ONCOLOGY

Comparative Enzyme Immunoassay of Matrix Metalloproteinases-2, -7, -9 and Their Tissue Inhibitor-2 in Tumors and Plasma of Patients with Gastric Cancer

E. S. Gerstein, Li Sini, A. B. Ryabov, E. K. Dvorova, A. A. Yurchenko, I. S. Stilidi, N. E. Kushlinskii, and M. I. Davydov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 12, pp. 660-663, December, 2009
Original article submitted August 6, 2009

The content of matrix metalloproteinases (MMP) -2, -7, and -9 was significantly higher in tumors in comparison with the adjacent histologically intact gastric mucosa in 80, 70, and 72% patients with gastric cancer, respectively, the increase in the level of MMP tissue inhibitor-2 (TIMP-2) detected in 61% tumors was insignificant. Only plasma level of MMP-7 was elevated in primary patients in comparison with the control and positively correlated with the expression of this protein in the tumor. The concentration of MMP-7 was maximum in the blood of patients with tumor invasion in lymph vessels. These data suggest MMP-7 as a possible serological marker of gastric cancer.

Key Words: *matrix metalloproteinase-2, matrix metalloproteinase-7, matrix metalloproteinase-9; matrix metalloproteinase tissue inhibitor-2; gastric cancer*

The capacity of invasion into adjacent tissues and metastasizing into distant organs is a basic property of malignant tumors. The most important mechanism of these processes is destruction of the adjacent basal membrane and extracellular matrix (ECM) by tumor-associated proteases. Several classes of proteases are involved in invasion and metastasizing, the most important of them being the multigenic family of matrix metalloproteinases (MMP) or matrixines, called so due to their capacity to hydrolyze specifically all main extracellular matrix proteins, primarily collagen [3]. In addition, MMP are involved in the regulation of tumor angiogenesis. The MMP family consists of more than 20 secreted or cell surface-related zinc-dependent endopeptidases. Their substrates are, in addition to the majority of ECM components, other proteases, chemotaxic molecules, latent forms of growth factors, soluble and membrane-associated proteins binding the growth factors [7,12]. Activities of MMP in the extracellular space is specifically inhibited by tissue inhibitors (TIMP), structurally related proteins; three of these inhibitors (TIMP-1, -2, and -4) are secreted in the soluble form and one (TIMP-3) is bound to ECM [8].

Elevated expression in tumors of different origin was demonstrated for many MMP. They are stimulated by the paracrine mechanism with involvement of growth factors and cytokines released by macrophages and lymphocytes infiltrating the tumor and by the tumor stroma cells [12]. Recent studies showed that the important independent role in the regulation of growth and differentiation of tumor and normal cells is played by TIMP, which are characterized by antiangiogenic effects [10]. Hence, various MMP and TIMP are now

N. N. Blokhin Cancer Research Center, Moscow, Russia. ***Address for correspondence:*** esgerstein@gmail.com. E. S. Gerstein

0007-4888/09/1486-0899

regarded as possible biological markers for disease prognosis and evaluation of drug sensitivity of malignant tumors, for example, gastric cancer [2,4,5,9,14], while the use of natural and synthetic inhibitors of MMP is regarded as a promising approach to drug therapy of tumors little sensitive to classical antitumor drugs [11,15].

We compared the levels of some representatives of the MMP family (MMP-2, -7, and -9) and one of their tissue inhibitors (TIMP-2) in tumors, histologically intact gastric mucosa, and blood plasma of patients with gastric cancer and analyzed the relationship between these parameters and the main clinical morphological features of the disease.

MATERIALS AND METHODS

The study was carried out in 89 primary patients with gastric cancer and 6 patients with disease relapse (50 men and 45 women aged 42-77 years, median 62 years). Control group consisted of 22 age- and sex-matched donors. The proteins were measured in the plasma, obtained by standard method before specific therapy, and in extracts of tumors and histologically intact gastric mucosa of 67 patients with operable gastric cancer.

Extracts of tumor and intact tissues of the stomach for EIA were prepared as described previously [1]. The markers were measured using standard kits for direct EIA: Human/Mouse/Rat MMP-2 (total), Human MMP-7 (total), Human MMP-9 (total), and Human TIMP-2 (Quantikine®, R&D Systems) according to the instruction. The measurements were carried out on an EL_X800 automated universal reader for microplates (Bio-Tek Instruments Inc.). Plasma concentration of the studied parameters was expressed in ng/ml, tissue concentrations in ng/mg total protein, measured by the method of Lowry.

Comparison and analysis of relationships between the parameters were carried out using Student *t* test, Mann—Whitney test, paired Wilcoxon test, and Spearman rank correlation test (*R*). The data were processed using Statistica 6.0 software (StatSoft Inc.).

RESULTS

Measurable levels of MMP-2 were found in all studied samples of gastric cancer and histologically intact mucosa. In 66 of 67 patients (98.5%), MMP-9 was detected in the tumor and mucosa, TIMP-2 was not detected in only one specimen of gastric cancer, while MMP-7 was detected in 75% tumors and 40% specimens of normal mucosa. The levels of MMP-2, -7, and -9 were significantly higher than in histologically intact tissue in 80, 70, and 72% patients, respectively (Table 1). The level of TIMP-2 was elevated in 61% patients, the mean values and medians of this parameters did not significantly differ in the tumor and intact mucosa (Table 1). A significant positive correlation between the values in the tumors and histologically intact tissues was detected for three markers: MMP-7 (R=0.39; p=0.0012), MMP-9 (R=0.38; p=0.0024), and TIMP-2 (R=0.35; p=0.028). In addition, the levels of MMP-2 and TIMP-2 formed a positive correlation in the tumor (R=0.58; p<0.0001) and in intact gastric tissue (R=0.59; p<0.0001).

It is practically important to know to what measure changes in MMP and TIMP production in tu-

TABLE 1. Content of MMP-2, -7, and -9 and TIMP-2 (ng/mg protein) in Tumors and Histologically Intact Gastric Mucosa of 67 Patients

Parameter	Tumor (T)		Mucosa (N)		T<N, % patients
	M±*m*	median	*M*±*m*	median	
MMP-2	36.8±3.2	32.6* (4.4-127)	23.3±3.8	15.9 (4.0-249)	80
MMP-7	2.4±0.5	1.1* (0-16.6)	0.3±0.1	0 (0-4.1)	70
MMP-9	246.0±41.3	150** (0-2000)	104.0±14.1	77.9 (0-563)	72
TIMP-2	23.2±2.6	18.8 (0-100)	20.1±1.9	16.7 (5.4-56.3)	61

Note. Range of values is shown in parentheses. *p<0.0001, **p<0.01 compared to intact mucosa (paired Wilcoxon test).

TABLE 2. Plasma Concentrations of MMP-2, -7, -9, and TIMP-2 (ng/ml) in Healthy Individuals and Patients with Gastric Cancer

Parameter	Gastric cancer				Control group	
	primary		relapse			
	M±*m*	median	*M*±*m*	median	*M*±*m*	median
MMP-2	271.0±8.6	268 (105-576)	236.0±16.7	228 (197-289)	282.0±22.8	281 (161-370)
MMP-7	3.5±0.4	2.9* (0-25.4)	1.6±0.2	1.6 (0.9-2.4)	1.6±0.4	1.2 (0-6.9)
MMP-9	178.0±13.1	167* (0-626)	155.0±50.3	141 (19.4-331)	279.0±41.4	267 (50.4-647)
TIMP-2	77.1±4.1	71.5 (23.5-131)	68.1	68.1	74.2±10.8	74.5 (39.6-114)

Note. Range is shown in parentheses. *$p<0.01$ compared to the control group.

mor tissue are reflected by their concentrations in the peripheral blood; if these parameters correlate, it is possible to evaluate the metastatic and invasive potential of the tumor without surgical intervention. The concentrations of these proteins were measured in the plasma of patients with gastric cancer and normal controls (Table 2). The level of MMP-7 was significantly higher in patients with primary gastric cancer in comparison with the control group ($p<0.01$). However, in only 19 of 89 patients (21%) this parameter surpassed the arbitrary upper threshold of normal (4.1 ng/ml, the level observed in 90% healthy subjects). Plasma level of MMP-7 in patients with cancer relapses virtually did not differ from the control. On the other hand, MMP-9 level in patients with gastric cancer (with primary disease and relapses) was almost 2-fold reduced in comparison with the control ($p<0.01$). This result disagrees with the previous data [14] indicating increased plasma levels of this parameter and its clinical significance in gastric cancer, but is in line with our previous data on colorectal cancer [1].

A significant positive correlation between the tumor and circulating concentrations was detected only for MMP-7 ($R=0.38$; $p=0.0017$). Similarly as in tissues, plasma concentrations of MMP-2 and TIMP-2 formed a significant positive correlation ($R=0.41$; $p=0.004$).

In order to evaluate clinical significance of measuring these markers in the tumors and peripheral blood of patients with gastric cancer, we analyzed their relationships with the main clinical morphological characteristics of the disease: primary tumor location, growth type, histological structure, size, and dissemination (T), involvement of regional lymph nodes (N), presence of distant metastases (M) and their location. No significant associations with the majority of prognostic factors were detected, except increased plasma concentration of MMP-7 in patients with tumor invasion in lymph vessels (5.6±1.1 ng/mg protein) in comparison with patients without invasion of this kind (3.0±0.3 ng/mg protein; $p<0.05$). In addition, significant increase in the levels of MMP-2 and TIMP-2 in tumor tissue was associated with an increase of N index (involvement of regional lymph nodes). The level of MMP-2 in cases without metastases into lymph nodes (N_0) was 37.8±5.3 ng/mg protein, with metastases at a distance <3 cm from the primary tumor (N_1) 26.1±3.0 ng/mg protein (the difference between these groups being negligible), and with metastases in more distant lymph nodes (N_2) 53.9±7.8 ng/mg protein ($p<0.05$ compared to values in two other groups). For TIMP-2 these values were 20.1±2.5 (N_0), 21.0±2.9 (N_1), and 37.1±11.2 (N_2) ng/mg protein ($p<0.05$ *vs.* N_0 and N_1).

Hence, expression of three representatives of the MMP family (MMP-2 collagenase/gelatinase, MMP-9 gelatinase, and MMP-9 matrilysin) in the tumors was elevated significantly in comparison with histologically intact tissue in the majority of gastric cancer patients. Only MMP-2 level correlated with the intensity of regional lymph node involvement. These results are in line with the data obtained by different methods, indicating increased expression of MMP in gastric cancer tissue and the absence of significant clinical morphological correlations [9,13]. It is noteworthy that

despite the absence of relationship with the classical prognostic factors, an important role of some MMP, for example, MMP-2, for prediction of the survival of gastric cancer patients was demonstrated [5,6].

On the other hand, the only of the studied markers, whose plasma level was elevated in primary patients with gastric cancer, was MMP-7. Its elevation was maximum in patients with tumor invasion in the lymph nodes. In addition, MMP-7 concentration in the peripheral blood positively correlated with expression of this protein in the tumor. Hence, this metalloproteinase can be regarded as a possible serological marker for evaluating dissemination and monitoring of gastric cancer. MMP-2 seems to be the most prospective tissue marker; its level is increased in tumors of 80% patients and it correlates with the process dissemination in the lymph system.

REFERENCES

1. E. S. Gerstein, E. A. Korotkova, A. M. Shcherbakov, *et al.*, *Byull. Eksp. Biol. Med.*, **143**, No. 3, 438-441 (2007).
2. H. Ajisaka, Y. Yonemura, and K. Miwa, *Hepatogastroenterology*, **51**, No. 57, 900-905 (2004).
3. E. I. Deryugina and J. P. Quigley, *Cancer Metastasis Rev.*, **25**, No. 1, 9-34 (2006).
4. A. Elnemr, Y. Yonemura, E. Bandou, *et al.*, *Gastric Cancer*, **6**, No. 1, 30-38 (2003).
5. F. J. Kubben, C. F. Sier, W. van Duijn, *et al.*, *Br. J. Cancer*, **94**, No. 7, 1035-1040 (2006).
6. J. Mrena, J. P. Wiksten, S. Nordling, *et al.*, *J. Clin. Pathol.*, **59**, No. 6, 618-623 (2006).
7. A. R. Nelson, B. Fingleton, M. L. Rothenberg, and L. M. Matrisian, *J. Clin. Oncol.*, **18**, No. 5, 1135-1149 (2000).
8. N. Ramnath and P. J. Creaven, *Curr. Oncol. Rep.*, **6**, No. 2, 96-102 (2004).
9. K. N. Shim, S. A. Jung, Y. H. Joo, and K. Yoo, *J. Gastroenterol.*, **42**, No. 2, 120-128 (2007).
10. N. E. Sounni, M. Janssen, J. M. Foidart, and A. Noel, *Matrix Biol.*, **22**, No. 1, 55-61 (2003).
11. S. A. Watson, T. M. Morris, H. M. Collins, *et al.*, *Br. J. Cancer*, **81**, No. 1, 19-23 (1999).
12. J. Westermarck and V. M. Kahari, *FASEB J.*, **13**, No. 8, 781-792 (1999).
13. C. Y. Wu, M. S. Wu, Y. J. Chen, *et al.*, *Eur. J. Cancer*, **43**, No. 4, 799-808 (2007).
14. C. Y. Wu, M. S. Wu, E. P. Chiang, *et al.*, *Clin. Cancer Res.*, **13,** No. 7, 2054-2060 (2007).
15. Y. Yonemura, Y. Endo, H. Fujita, *et al.*, *J. Exp. Clin. Cancer Res.*, **20**, No. 2, 205-212 (2001).