Matrix Metalloproteinases 2, 3, 13 and Their Type 2 Tissue Inhibitor in Tumors and Plasma of Patients with Colorectal Cancer

E. S. Gershtein, E. A. Korotkova, V. V. Prorokov, and N. E. Kushlinsky

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Enzyme immunoassay studies revealed increased content of matrix metalloproteinases 2, 3 and 13 in tumors compared to the adjacent histologically unchanged mucosa in 70-90% patients with colorectal cancer, while the increase in the content of type 2 metalloproteinase tissue inhibitor did not reach the level of statistic significance. Plasma concentrations of these proteins did not correlate with the corresponding values in the tumors and did not surpass the normal levels, while their decrease after removal of the primary tumor was observed only in patients with initially high levels of this parameter.

Key Words: *matrix metalloproteinase 2; matrix metalloproteinase 3; matrix metalloproteinase 13; type 2 matrix metalloproteinase tissue inhibitor; colorectal cancer*

Destruction of the basal membrane surrounding the tumor, and extracellular matrix by tumor-associated proteases is one of the main biochemical mechanisms of invasion, metastases, and neoangiogenesis [3,6,14]. Several protease classes are involved in these processes, the most important of which is the multigene family of matrix metalloproteinases (MMP) or matrixins, called so due to their capacity to specifically hydrolyze all main proteins of extracellular matrix, primarily collagen. The MMP family includes more than 20 secreted or cell surface-bound zinc-dependent endopeptidases; their substrates can be, in addition to the majority of the extracellular matrix components, other proteases, chemotactic molecules, latent forms of growth factors, and soluble and membrane-associated growth factor-binding proteins. The major MMP subfamilies are collagenases, stromelysines, gelatinases, and matrilysines. MMP activity in the extracellular space is specifically suppressed by tissue inhibitors (TIMP), structurally related proteins, three of which (TIMP-1, -2, and -4) are released in the soluble forms and one (TIMP-3) is linked to the extracellular matrix [3,11].

Increased expression in tumors of different origin was demonstrated for many MMP, activation being realized through the paracrine mechanism with the involvement of growth factors and cytokines released by macrophages and lymphocytes infiltrating the tumor and by the tumor stroma cells [12]. Recent findings indicate that TIMP play an important independent role in the regulation of tumor and normal cell growth and differentiation; in addition, TIMP exhibited antiangiogenic effects [11]. Therefore, various MMP and TIMP are now regarded as possible biological predictors and markers of drug sensitivity of malignant tumors, for example, colorectal cancer (CRC) [7,10,12,13,15], while the use of natural and synthetic inhibitors of MMP is assumed to be a promising approach to antitumor therapy [9,10].

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** esgershtein@gmail.com. E. S. Gershtein

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Our previous comparative study employing the method of enzyme immunoassay [1] showed that the content of MMP-7 and TIMP-1 in colorectal tumors was significantly higher than in adjacent histologically unchanged mucosa, while MMP-9 and TIMP-4 levels in the tumors and mucosa were virtually the same. Plasma concentrations of these proteins did not correlate with the corresponding values in tumors and did not decrease after tumor removal in the majority of patients.

The aim of this study was to compare the levels of 4 representatives of the MMP family (MMP-2, -3, -13, and TIMP-2) in the tumors and histologically unchanged mucosa of CRC patients and in the plasma of these patients before and after surgery and to detect the most perspective biological markers of this disease.

MATERIALS AND METHODS

The study was carried out in 20 patients with stages III-IV CRC (8 men and 12 women aged 42-77 years, median 63 years). Control group consisted of 10 donors of the same age. In addition, 4 patients with colonic polyps were examined. Proteins were measured in extracts from colonic tumors, sites of histologically unchanged colonic mucosa, and plasma obtained by the standard method before and 5-27 days after surgery.

Tissue extracts for EIA were prepared as described previously [1]. Standard kits for direct EIA Human/Mouse/Rat MMP-2 (total) and Human TIMP-2 (Quantikine®, R&D Systems), Human MMP-13 ELISA, and Human MMP-3 Instant ELISA (Bender Medsystems) were used in accordance with manufacturer's instructions. The measurements were carried out on an EL_X800 automated universal microplate reader (Bio-Tek Instruments Inc.). The concentrations of proteins in tissues were expressed in ng/mg total protein measured by Lowry's method.

The relationships between the parameters were compared and analyzed using Student's *t* test, Mann—Whitney test, paired Wilcoxon's test, and Spearman's rank correlation test (*R*). The data were processed using Statistics 6.0 (StatSoft Inc.) and OriginPro 7.5 (OriginLab Corporation) software.

RESULTS

The studied proteins were detected in all analyzed specimens of CRC and unchanged colonic mucosa, their levels varying within a wide range (Table 1). The concentrations of MMP-2, -3, and -13 were significantly elevated in comparison with histologically unchanged tissue in 89, 70, and 90% patients, respectively. The level of TIMP-2 was increased in 69% patients, but the means and median values of this parameter in the tumor and unchanged mucosa virtually did not differ (Table 1). The content of MMP-3, -13, and TIMP-2 in benign colonic polyps virtually did not differ from the values in malignant tumors and normal mucosa, while the level of MMP-2 (0-12.1; median 3.7 ng/ml protein) was significantly lower ($p<0.01$).

No correlations between the levels of each protein in the tumor and adjacent tissue were detected. On the other hand, the levels of MMP-2 and -3 in the tumor and unchanged colonic mucosa significantly correlated ($R=0.62$; $p<0.05$ and $R=0.74$; $p<0.01$, respectively). A significant correlation between MMP-2 and TIMP-2 levels in tumor tissue was detected ($R=0.57$; $p<0.01$).

From a practical viewpoint, it is essential, to what measure the changes in MMP and TIMP production in tumor tissue are reflected by changes in their concentrations in the peripheral blood, as if these parameters correlate, the metastatic and invasive potential of the tumor can be evaluated without surgical intervention. The results of measurements of these proteins in the plasma of CRC pa-

TABLE 1. The Content (ng/mg protein) of MMP-2, -3, -13, and TIMP-2 in Tumors and Histologically Unchanged Mucosa of CRC Patients

Parameter	Tumor (T)		Mucosa (N)		T>N, %
	M±*m*	Median, range	*M*±*m*	Median, range	
MMP-2	40.9±7.4*	32.0^{+} (12.9-128)	20.4±3.9	17.7 (2.8-66.4)	89
MMP-3	15.1±1.9**	13.1^{+} (1.2-30.5)	8.1±1.8	6.7 (0.3-35.1)	70
MMP-13	1.30±0.06*	1.2^{++} (0.7-1.9)	0.80±0.04	0.8 (0.5-1.1)	90
TIMP-2	25.7±2.9	24.3 (9.5-45.7)	19.4±1.8	20.5 (5.4-31.2)	69

Note. *$p<0.01$, **$p<0.05$ compared to unchanged mucosa (Student's test); $^{+}p<0.05$ and $^{++}p<0.001$ compared to unchanged mucosa (paired Wilcoxon's test).

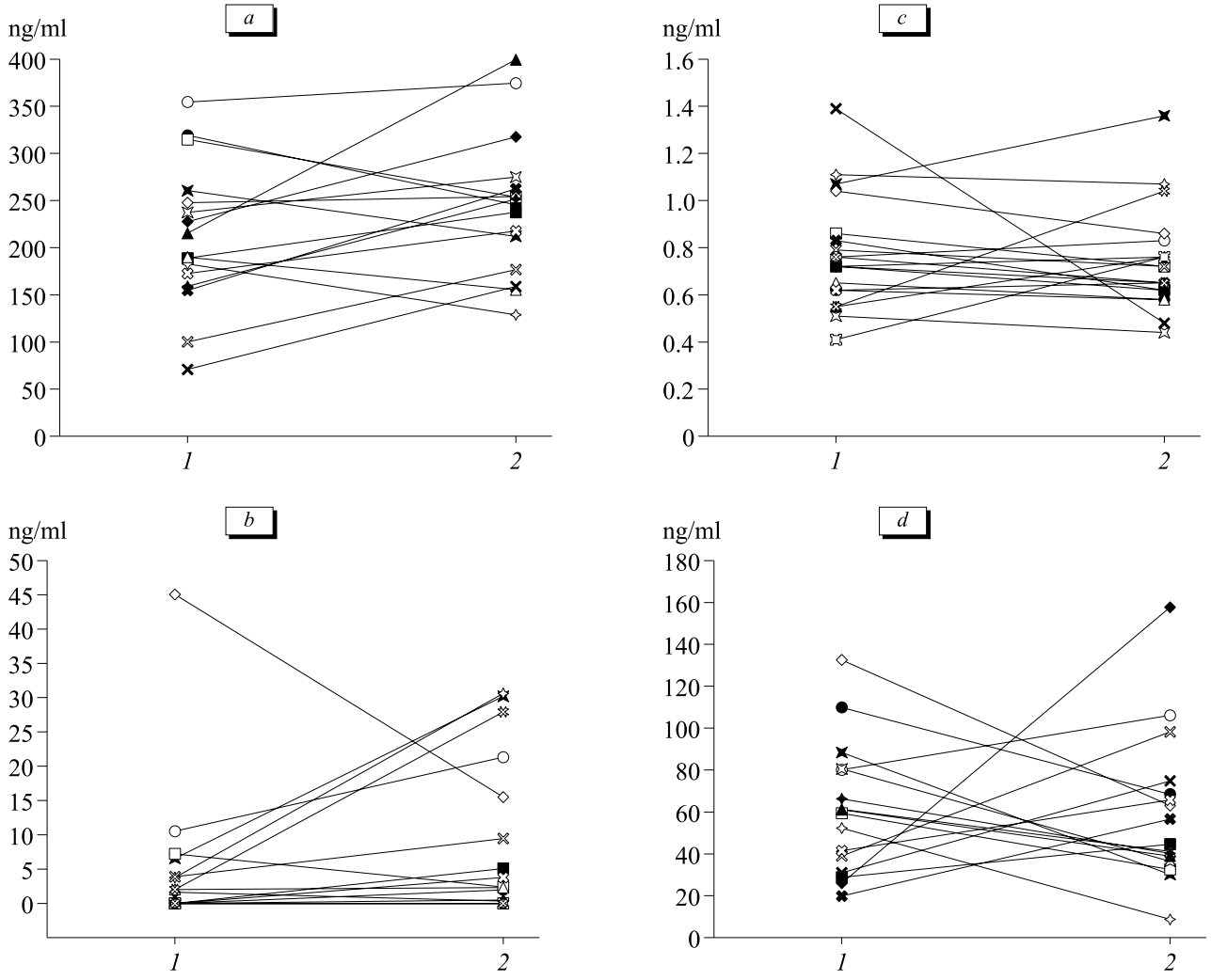

Fig. 1. Time course of plasma MMP-2 (*a*), -3 (*b*), -13 (*c*), and TIMP-2 concentrations (*d*) in CRC patients. Ordinate: protein concentration. *1*) parameter before surgery; *2*) parameter 5-27 days after surgery.

tients before specific therapy and after removal of the primary tumor and in control group are presented in Table 2. None of the parameters increased in comparison with the control. Plasma levels of the studied proteinases in patients with colonic polyps also did not differ from the normal. Significant positive correlations between plasma levels of MMP-3 and -13 in CRC patients before (R=0.50; p<0.05) and after surgery (R=0.69; p<0.001) and between the preoperative levels of MMP-2 and TIMP-2 (R=0.66; p<0.01) were detected. No significant relationship between the tumor and circula-

TABLE 2. Plasma Concentrations (ng/ml) of MMP-2, -3, -13, and TIMP-2 in Donors and CRC Patients before Therapy and 5-27 Days after Surgery

Parameter	Control group	CRC patients		Decrease, % of patients
		before surgery	after surgery	
MMP-2	274 (161-370)	202 (71-355)	248 (129-399)	31
MMP-3	25.5 (9.0-39.8)	0 (0-45.1)	2.3 (0-53.9)	16
MMP-13	0.9 (0-1.7)	0.7 (0.4-1.4)	0.7 (0.4-1.4)	63
TIMP-2	56.1 (39.6-111)	60.2 (19.9-132)	50.7 (8.7-158)	56

Note. The median and range of concentrations are presented in parentheses

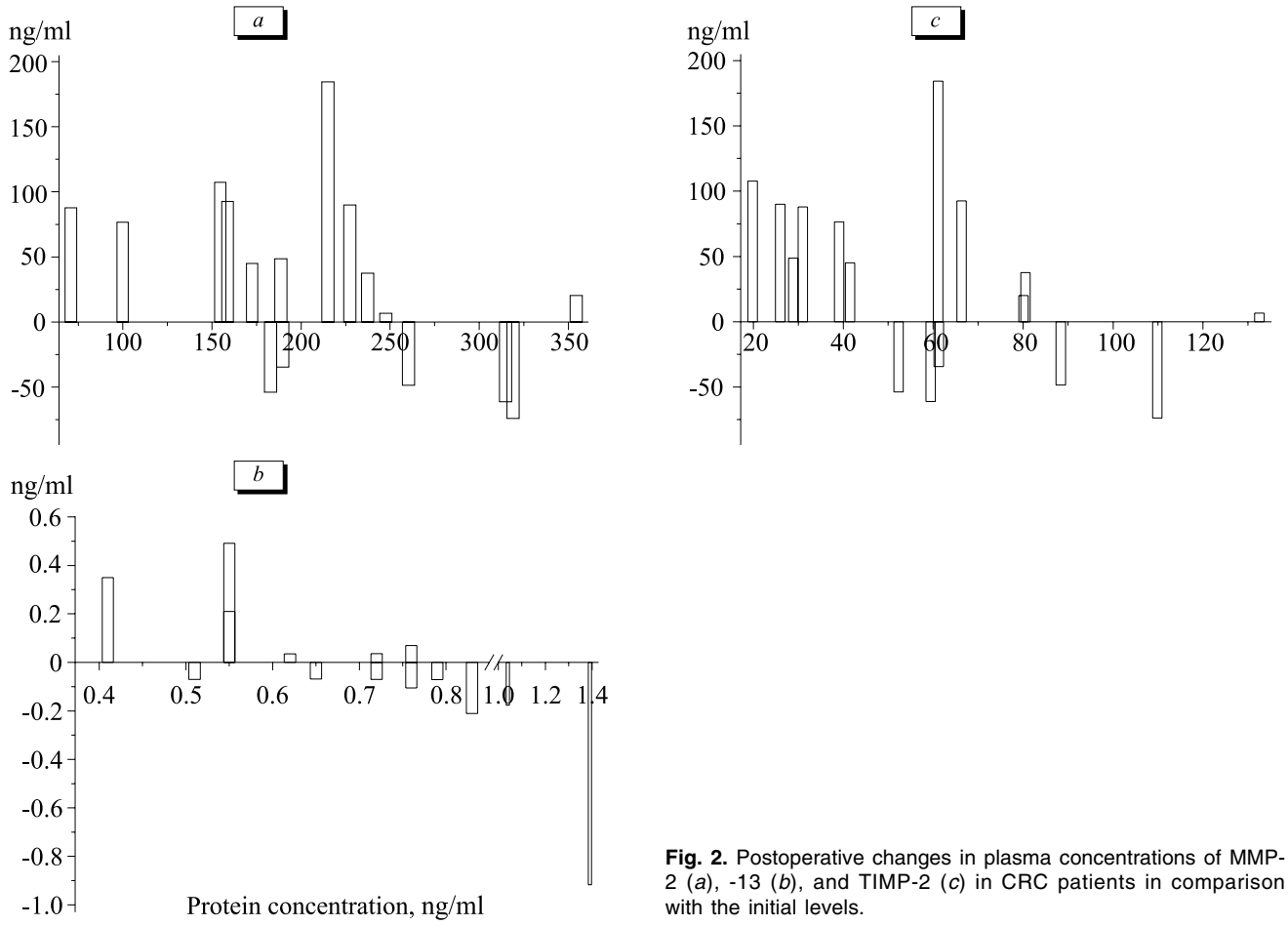

Fig. 2. Postoperative changes in plasma concentrations of MMP-2 (*a*), -13 (*b*), and TIMP-2 (*c*) in CRC patients in comparison with the initial levels.

ting concentrations were detected for any of the studied proteins.

Repeated analyses of the plasma 5-27 days after tumor removal showed opposite changes in the concentrations of the studied proteins (Fig. 1). The levels of MMP-3 and -13 increased after surgery in the majority of patients. No appreciable differences in the concentrations before and after surgery were detected (Table 2). No significant relationship between the direction and degree of changes and the interval between the studies were detected. An inverse correlation between postoperative shifts and the initial plasma concentrations of MMP-2, -13, and TIMP-2 (R=-0.60, R=-0.55, and R=-0.55, respectively, was detected; p<0.05): if the initial levels of these proteins were high, they more often decreased, while if they were low, their plasma concentrations increased (Fig. 2). No relationship between the values was detected for MMP-3.

Hence, a significant increase in the expression of three MMP, belonging to different subgroups: collagenase (MMP-13), gelatinase (MMP-2), and stromelysine (MMP-3) was detected in tumors of 70-90% examined patients with CRC. We previously demonstrated an increase in matrilysin (MMP-7) level in CRC tissue [1] and high expression of MMP-2 in the tumors of CRC patients with metastases [2]. Each of these parameters can be regarded as a candidate biomarker of CRC, characterizing its invasive and metastatic potential, and as the target for molecular directed therapy of the disease. Further clinical and morphological studies are needed for detecting the most perspective tissue markers of this group.

Unfortunately, no significant correlation between tissue expression and plasma concentration was detected for any of the studied ensymes. Moreover, no appreciable increase in their blood levels in comparison with the control or its significant reduction after removal of the primary tumor was detected. The latter result is in line with the findings of other authors, demonstrating elevated contents of some MMP and TIMP in the blood of CRC patients during early periods (up to 30 days) after removal of the primary tumor [4,8]. Hence, at present we can hardly regard the concentrations of MMP or their inhibitors as perspective markers for the diagnosis or monitoring of CRC. On the other

hand, the feedback between the initial level, direction, and degree of postoperative changes in plasma MMP concentrations prompts further studies of their possible use for monitoring of patients with initially high values and for longer follow-up.

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