

Prognostic Significance of Matrix Metalloproteinase-7 (MMP-7) Expression at the Invasive Front in Gastric Carcinoma

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To evaluate the clinicopathological significance of matrix metalloproteinase-7 (MMP-7) expression in gastric carcinoma, we investigated immunohistochemically MMP-7 expression in 214 gastric carcinomas, and examined its relations with the clinicopathologic parameters including patient prognosis. MMP-7 expressed predominantly in cancer cells, and MMP-7-positive tumor cells were preferentially found in deeply invading nests, especially at the invasive front. The mean MMP-7 labeling index (LI) at the invasive front was significantly higher in tumors invading or penetrating the muscularis propria and in stages II–IV than within the submucosal layer and in stage I, respectively ($P < 0.001$). Statistical analysis revealed that MMP-7 LI at the invasive front was related to lymph node metastasis, vascular invasion, and lymphatic permeation, when all 214 cases were examined as one group ($P < 0.05$ for all), and the cases with high MMP-7 expression at the invasive front showed significantly more unfavorable prognosis as compared with that of low MMP-7 expression tumors ($P < 0.01$). Multivariate analysis revealed that TNM stage and MMP-7 expression status at the invasive front were independent prognostic factors ($P = 0.0017$, relative risk (RR) = 3.12; $P = 0.0019$, RR = 2.67, respectively). Our findings indicated that expression of MMP-7 at the invasive front is closely associated with local invasiveness, and might be a reliable prognostic marker for patients with gastric carcinoma.

Key words: Gastric carcinoma — Matrix metalloproteinase-7 (MMP-7) — Immunohistochemistry — Prognosis

Two major characteristics of malignant tumor cells are invasion and metastasis, for which proteolytic degradation of the extracellular matrix, including basement membrane, by matrix metalloproteinases (MMPs) is one of the essential events.^{1,2} MMP-7, one of the MMPs family members, has a broad spectrum of proteolytic activity against a variety of extracellular matrix substrates, including type IV collagens, proteoglycans, laminin, fibronectin and casein.^{3–5} It can also inactivate α 1-antitrypsin, which augments the serine protease activity, resulting in activation of MMPs themselves.⁶ MMP-7 is expressed predominantly by tumor cells in various cancers.^{7–9} Immunohistochemical studies have shown that the expression of MMP-7 correlates with tumor invasion/metastasis and prognosis in both colorectal adenocarcinoma and esophageal squamous cell carcinoma.^{8–10}

Gastric carcinoma is one of the most common cancers in Japan, and many patients die due to invasion or metastasis of cancer cells. Previous studies concerning MMP-7 expression in gastric carcinoma showed that the expression of MMP-7 mRNA and protein was higher in cancer tissue than in the surrounding normal tissue, and

that it might be associated with tumor invasiveness and/or metastasis.^{7,11–13} However, the prognostic significance of MMP-7 expression in gastric carcinoma has not been reported yet. In the present study, we examined immunohistochemically MMP-7 expression in 214 gastric carcinomas to assess the relationships of MMP-7 expression to clinicopathologic parameters including patient prognosis in gastric carcinoma.

MATERIALS AND METHODS

Patients In this study, we used 214 cases of gastric carcinoma. All patients underwent curative surgery in the Tsushimi Hospital, Hagi, Yamaguchi, between January 1991 and December 1997, and received neither chemotherapy nor radiation therapy before surgery. Of these patients, 126 (58.9%) were men and 88 (41.1%) were women, with the mean age of 67 years (range: 30–93 years). All patients were followed-up after surgery until March 1, 2001. During the follow-up period, 20 patients (17 died of unrelated causes and 3 died within 30 days after surgery) were excluded, leaving 194 patients for survival analyses.

Pathological diagnosis including the depth of tumor invasion, vascular invasion, lymphatic permeation, and

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lymph node metastasis, was made according to the general rules for gastric cancer outlined by the Japanese Research Society for Gastric Cancer.¹⁴⁾ The tumor stage was determined according to the 1997 TNM Classification System of the UICC,¹⁵⁾ stage I ($n=73$), stage II ($n=35$), stage III ($n=60$), and stage IV ($n=46$). Histologic differentiation was graded well ($n=53$), moderate ($n=74$), or poor ($n=87$). The clinicopathologic findings were diagnosed by reviewing all hematoxylin and eosin (H & E) stained tissue sections.

Immunohistochemistry In this study, 4- μm thick paraffin sections were cut from each block of resected specimens, and mounted on slides. In each case, 1–3 paraffin sections containing the invasive front were selected for immunohistochemical staining. Immunostaining for MMP-7 was performed using a standard avidin-biotin peroxidase (ABC) technique. Sections were dewaxed in xylene, rehydrated in alcohol, and then heated in 10 mmol/liter sodium citrate buffer (pH 6.0) by a microwave oven (650 W) for 20 min to retrieve the antigen. The endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 5 min. After blocking of non-specific binding sites with 10% normal rabbit serum, slides were incubated overnight at 4°C with monoclonal antibody against MMP-7 (10 $\mu\text{g}/\text{ml}$, clone 141-7B2, Daiichi Fine Chemicals, Ltd., Takaoka), in 1% bovine serum albumin (BSA)/phosphate-buffered saline (PBS), pH 7.6. Subsequent reaction, using a HISTOFINE SAB-PO (M) Immunohistochemical Staining Kit (Nichirei, Tokyo), was based on the streptavidin-biotin complex/horseradish peroxidase method. Positive reaction was visualized with hydrogen peroxide containing 3,3'-diaminobenzidine (DAB)/PBS. Sections were counterstained with Mayer's hematoxylin for nuclei and mounted. Negative control sections was made by omitting the primary antibody.

Evaluation of MMP-7 staining MMP-7 labeling index (MMP-7 LI) at the invasive front was calculated as the percentage of cells stained positively for MMP-7 among tumor cells counted. Ten high-power fields were selected, and at least 1000 tumor cells were observed for evaluation.

All calculations were independently performed twice and cases of twice-counted disagreements were reviewed, followed by a conclusive judgement.

Statistical analysis Data are shown as the mean \pm standard deviation ($\pm\text{SD}$). MMP-7 LI was compared between different clinicopathological subgroups using a nonparametric Mann-Whitney U test or Kruskal-Wallis test. Univariate and multivariate survival analyses were performed according to the Kaplan-Meier method and proportional hazard regression model, respectively. For all statistical tests, a P value less than 0.05 was defined as statistically significant.

RESULTS

Features of MMP-7 expression The cytoplasm and cell membrane were stained for MMP-7 in tumor cells (Fig. 1). The antral gland epithelium was rarely positive and stromal cells were negative for MMP-7 staining. The distribution of MMP-7-positive tumor cells varied from area to area, but the cells were frequently located in deeply invading tumor cell nests, especially at the invasive front (Fig. 2). MMP-7-positive tumor cells were found in 8 of 46 mucosal cancers (17.4%), 14 of 38 submucosal cancers (36.8%), 22 of 33 cases invading the muscularis propria (66.7%), 28 of 39 tumors invading subserosa (71.8%), and 43 of 58 cancers invading serosa (74.1%).

Relationship of MMP-7 expression with the depth of tumor invasion The mean MMP-7 LI at the invasive front increased with the depth of tumor invasion. The mean MMP-7 LI of cancers invading mucosa, submucosa, muscularis propria, subserosa and serosa, determined based on histologic evaluation, was $6.7 \pm 10.7\%$ ($n=46$), $7.7 \pm 13.7\%$ ($n=38$), $23.6 \pm 14.4\%$ ($n=33$), $26.9 \pm 14.6\%$

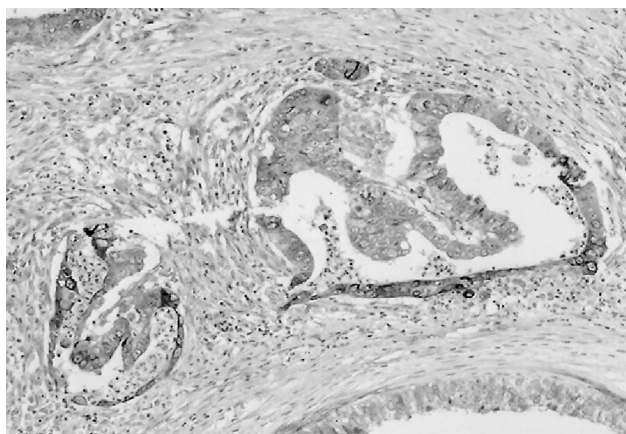


Fig. 1. MMP-7 positive staining of cancer cells.

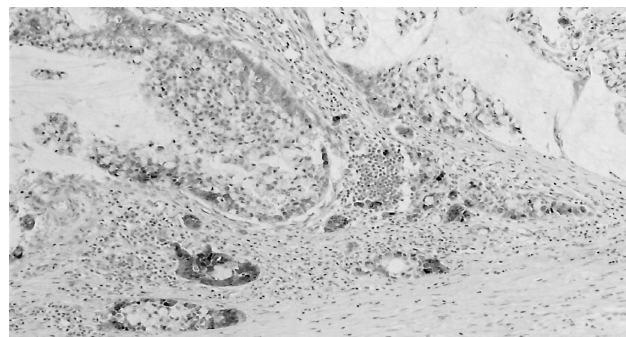


Fig. 2. MMP-7-positive cells are located frequently in deeply invading nests, especially at the invasive front.

($n=39$), and $28.2\pm 13.9\%$ ($n=58$), respectively. The mean MMP-7 LI at the invasive front was significantly higher in “advanced cancers” (tumors of muscularis propria or beyond) than in “so-called early cancers” (mucosal or submucosal tumors) ($P<0.001$).

Relationships of MMP-7 expression to clinicopathologic features Table I summarizes the relationships of MMP-7 LI at the invasive front to clinicopathological features in 214 gastric carcinomas. The mean MMP-7 LI was significantly higher in stage II–IV and T2–4 tumors, and cancers with lymph node metastasis, vascular invasion, and/or lymphatic permeation than in stage I and T1 tumors, and cancers with none of them ($P<0.05$ for all). However, histological differentiation of tumors did not affect MMP-7 LI.

Based on T classification of tumors, the 214 cases were classified into 4 groups. The relationships between MMP-7 LI at the invasive front and lymph node metastasis, vascular invasion, and lymphatic permeation were further analyzed in each group, but no significant relationship between them was found in any group (data not shown).

Table I. Associations of MMP-7 Expression at the Invasive Front with Clinicopathologic Factors

Factor	No.	MMP-7	
		MMP-7 LI (%) (mean \pm SD)	P value
Total no. of patients	214		
Gender			
Male	126	16.7 \pm 18.4	NS
Female	88	18.9 \pm 21.6	
Age			
≥ 67	130	17.5 \pm 19.9	NS
< 67	84	17.8 \pm 19.3	
Lymph node metastasis			
Negative	108	12.8 \pm 16.7	<0.05
Positive	106	24.1 \pm 21.3	
TNM stage			
I	73	11.4 \pm 15.4	<0.05
II	35	24.6 \pm 24.2	
III	60	26.1 \pm 21.2	
IV	46	29.3 \pm 20.3	
Histologic differentiated			
Well differentiated	53	20.6 \pm 18.5	NS
Moderately differentiated	74	20.4 \pm 21.1	
Poorly differentiated	87	24.9 \pm 10.1	
Lymphatic permeation			
Negative	70	9.8 \pm 15.4	<0.001
Positive	144	21.4 \pm 20.56	
Vascular invasion			
Negative	114	13.4 \pm 17.7	<0.05
Positive	100	22.3 \pm 20.8	

SD, standard deviation; LI, labeling index.

Relationship of MMP-7 expression to patients' survival

Thirty percent was selected as the cutoff value of MMP-7 LI at the invasive front by testing the most significant cut-off value referring to the distribution chart of the MMP-7 LI at the invasive front and survival period.^{16, 17} Using the log-rank test, disease-specific survival time of patients with gastric carcinoma having high MMP-7 expression at the invasive front (MMP-7 LI \geq 30%) was shorter than that of patients with low MMP-7-expressing tumors (MMP-7 LI $<$ 30%) (Fig. 3; $P<0.01$). Univariate survival analysis revealed that in addition to MMP-7 LI, tumor size, histologic differentiated type, depth of tumor invasion, TNM stage, lymph node metastasis, vascular invasion, and lymphatic permeation were significantly associated with disease-specific survival (Table II, $P<0.05$ for all). Multivariate survival analysis disclosed that of these parameters, only tumor stage ($P=0.0017$, relative risk (RR)=3.12) and expression status of MMP-7 at the invasive front ($P=0.0019$, RR=2.67) were independent prognostic factors (Table II).

DISCUSSION

MMP-7 was expressed predominantly in deeply invading tumor cell nests, especially at the invasive front. This is in accordance with the report by Adachi *et al.* in gastric carcinomas.¹¹ MMP-7 is known to play an important role in tumor invasion,^{7–10} and MMP-7 expression at the invasive front should more accurately represent tumor invasive activity. Therefore, we selected MMP-7 LI at the invasive front for analyzing the association of MMP-7 expression with clinicopathologic features in this study. Our results demonstrated that the percentage of MMP-7-positive cases increased with the depth of tumor invasion, and the mean MMP-7 LI at the invasive front was significantly higher in advanced cancer than in early cancer. These observations suggest that MMP-7 expression affords invasive character

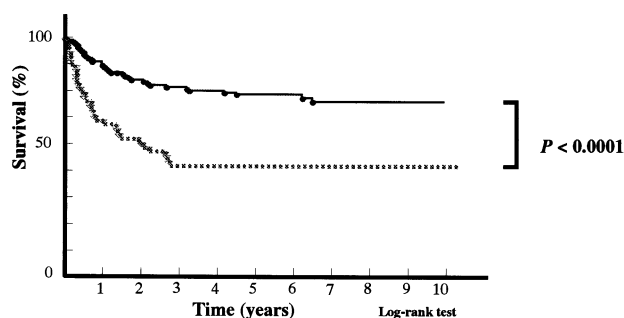


Fig. 3. Kaplan-Meier survival curve of cases with high MMP-7 LI (— MMP-7 LI at the invasive front \geq 30%, $n=60$) or low MMP-7 LI (..... MMP-7 LI at the invasive front $<$ 30%, $n=134$) gastric carcinomas.

Table II. Univariate and Multivariate Analyses of Disease-specific Survival for 194 Patients with Gastric Carcinoma

Prognostic factors	Univariate	Multivariate		
	P value ^{a)}	P value ^{a)}	Relative risk	95% CI
Age (≥67 vs. <67)	NS	Not included		
Sex (male vs. female)	NS	Not included		
Tumor size (<4.5 vs. ≥4.5)	<0.0001	NS		
Histologically differentiated	0.0107	NS		
Depth of invasion	<0.0001	NS		
Lymph node metastasis (pos vs. neg)	<0.0001	NS		
TNM stage ^{b)}	<0.0001	0.0017	3.12	1.52–5.36
Lymphatic permeation (pos vs. neg)	<0.0001	NS		
Vascular invasion (pos vs. neg)	<0.0001	NS		
MMP-7 expression (high vs. low)	<0.0001	0.0019	2.67	1.44–4.95

a) P<0.05 was considered statistically significant.

b) Stage was determined according to 1997 TNM Classification System of UICC.
pos, positive; neg, negative; CI, confidence interval; NS, not significance.

to tumor cells, resulting in the progression of gastric carcinoma.

In some reports concerning MMP-7 in gastric carcinoma, MMP-7 expression was significantly associated with clinicopathologic features such as the depth of tumor invasion, lymph node metastasis, vascular vessel invasion, and/or lymphatic permeation.^{7, 11, 12)} However, Senota *et al.* reported that the level of MMP-7 mRNA was linked only to tumor invasion depth at the gastric wall.¹³⁾ In our study of 214 cases, MMP-7 LI at the invasive front correlated significantly with the invasive depth at the gastric wall. The mean MMP-7 LI at the invasive front was significantly higher in carcinomas with lymph node metastasis, vascular vessel invasion, and/or lymphatic permeation than in those without any of them. However, when 214 cases were classified into four groups based on T classification of tumor, such a relationship was not found in the separate groups. This may support the view that expression of MMP-7 well represents tumor invasiveness, especially local invasiveness. Some researchers have indicated that the changes of other molecules, such as adhesion molecules,^{18, 19)} motility factors,²⁰⁾ and sugar chains²¹⁾ are required for lymph node metastasis, vascular vessel invasion, and lymphatic permeation. Yamashita *et al.* reported that the positive ratio of MMP-7 was significantly higher in intestinal-type than in diffuse-type gastric carcinoma.¹²⁾ Data from Honda *et al.*⁷⁾ and our studies, however, indicate that MMP-7 expression was not affected by histological type.

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To our knowledge, this is the first report to assess the correlation between MMP-7 expression and survival of patients with gastric carcinoma. Survival time of patients with gastric carcinoma expressing high levels of MMP-7 at the invasive front was shorter than that of patients with low MMP-7 expression. Moreover, multivariate survival analysis revealed that both TNM stage and MMP-7 expression status were independent prognostic factors. This was also reported to be the case in colorectal cancer and esophageal squamous cell carcinoma.^{8–10)} These results suggest that the level of MMP-7 expression is a reliable prognostic marker, at least in gastric cancer. In addition, detection of MMP-7 would offer the opportunity for a novel therapeutic approach, i.e., MMP-7 could be a potential target for therapeutic intervention. The use of synthetic broad-spectrum MMP inhibitors is one of the possible strategies.²²⁾ Taking our results into consideration, MMP-7 could be a primary target of such inhibitors in gastric carcinoma. Thus, the immunohistochemical analysis of MMP-7 in gastric carcinoma tissues could provide basic data for a new therapeutic strategy via broad-spectrum and/or selective MMP inhibitors.

In conclusion, we demonstrated that MMP-7 expression at the invasive front is linked with the invasive depth at the gastric wall. The expression of MMP-7 was found to be an independent prognostic factor in patients with gastric carcinoma.

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