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CLINICOPATHOLOGIC AND PROGNOSTIC SIGNIFICANCE OF MATRILYSIN EXPRESSION AT THE INVASIVE FRONT IN HUMAN COLORECTAL CANCERS

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Matrix metalloproteinases (MMPs) have been implicated in tumor progression. Matrilysin, one of the matrix metalloproteinases, is frequently overexpressed in gastrointestinal cancers. The aim of our study was to assess the validity of matrilysin as a prognostic marker of colorectal cancers. Matrilysin expression was immunohistochemically analyzed using formalin-fixed, paraffin-embedded specimens from 113 colorectal cancer patients who had undergone curative surgery. The lumenal surface of neoplastic glands in the superficial layer was apically stained, while the cytoplasm of cancer cells at the invasive front was diffusely stained for matrilysin. Sections with immunostaining signals in more than 30% of carcinoma cells at the invasive front, which were observed in 47 (42%) cases, were judged as being positive for matrilysin. Matrilysin positivity was significantly correlated with the depth of invasion, lymph node metastasis, lymphatic invasion, advanced Dukes' stage and poor outcome. Patients with matrilysin-positive cancer had a significantly shorter overall survival time than those with matrilysin-negative cancer. For patients with intermediate invasive tumor (T2 or T3), only matrilysin was a significant prognostic variable for predicting overall survival in multivariate analysis. Matrilysin expression at the invasive front could be an important marker, predicting an unfavorable prognosis after surgical treatment in patients with colorectal cancer.

Key words: *matrix metalloproteinase (MMP); matrilysin; colorectal cancer; invasive front; tumor progression; prognosis*

Matrix metalloproteinases (MMPs) have been implicated in tumor progression as well as in various normal processes of tissue remodeling.[1–3] The proteolytic degradation of an extracellular matrix by MMPs is one of the most important mechanisms in tumor invasion and metastasis. MMPs are often overexpressed during tumor progression.

Matrilysin (MMP-7) is a member of the MMP gene family. As an MMP, matrilysin is unique in its minimum MMP structure, wide spectrum of substrate specificity, potency to start an activation cascade of MMPs and, most notably, in production by cancer cells themselves.[4,5] Matrilysin is overexpressed in a variety of cancer tissues such as adenocarcinomas of the breast, prostate, colon, stomach, glioblastoma, squamous cell carcinomas of the lung, head and neck and esophagus and hepatocellular carcinomas.[4–11] The production by cancer cells themselves could be an advantage as a biologic marker of the malignant phenotype. Indeed, we have found that matrilysin expression at the invasive front correlates with the progression of gastric adenocarcinoma and esophageal squamous cell carcinoma.[9–11] These results suggest that matrilysin in the invading cells may have a direct role in the progression of cancer and support the notion that tumor cells at the invasive front represent the highest malignant potential, thus ultimately spreading and metastasizing.[12]

The pathologic relevance of matrilysin in the malignant phenotype of colorectal cancer cells has been investigated extensively in clinical cases,[6,7,13] *in vitro* assays[14–16] and animal models.[13,17–20] More recently, we have reported that the immunohistochemic expression of matrilysin is correlated with lymph node or distant metastasis in patients with colorectal cancer.[13] However, the prognostic significance of matrilysin in colorectal cancer has not been explored. Aside from its possible clinicopathologic and prognostic significance, matrilysin could be a target for anti-MMP therapy.[21] Inhibition of matrilysin by an antisense expression vector or antisense oligonucleotides has been demonstrated to suppress the *in vitro* invasive potential or *in vivo* metastatic potential of colon cancer cells.[14,16,19] Thus, it seems promising to assess the validity of matrilysin as a prognostic marker of colorectal cancers.

Based on these observations, we immunohistochemically analyzed specimens from 113 colorectal cancer patients who had undergone curative surgery.

MATERIAL AND METHODS

Patients and tissue samples

Tumor specimens were obtained from 113 Japanese patients with colorectal cancer who had undergone curative surgery in our university hospital or related hospitals from 1988–93. Each sample was fixed in formalin and embedded in paraffin wax. All of the tumors were adenocarcinoma, and the histopathologic and clinical features of the specimens were classified according to "the Japanese classification of colorectal carcinoma" and Dukes' stage. Informed consent was obtained from each patient and the study was conducted after Human Experimentation Review by the institutional committee.

Immunohistochemistry

Sections of 5 μm in thickness were dewaxed in xylene and rehydrated in alcohol, then heated to 105°C in an autoclave for 10 min. The endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 5 min. After being rinsed twice in PBS, the sections were treated for 18 hr with an antihuman matrilysin monoclonal antibody (141-7B2; Fuji Chemical, Toyama, Japan) at the concentration of 10 μg/ml. The characteristics of this antibody have been described previously.[13] An antiidiotypic monoclonal antibody AI-206 was used as a negative control. After washing 3 times in PBS, the sections were treated with biotinylated goat antimouse immunoglobulin (Dako, Glostrup, Denmark) for 10 min and then by horseradish peroxidase-avidin complex, diluted as recommended by the manufacturer, for 10 min. The slides were then washed in PBS and developed in 0.05 M tris-HCl (pH 7.5) containing 0.6 mg/ml 3-3′ diaminobenzidine at room temperature. The sections were counterstained in Mayer's hematoxylin and mounted. Immunostaining signals at the invasive front were scored in 2 sections each by 2 independent observers. The scores were calculated as the number of stained cells divided by the total number of carcinoma cells, as described previously.[22]

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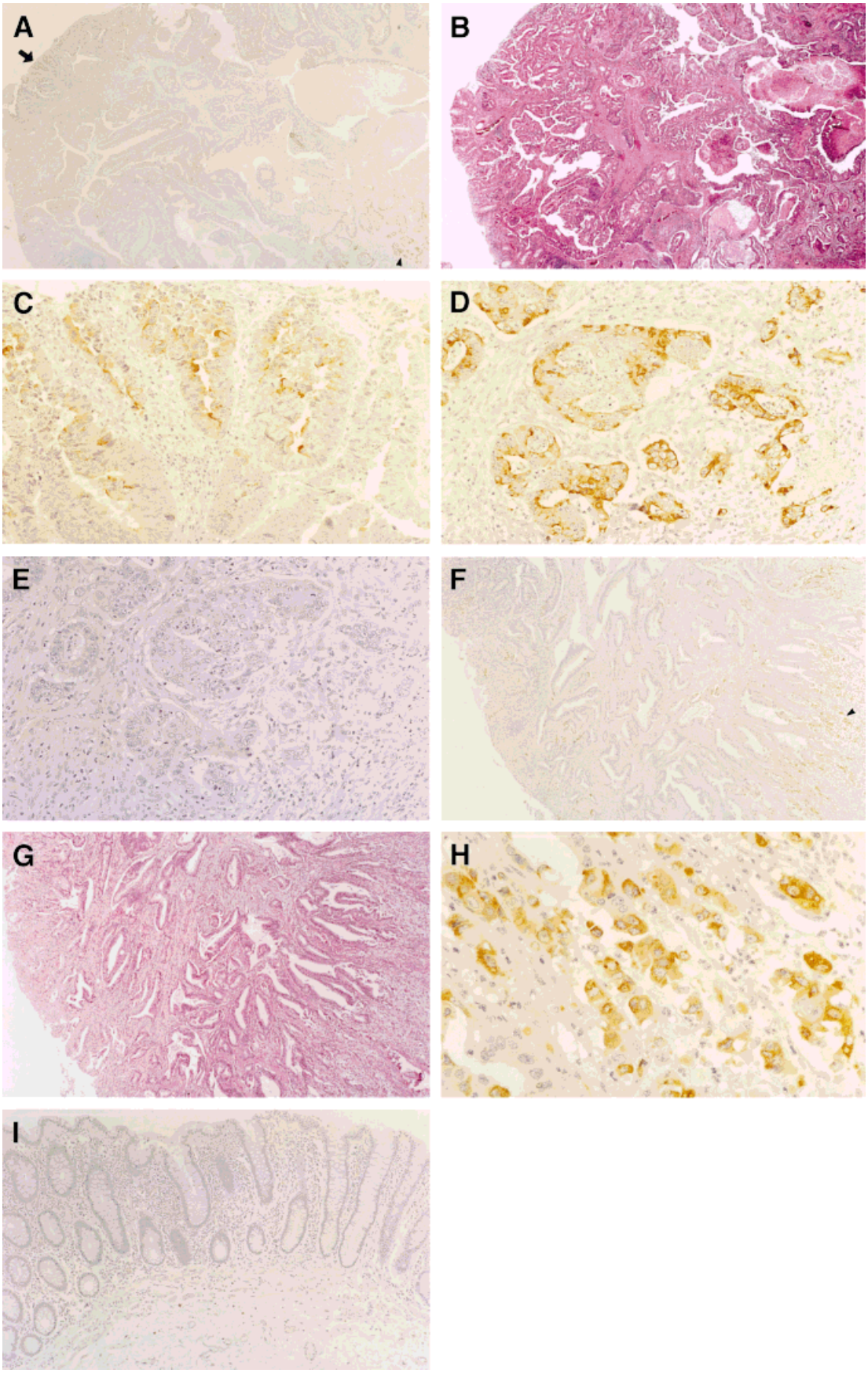

FIGURE 1 (Overleaf.)

Statistical analysis

Statistical significance of associations between matrilysin expression and clinicopathologic factors was determined by the χ^2 test or Mann-Whitney's *U*-test. Survival curves were constructed according to the method of Kaplan and Meier and compared by the log-rank test. Conditional logistic regression analysis was used for the analysis of confounding factors. *p*-values of less than 0.05 were considered significant.

RESULTS

Formalin-fixed sections of 113 resected primary colorectal cancers were immunohistochemically analyzed for matrilysin expression. The positively stained cancer cells were distributed heterogeneously in the tumor nests and were frequently located at the invasive front (Fig. 1*a*,*f*). The lumenal surface of neoplastic glands in the superficial layer was apically stained (Fig. 1*c*), while the cytoplasm of cancer cells at the invasive front was diffusely stained for matrilysin (Fig. 1*d*,*h*). There was no detectable immunoreactivity with the control AI-206 antibody (Fig. 1*e*). Stromal cells other than some monocytes were not stained (Fig. 1*i*). Sections with immunostaining signals in more than 30% of cancer cells at the invasive front, which were observed in 47 (42%) cases, were judged as being positive for matrilysin. We set the cutoff value as 30%, the lowest value that reflected clinicopathologic characteristics and patients' survival. Matrilysin positivity was significantly correlated with the depth of invasion ($p = 0.0087$), lymph node metastasis ($p = 0.0023$), lymphatic invasion ($p = 0.0052$), advanced Dukes' stage ($p = 0.0018$) and poor outcome ($p = 0.0003$) (Table I). Patients with matrilysin-positive cancer had a significantly shorter overall survival time than those with matrilysin-negative cancer ($p = 0.0004$, Fig. 2*a*).

The 5-year survival rates were 100% for patients with Tis (intraepithelial or invasion of the lamina propria) or T1 (tumor invading the submucosa), 55.6% for patients with T2 (tumor invading muscularis propria) or T3 (tumor invading through muscularis propria into subserosa or into nonperitonealized pericolic or perirectal tissues) and 23.8% for patients with T4 (tumor directly invading other organs or structures and/or perforating the visceral peritoneum) (Fig. 2*b*). The median follow-up times were 65.5, 46.5 and 28.4 months, respectively. Since the clinical outcome is obviously good for patients with T1 tumors and worse for patients with T4 tumors (Fig. 2*b*), we then focused on the intermediate invasive tumors (T2 or T3). A Kaplan-Meier life-table analysis of the survival rate of patients showed that the 5-year survival rate of patients with matrilysin-positive cancer was 21.8%, while it was 77.3% for patients with matrilysin-negative cancer (Fig. 2*c*). The median follow-up times were 32.2 and 54.9 months, respectively. The clinical outcome for patients with matrilysin-positive cancer was poorer than that for patients with matrilysin-negative cancer, and the former was similar to that for patients with T4 tumor (Fig. 2*c*). We then carried out conditional logistic regression analysis, employing eight factors as explanatory variables and the patient mortality rate 60 months after surgery as the outcome variable (Table II). Interestingly, only matrilysin was significant in this multivariate analysis ($p < 0.05$).

TABLE I – RELATIONSHIP BETWEEN CLINICOPATHOLOGIC CHARACTERISTICS AND MATRILYSIN EXPRESSION IN PATIENTS WITH COLORECTAL CANCER

Parameters		Matrilysin at the invasive front ($n = 113$)	
		Positive	*p*-value
Depth of invasion	Tis–T1	1/10	0.0087[1]
	T2–T3	22/59	
	T4	24/44	
Metastasis to lymph nodes	n(−)	17/60	0.0023[2]
	n(+)	30/53	
Lymphatic invasion	ly(−)	4/24	0.0052[2]
	ly(+)	43/89	
Venous invasion	v(−)	26/63	NS[3]
	v(+)	17/40	
Dukes' stage	A	2/22	0.0018[1]
	B	14/32	
	C	19/38	
	D	12/21	
Prognosis	Alive	13/54	0.0003[2]
	Dead	34/59	

[1]Mann-Whitney U-test.–[2]χ^2.–[3]Not significant.

DISCUSSION

Matrilysin-positive cancer cells showed a unique distribution in the tumor nests. First, the immunoreactivity was polarized to the lumenal surfaces of neoplastic glands in the superficial layer. This staining pattern is similar to that reported in several exocrine glands[23] and supports the notion that matrilysin may play a role in exocrine functions. MMPs reportedly activate lumenal or membrane-bound cytokines or growth factors, such as tumor necrosis factor α and heparin-binding epidermal growth factor, to locally perturb the growth of responsive cells.[24,25] Therefore, it is tempting to speculate that matrilysin may play a role in early colorectal carcinogenesis,[18,26] and this possibility warrants further study.

In contrast, cancer cells at the invasive front showed depolarized diffuse cytoplasmic staining. Because cancer cells at the invasive front are thought to represent the highest malignant potential, these results suggest that matrilysin at the invasive front plays a direct role in tumor progression. In support of this hypothesis, matrilysin expression at the invasive front was correlated with the more aggressive phenotype. With respect to the mechanism(s) underlying the depolarized matrilysin expression in cancer cells at the invasive front, genetic alterations inducing matrilysin expression and/or tumor-host interactions may play a key role.[27,28] In this regard, it is of interest that expression of matrilysin could be regulated by β-catenin,[29] and overexpression of β-catenin has often been found predominantly at the invasive front in colorectal cancer.[30]

The implication of the significance of matrilysin expression at the invasive front was further substantiated by its correlation with a poor clinical outcome for the patients with colorectal cancer. This was more significant for those with intermediate invasive cancer, and only matrilysin was a significant prognostic factor in multivariate analysis. These results further support the notion that matrilysin plays an important role in the progression of colorectal cancers and that matrilysin could become a reliable marker of cancer aggressiveness and poor prognosis, independently of the conventional clinicopathologic criteria. Immunohistochemical analysis of matrilysin expression would make it possible to iden-

FIGURE 1 (Overleaf.) – Immunohistochemical analysis of matrilysin in colorectal cancers. (*a–e*) Well-differentiated adenocarcinoma. (*a*) Heterogeneous distribution of matrilysin-positive cancer cells in the tumor nests. (*b*) Hematoxylin-eosin-stained section of (*a*). (*c*) High-magnification view of arrow in (*a*). Matrilysin expression in the lumenal surface of malignant ducts in the superficial layer. (*d*) High-magnification of arrowhead in (*a*). Depolarized diffuse expression of matrilysin in the tumor cells at the invasive front. (*e*) No detectable immunoreactivity with the control AI-206 antibody in the malignant cells in the serial section of (*d*). (*f–h*) Moderately differentiated adenocarcinoma. (*f*) Heterogeneous distribution of matrilysin-positive cancer cells in the tumor nests. (*g*) Hematoxylin-eosin stained section of (*f*). (*h*) High-magnification view of arrowhead in (*f*). Depolarized diffuse expression of matrilysin in the tumor cells at the invasive front. (*i*) Normal epithelial cells adjacent to the cancer nests were negative for matrilysin. Original magnification ×20 (*a*,*b*), ×40 (*f*,*g*), ×100 (*i*), ×200 (*c–e*), ×400 (*h*).

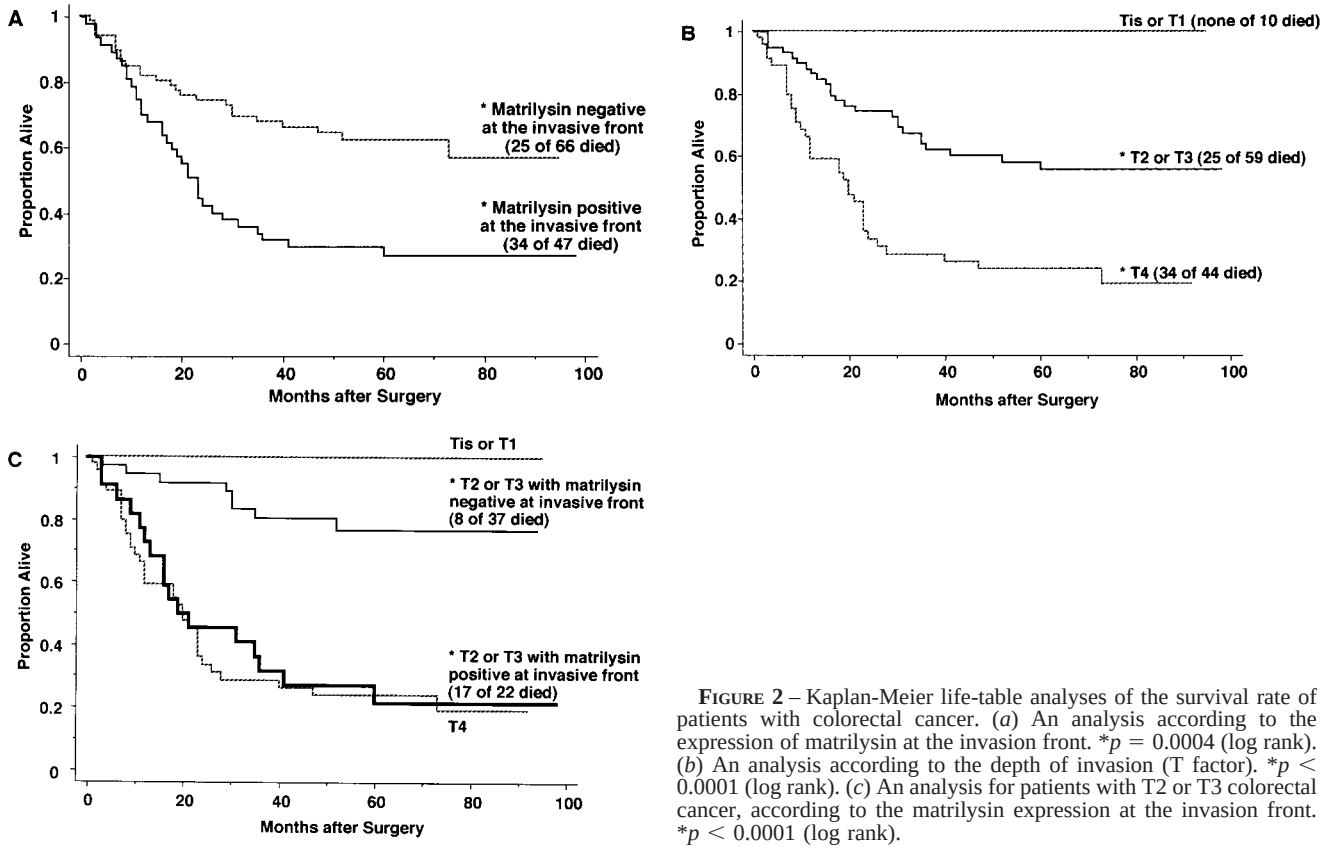

FIGURE 2 – Kaplan-Meier life-table analyses of the survival rate of patients with colorectal cancer. (*a*) An analysis according to the expression of matrilysin at the invasion front. $*p = 0.0004$ (log rank). (*b*) An analysis according to the depth of invasion (T factor). $*p < 0.0001$ (log rank). (*c*) An analysis for patients with T2 or T3 colorectal cancer, according to the matrilysin expression at the invasion front. $*p < 0.0001$ (log rank).

TABLE II – CONDITIONAL LOGISTIC REGRESSION ANALYSIS FOR PATIENTS WITH T2 OR T3 COLORECTAL CANCER

Factor	Coefficient	Standard error	t
Age	−0.06	0.07	−0.86
Gender	1.24	1.47	0.84
Lymphatic invasion	−2.17	1.41	−1.54
Venous invasion	−0.86	1.14	−0.75
Metastasis to lymph nodes	−1.93	3.90	−0.49
Histological stage	3.27	6.08	0.54
Dukes' stage	2.39	3.88	0.62
Matrilysin	3.86	1.97	1.96[1]

[1] $p < 0.05$.

tify high-risk patients more precisely and to choose an appropriate postoperative follow-up schedule.

The use of matrilysin as a molecular marker may become a routine part of the management of patients with colorectal cancer, thus helping to define appropriate therapeutic and follow-up protocols. It is important that matrilysin could be a target of anti-MMP therapy.[21] Some synthetic MMP inhibitors have been proven to be of therapeutic significance in clinical trials. However, currently ongoing clinical trials are not based on the MMP expression in given tumors. For a specific selection of patients who would benefit from those therapies, examination of MMP expression will be necessary in the near future. In this regard, immunohistochemic analysis of matrilysin could form the basis of a new therapeutic strategy via a broad or selective MMP inhibitor.

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