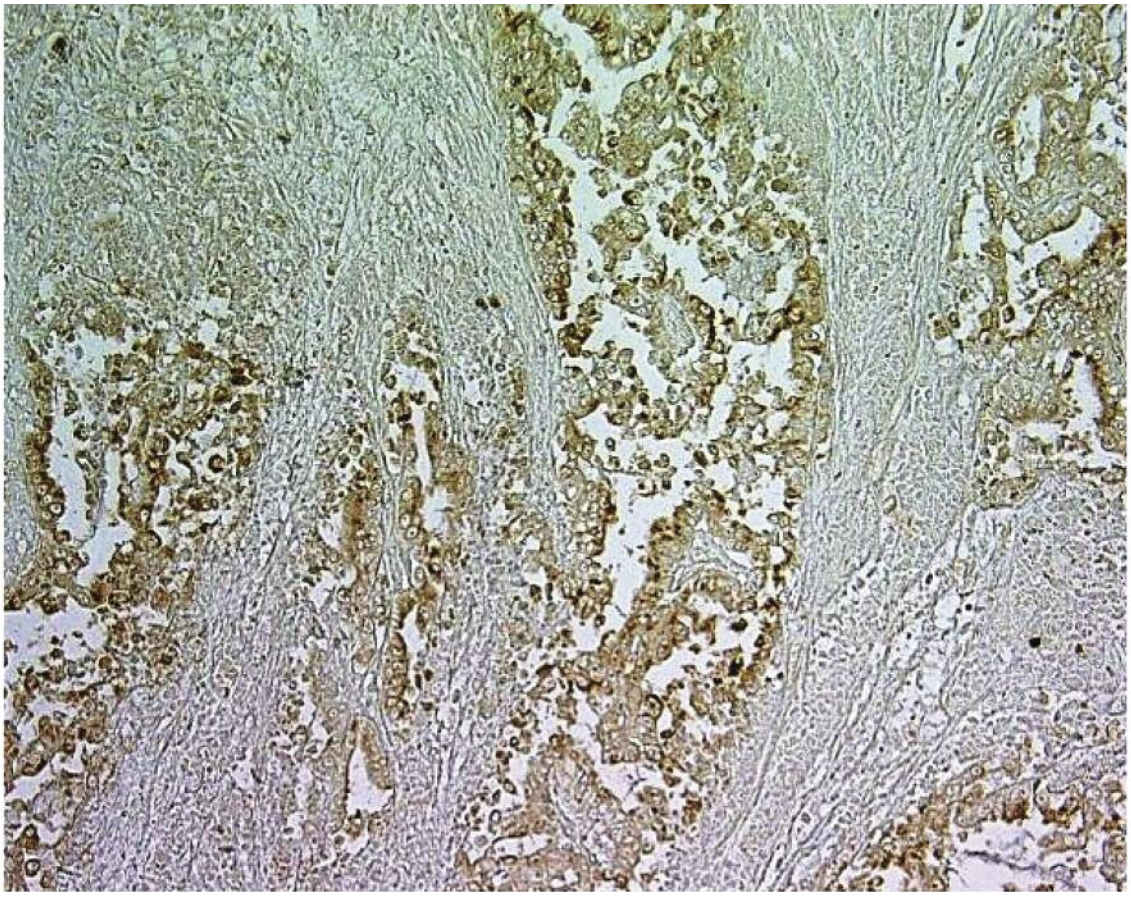

Figure 4. *Immunohistochemical staining of MMP-1 in human gastric cancer. The immunoreaction pattern of MMP-1 positive cells was detected in the tumor cells strongly. We confirmed MMP-1 positive cells at the same area where we found PAR-1 positive cells. Staining was judged as +++. Original magnification x100.*

facilitate the ability of the proliferating tumor front to invade the stroma, as well as to degrade and remodel the extracellular matrix (21). While we think that these sequential phenomena need various proteinases, those such as matrix metalloproteinases and urokinase-type plasminogen activator (u-PA) play an important role in the invasion by cancer cells into the extracellular matrix (23-25). The tumor cells secrete several factors including interleukins, cytokines and angiogenesis factors, to induce the stromal cells around the cancer cells to produce MMPs (26). Stromal cells which brought out active MMP-1 by various cytokines produced by neighboring cancer cells may present high local concentration of MMP-1 to PAR-1 on the same tumor cells surface. The endogenous MMP-1 activity generated *in situ* from the fibroblast media activates PAR-1 and is sufficient to cause robust migration and invasion of breast cancer cells *via* PAR-1 (12). We showed that PAR-1 expression was intensely strong on the cell membrane of primary cancer tissues, and MMP-1-positive cells were detected in the tumor and stromal cells. We believe that the gastric cancer tissue produces MMP-1, cleaving PAR-1 to generate a new receptor *N*-terminus in the autocrine and paracrine manner, and activated PAR-1 causes gastric cancer cell invasion and metastasis *in vitro*. Thus, patients with both PAR-1 and MMP-1 expression-positive tumors have a poorer prognosis than the other patients.

Table III. *Associated between PAR-1 expression and MMP-1 expression.*

	PAR-1		
Variable	Negative	Positive	*P*-value*
MMP-1			
Negative	48	16	<0.001
Positive	23	42	

*Chi-square test.

Table IV. *Association between concomitant expression of PAR-1 and MMP-1 and clinicopathological findings.*

Variable	Negative[a]	Positive[b]	*P*-value*
Histopathological stage			
IA	10	2	0.008
IB	25	4	
II	16	4	
IIIA	8	7	
IIIB	7	3	
IV	21	22	
Histological differentiation			
Differentiated (pap, tub1, tub2)	50	23	0.085
Undifferentiated (por1, por2, sig, muc)	37	19	
Macroscopic type			
Infiltrating	28	20	0.1899
Localized	59	22	
Depth of wall invasion			
T1	14	2	<0.001
T2	51	18	
T3	16	6	
T4	6	16	
Lymph node metastasis			
n0	39	7	0.0074
n1	21	10	
n2	16	15	
n3	11	10	
Peritoneal dissemination			
Negative	80	33	0.0307
Positive	7	9	
Liver metastasis			
Negative	83	37	0.1269
Positive	4	5	

*Chi-square test. [a]Negative for either or both PAR-1 and MMP-1 expression, [b]Positive for both PAR-1 and MMP-1 expression.

Finally, the afore-mentioned results suggest that blocking MMP-1 may prove beneficial in the treatment of a variety of tumor invasive and proliferative conditions. Unfortunately, clinical trials with broad-spectrum MMP inhibitors for treatment of diverse types of cancer have suffered from dose-limiting joint toxicity thought to be due to inhibition of MMP-1 (27). PAR-1 can be directly activated by the interstitial collagenase MMP-1 (12), hence we believe that PAR-1 may be a more an attractive target as a novel therapeutic approach for blocking the progression of invasive and metastatic gastric cancer, rather than achieving this through inhibition of MMP-1 itself.

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Table V. *Univariate analysis of PAR-1 and MMP-1 expression and clinicopathological findings.*

Univariate Variable	Hazard ratio (95% CI)	*P*-value
PAR-1 expression	2.862(1.668-4.911)	0.0001
MMP-1 expression	2.639(1.535-4.538)	0.0004
Concomitant PAR-1 and MMP-1 expression	3.210(1.919-5.372)	<0.0001
Histological differentiation	1.590(0.954-2.652)	0.0753
Macroscopic type	1.998(1.198-3.331)	0.0079
Depth of wall invasion ≥T3	5.662(3.306-9.698)	<0.0001
Lymph node metastasis ≥n1	3.967(1.948-8.078)	<0.0001
Peritoneal dissemination	5.496(3.021-9.996)	<0.0001
Liver metastasis	3.782(1.776-8.051)	0.0006
Lymphatic invasion	2.446(0.978-6.121)	0.558
Vascular invasion	2.839(1.652-4.878)	0.0002

Table VI. *Multivariate analysis of PAR-1 and MMP-1 expression and clinicopathological findings.*

Variable	Hazard ratio (95% CI)	*P*-value
PAR-1 expression	1.855(1.048-3.283)	0.0340
Macroscopic type	0.672(0.345-1.308)	0.2419
Depth of wall invasion ≥T3	3.757(1.935-7.297)	<0.0001
Lymph node metastasis ≥n1	1.764(0.772-4.028)	0.1779
Peritoneal dissemination	2.453(1.185-5.078)	0.0157
Liver metastasis	2.877(1.274-6.494)	0.0110
Vascular invasion	1.343(0.734-2.459)	0.3382
MMP-1 expression	1.610(0.904-2.867)	0.1061
Macroscopic type	0.694(0.904-1.332)	0.694
Depth of wall invasion ≥T3	3.552(1.843-6.844)	0.0002
Lymph node metastasis ≥n1	1.824(0.802-4.147)	0.1515
Peritoneal dissemination	2.889(1.413-5.908)	0.0036
Liver metastasis	2.865(1.267-6.476)	0.0114
Vascular invasion	1.203(0.655-2.209)	0.5511
Concomitant PAR-1 and MMP-1 expression	1.818(1.025-3.226)	0.041
Macroscopic type	0.635(0.324-1.242)	0.1841
Depth of wall invasion ≥T3	3.823(1.960-7.456)	<0.0001
Lymph node metastasis ≥n1	01.782(0.775-4.101)	0.1740
Peritoneal dissemination	2.603(1.264-5.363)	0.0095
Liver metastasis	2.758(1.215-6.265)	0.0153
Vascular invasion	1.156(0.62-2.157)	0.6477

References

1 Coughlin SR: Thrombin signaling and protease-activated receptors. Nature *407*: 258-264, 2000.

2 Vu TKH, Hung DT, Wheaton IV and Coughlin SR: Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor action. Cell *64*: 1057-1068, 1991.

3 Kuliopulos A, Covic L, Seeley SK, Sheridan PJ, Helin J and Costello CE: Plasmin desensitization of the PAR1 thrombin receptor: kinetics, sites of truncation, and implications for thrombolytic therapy. Biochemistry *38*: 4572-4585, 1999.

4 Riewald M, Kravchenko VV, Petorovan RJ, O'Brien PJ, Brass LF, Ulevitch RJ and Ruf W: Gene induction by coagulation factor Xa is mediated by activation of protease-activated receptor1. Blood *97*: 3109-3116, 2001.

5 Riewald M, Petrovan RJ, Donner A, Muller BM and Ruf W: Activation of endothelial cell protease receptor1 b the protein C pathway. Science *296*: 1880-1882, 2002.

6 Seeley S, Covic L, Jacques SL, Sudmeier J, Baleja JD and Kuliopulos A: Structural basis for thrombin activation of a protease-activated receptor: Inhibition of intramolecular liganding. Chem Biol *10*: 1033-1041, 2003.

7 Wojtukiewicz MZ, Tang DG, Ben-Josef E, Renaud C, Walz DA and Honn KV: Solid tumor cells express functional 'tethered ligand' thrombin receptor. Cancer Res *55*: 698-704, 1995.

8 Rudroff C, Schafberg H, Nowak G, Weinel R, Scheele J and Kaufmann R: Characterization of functional thrombin receptor in human pancreatic tumor cells (MIA PACA-2). Pancreas *16*: 189-194, 1998.

9 Kaufmann R, Schaberg H, Rudroff C and Nowak G: Thrombin receptor activation results in calcium signaling and protein kinase C-dependent stimulation of DNA synthesis in Hep-2g laryngeal carcinoma cells. Cancer *80*: 2068-2074, 1997.

10 Even-Ram S, Uziely B, Cohen P, Grisaru-Granovsky S, Maoz M, Ginzburg Y, Reich R, Vlodavsky I and Bar-Shavit R: Thrombin receptor overexpression in malignant and physiological invasion processes. Nat Med *4*: 909-914, 1998.

11 Henrikson KP, Salazar SL, Fenton JW 2nd and Pentecost BT: Role of thrombin receptor in breast cancer invasiveness. Br J Cancer *79*: 401-406, 1999.

12 Boire A, Covic L, Agarwal A, Jacques S, Sherifi S and Kuliopulos A: PAR1 is a Matrix metalloproteinase-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell *120*: 303-313, 2005.

13 Nelson AR, Fingleton B, Rothenberg ML and Matrisian LM: Matrix metalloproteinases: biologic activity and clinical implications. J Clini Oncol *18*: 1135-1149, 2000.

14 Collins HM, Morris TM and Watson SA: Spectrum of matrix metalloproteinase expression in primary and metastatic colon cancer: relationship to the tissue inhibitors of metalloproteinase and membrane type-1 matrix metalloproteinase. Br J Cancer *84*: 1664-1670, 2001.

15 D'Errico A, Garbisa S, Liotta LA, Castronovo V, Stetler-Stevenson WG and Grigioni WF: Augmentation of type IV collagenase, laminin receptor, and Ki67 proliferation antigen associated with human colon, gastric, and breast carcinoma progression. Mod Pathol *4*: 239-246, 1991.

16 Grigioni WF, D'errico A, Fortunato C, Fiorentino M, Mancini AM and Stetler-Stevenson WG: Prognosis of gastric carcinoma revealed by interactions between tumor cells and basement membrane. Mod Pathol *7*: 220-225, 1994.

17 Sakurai Y, Otani Y, Kameyama K, Igarashi N, Kubota T, Kumai K and Kitajima M: The role of stromal cells in the expression of interstitial collagenase (matrix metalloproteinase-1) in the invasion of gastric cancer. J Surg Oncol *66*: 168-172, 1997.

18 Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma -2nd English edition. Gastric Cancer *1*: 10-24, 1998.

19 Migita T, Sato E, Saito K, Mizoi T, Shiiba K, Matsuno S, Nagura H and Ohtani H: Differing expression of MMPs-1 and -9 and urokinase receptor between diffuse- and intestinal-type gastric carcinoma. Int J Cancer *84*: 74-79, 1999.

20 Fujimoto D, Hirono Y, Goi T, Katayama K, Hirose K and Yamaguchi A: Expression of protease activated receptor-2 (PAR-2) in gastric cancer J Surg Oncol *93*: 139-144, 2006.

21 Gregiore M and Lieubeau B: The role of fibroblasts in tumor behavior. Cancer Metastasis Rev *14*: 339-350, 1995.

22 O'Meara RAQ: Coagulative properties of cancers. Ir J Med Sci *394*: 474-479, 1958.

23 Hou L, Kapas S, Cruchley AT, Macey MG, Harriott P, Chinni C, Stone SR and Howells G: Immunolocaliozation of protease-activated receptor-2 in skin: receptor activation stimulates interleukin-8 secretion by keratinocytes *in vitro*. Immunology *94*: 356-362, 1998.

24 Koshikawa N, Hasegawa S, Nagashima Y, Mitsuhashi K, Tsubota Y, Miyata S, Miyagi Y, Yasumitsu H and Miyazaki K: Expression of trypsin by epithelial cells of various tissues, leukocytes, and neurons in human and mouse. Am J Pathol *153*: 937-944, 1998.

25 Koshikawa N, Nagashima Y, Miyagi Y, Mizushima H, Yanoma S, Yasumitsu H and Miyazaki K: Expression of trypsin in vascular endothelial cells. FEBS Lett *499*: 442-448, 1997.

26 Zucker S, Mirza H, Conner CE, Lorenz AF, Drews MH, Bahou WF and Jesty J: Vascular endothelial growth factor induces tissue factor and matrix metalloproteinase production in endothelial cells: conversion of prothrombin to thrombin results in progelatinase A activation and cell proliferation. Int J Cancer *75*: 780-786, 1998.

27 Yamamoto A, Yano S, Shiraga M, Ogawa H, Goto H, Miki T, Zhang H and Sone S: A third-generation matrix metalloproteinase (MMP) inhibitor (ONO-4817) combined with docetaxel suppresses prognosis of lung micrometastasis of MMP-expressing tumor cells in nude mice. Int J Cancer *103*: 822-828, 2003.

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