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Prognostic Impact of Tissue Inhibitor of Matrix Metalloproteinase- 1 in Plasma of Patients with Colorectal Cancer

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Abstract. *Background: Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in plasma has been reported to be related to disease progression in patients with colorectal cancer. However, the prognostic significance of plasma TIMP-1 has not been clarified. Patients and Methods: Concentrations of TIMP-1 protein were measured by enzyme-linked immunosorbent assay in plasma samples of 87 preoperative patients who subsequently underwent resection, and prognosis was compared. The cut-off value of plasma TIMP-1 was defined as 170 ng/ml. Results: When clinicopathological factors between patients with positive and those with negative plasma TIMP-1 were analyzed, significant differences were observed in lymph node metastasis, serosal invasion, curability and Dukes' classification. Univariate analysis of these factors demonstrated that depth of invasion, metastases to lymph nodes, peritoneum, liver and distant organ, lymphatic and vessel invasions, curability, Dukes' classification and plasma TIMP-1 concentration were significant. By multivariate analysis excluding patients with distant or peritoneal metastases, histological type, lymphatic invasions, lymph node metastasis and plasma TIMP-1 were retained in the final model. Conclusion: These results suggested that plasma TIMP-1 may be a useful prognostic marker for survival in patients with colorectal carcinoma.*

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The activities of both matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) play a significant role in invasion and metastases of cancer cells(1,2). TIMPs act as negative regulators of the degradation process (1), and it has been reported that their expression parallels that of MMPs (3). Among TIMPs, TIMP-1 has strong inhibitory effects on various types of MMPs (4). Several studies have reported an additional function of TIMP-1 as a growth-stimulating factor for normal and malignant cells (5).

Recently, the clinical significance of TIMP-1 in colon cancers has been examined. Zeng demonstrated a correlation between TIMP-1 mRNA level and advanced stage (6). Holten-Andersen documented that plasma TIMP-1 was higher in patients with advanced disease than that of healthy controls (7). Recently, we examined TIMP-1 protein concentrations in plasma of patients with colorectal carcinoma and found a correlation between elevated plasma TIMP-1 and tumor invasiveness and metastasis (8). To further clarify the clinical significance of plasma TIMP-1 in patients with colorectal cancer, we examined the prognostic value of plasma TIMP-1 by uni- and multivariate analyses in this study.

Patients and Methods

Patients. Peripheral blood samples were collected from 87 colorectal cancer patients at the Kanagawa Cancer Center, Japan, between June 1999 and July 2000. All the patients underwent surgical resection and all samples were obtained prior to treatment. Clinical and pathological classifications followed the General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum, and Anus in Japan (9). Metastases to liver, lymph node and peritoneum were diagnosed by computed tomography (CT) or

Table I. *Patients' backgrounds.*

	TIMP-1 negative(n=42)	TIMP-1 positive(n=45)	*P*-value**
Sex(male/female)	25/17	29/16	0.636
Age(years)	63.17±2.02	65.4±1.44	0.365
*Macroscopic type**			
Type 0(early cancer)	4	0	0.214
Type 1	8	6	
Type 2	26	32	
Type 3	3	6	
Type 5	1	1	
Histological type			
Well-differentiated	20	16	0.254
moderately-and poorly-differentiated	22	29	
Lymph node metastasis			
Negative	23	13	0.014
Positive	19	32	
Peritoneal metastasis			
Negative	41	43	0.598
Positive	1	2	
Liver metastasis			
Negative	38	36	0.171
Positive	4	9	
Serosal invasion			
Negative	19	9	0.012
Positive	23	36	
Lymphatic invasion			
Negative	29	23	0.088
Positive	13	22	
Venous invasion			
Negative	14	7	0.053
Positive	28	38	
Curability			
Curative resection	40	34	0.010
Non-curative resection	2	11	
Stage			
I	9	2	0.103
II	11	9	
IIIA	9	14	
IIIB	7	8	
IV	6	12	
Dukes' clasification			
Dukes' A or B	22	11	0.007
Dukes' C	20	34	

*Pathological measures according to Japanese Research Society for Cancer of the Colon and Rectum General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum, Anus in Japan, 1998
**Comparisons between the two group were performed by unpaired Student's *t*-test and by Chi-square method

during surgery with confirmation by pathological examination of biopsied specimens.

Measurement of TIMP-1. Plasma TIMP-l was measured by a commercial, one-step sandwich enzyme-linked immuno-sorbent assay (ELISA) kit (Fuji Chemical, Takaoka, Japan) (10). For the assay, 5 μl of properly diluted sample or serially diluted human TIMP-1 (standard) and 75 μl of peroxidase-conjugated anti-human TIMP-1 antibody were added to wells of a microtiter plate. After a 30-min incubation at 30˚C, the wells were washed with 0.05% Tween 20 in phosphate-buffered saline (PBS) for effective washing. Bound complexes were then incubated with o-phenylenediamine containing H_2O_2 for 15 min at 30˚C. The reaction was stopped by adding 1 M H_2SO_4 and the absorbance at 492 nm was measured with microtiter-plate reader. The limit of quantitation for the assay was 51 ng/ml, and a linear response was seen from 51 to 2000 ng/ml, with a coefficient of variation less than 5.0%.

Statistical analysis. Comparisons between the two groups were examined by paired and unpaired Student's *t*-test and by the Chi-square method. For more than three groups, one-way analysis of variance and Duncan's multiple range test were used. Univariate and multivariate survival analyses were performed by Cox's proportional hazard model. Survival curves were calculated by the Kaplan-Meier method and compared by the log-rank test. $P<0.05$ was considered statistically significant and data were expressed as mean ± standard deviation (SD).

Results

Plasma TIMP-1 levels were 126.2±33.7 in the 5 patients with early colorectal carcinoma and 183.5±82.6 in the 82 patients with advanced disease. We defined the cut-off value of plasma TIMP-1 concentrations as 170.0 ng/ml, because all the patients with early disease had a value less than 170.0 ng/ml (ranging from 68.0 ng/ml to 166.4 ng/ml).

Using this cut-off value, we found positive plasma TIMP-1 concentrations in 45 of the 87 patients (51.7%). When clinicopathological factors between patients with positive and those with negative plasma TIMP-1 concentrations were analyzed, significant differences were observed in serosal invasion, lymph node metastasis, lymphatic invasion, venous invasion, curability and Dukes' classification (Table I).

Univariate analysis of these factors demonstrated that depth of invasion, metastases to the lymph nodes, peritoneum, liver, and distant organ, lymphatic and vessel invasions, curability, Dukes' classification and plasma TIMP-1 concentration were significant (Table II). Kaplan-Meier survival curves were significantly different between patients with positive and those with negative plasma TIMP-1 (Figure 1). By multivariate analysis, metastases to

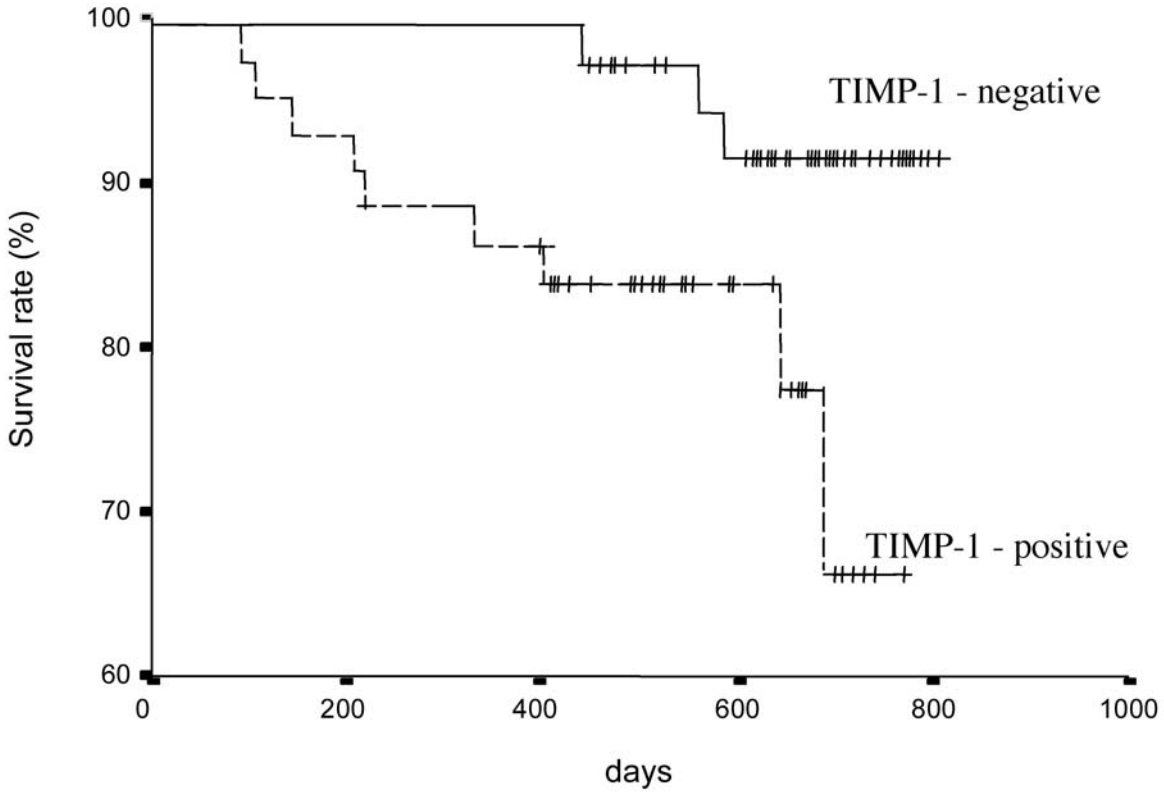

Figure 1. *Survival curves in patients with negative or positive plasma TIMP-1.*

Table II. *Univariate analysis of clinicopathological factors.*

Factors		*p*-value	Hazard ratio
Sex	male *vs*. female	0.3921	0.73
Age	-59 *vs.* 60-	0.4377	0.60
Histological type	well-*vs*. moderately- and poorly- differentiated	0.0548	3.69
Depth of invasion	-ss *vs.* se-	0.0116	6.36
Lymph node metastasis	negative *vs.* positive	0.0079	7.05
Peritoneal metastasis	negative *vs.* positive	0.0003	12.80
Liver metastasis	negative *vs.* positive	0.0000	45.79
Metastasis of distant organ	negative *vs.* positive	0.0000	16.78
Lymphatic invasion	negative *vs.* positive	0.0307	4.67
Venous invasion	negative *vs.* positive	0.0454	4.00
Curability	curative *vs.* non-curative	0.0000	43.10
Dukes' classification	A or B *vs.* C	0.0156	5.84
Plasma TIMP-1	negative *vs.* positive	0.0259	4.96

the peritoneum and liver were significant factors (Table III). By multivariate analysis excluding patients with distant or peritoneal metastases, histological type, lymphatic invasions, lymph node metastasis and plasma TIMP-1 were retained in the final model (Table IV).

Discussion

The various matrix metalloproteinases (MMPs), such as MMP-1, -2, -3, -7 and -9, and MT-MMP-1 and –2, play a dominant role in invasion and metastases by the tumor

Table IV. *Multivariate analysis of clinicopathological factors without distant and peritoneal metastases.*

Factors		*p*-value	Hazard ratio
Histological type	well- *vs.* moderately- and poorly- differentiated	0.311	5.142
Lymph node metastasis	negative *vs.* positive	0.909	0.855
Lymphatic invasion	negative *vs.* positive	0.231	7.394
Plasma TIMP-1	negative *vs*. positive	0.221	5.844

Table IV. *Multivariate analysis of clinicopathological factors without distant and peritoneal metastases.*

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(1,2). When MMPs are activated, specific TIMPs are induced in stromal cells to control the degradation process (11). Of known TIMPs, TIMP-1 has strong inhibitory effects on many MMPs (4). Recently, TIMP-1 expression has been reported to correlate with disease progression in patients with colorectal carcinoma (12). Moreover, plasma TIMP-1 levels were significantly higher in patients with far advanced colorectal cancer than those in healthy controls (7). More recently, we have clearly demonstrated that plasma concentrations of TIMP-1 were associated with tumour progression (13). To further clarify the clinical role of plasma TIMP-1 in patients with colorectal carcinoma, we examined its prognostic value by both univariate and multivariate analyses.

A cut-off value for plasma TIMP-1 was determined based on the value found in patients with early colorectal carcinoma. TIMP-1 would be less likely to be induced in early colorectal cancer because degradation reactions are thought to be minimum in early disease. In fact, Nomura *et al.* demonstrated that MMP-2 was not elevated in early gastric cancer (14). Also no change in TIMP-1 mRNA in the early disease has been confirmed (15). In this study, the cut-off value we set separated patients with good and bad prognosis. Interestingly, positive TIMP-1 strongly correlated with Dukes' stage ($p=0.007$), but not with metastases to liver ($p=0.171$) and peritoneum ($p=0.598$). TIMP-1 might reflect not the specific metastases but the total amount of degradation reactions.

It has been generally accepted that the most important factors affecting survival are depth of tumor invasion (T), extent of lymph node metastasis (N) and presence of distant metastases (M) (9,16). The significance of T and N for TNM classification was calculated from a subset of patients without distant metastases (17). So far, a variety of new biomarkers including oncogenes, tumor-suppressor genes, angiogenic factors and proteinases have been examined and discussed in relation to tumor progression and patient survival. However, none of them have proven superior to the conventional pathological markers (T, N and M) in determining prognoses. In our results, metastases to the liver and the peritoneum are the significant independent prognosticators for survival in patients with colorectal carcinoma. When analyzing only patients without distant metastases, TIMP-1 became an independent prognostic factor for survival. Although its *p* value did not reach statistical significance, its prognostic value was superior to lymph node metastasis and serosal invasion.

Thus, plasma TIMP-1 concentration could be a significant prognostic marker for survival in patients with colorectal carcinoma.

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