Matrix Metalloproteinases 7 and 9 and Their Types 1 and 4 Tissue Inhibitors in Tumors and Plasma of Patients with Colorectal Cancer

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Enzyme immunoassays showed significantly elevated content of matrix metalloproteinase 7 and type 1 tissue inhibitor of metalloproteinases in tumors compared to adjacent histologically unchanged mucosa of patients with colorectal cancer; the levels of metalloproteinase 9 and type 4 tissue inhibitor of metalloproteinases were virtually the same in the tumors and mucosa. Plasma concentrations of the studied proteins did not correlate with their levels in the tumor, did not surpass the normal, and did not decease after removal of the primary tumor in the majority of patients.

Key Words: *matrix metalloproteinase 7; matrix metalloproteinase 9; type 1 tissue inhibitor of matrix metalloproteinases; type 4 tissue inhibitor of matrix metalloproteinases; colorectal cancer*

Destruction of the basal membrane and extracellular matrix (ECM) by tumor-associated proteases playing also an important role in the metastasizing and neoangiogenesis is one of the main mechanisms of malignant tumor invasion [1,2,13,14]. Several classes of proteases are involved in tumor invasion; of particular importance is the group of matrix metalloproteinases (MMP) or matrixines, called so due to their capacity to specifically hydrolyze all main proteins of ECM and primarily collagen [2]. MMP are a multigenic family including more than 20 secreted cell surface-associated zinc-dependent endopeptidases [2,14]. In addition to the majority of ECM components, other proteases, chemotactic molecules, latent forms of growth factors, growth factor-binding soluble and membrane-associated proteins can serve as MMP substrates [9]. Important representatives of MMP are collagenases (MMP-1, MMP-8, and MMP-13), stromelysins (MMP-3 and MMP-10), matrilysin (MMP-7), and gelatinases A and B (MMP-2 and MMP-9). Activity of MMP in the intercellular space is specifically suppressed by tissue inhibitors (TIMP), four structurally related proteins, three of which (TIMP-1, TIMP-2, and TIMP-4) are secreted in a soluble form and one (TIMP-3) is associated with ECM [2,11].

Increased expression in tumors of different genesis was demonstrated for many MMP. Activation of this expression is realized by the paracrine mechanism with participation of growth factors and cytokines secreted by macrophages and lymphocytes infiltrating the tumor and by tumor stromal cells [5,7,12]. Recent findings indicate that TIMP play an important role in the regulation of growth and differentiation of tumor and normal cells and exhibit antiangiogenic properties [11]. Different MMP and TIMP are therefore regarded as posible biomarkers of malignant tumor prognosis and drug sensitivity, for example, for colorectal cancer [6,7, 12], while the use of natural and synthetic MMP

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inhibitors is considered as a prospective approach of antitumor therapy [3,6,8,10].

All this determined the aim of our study: to compare the levels of some representatives of MMP family (MMP-7 and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-4) in tumors and histologically unchanged mucosa of patients with colorectal cancer and in plasma of these patients before and after surgery.

MATERIALS AND METHODS

The study included 20 patients with stages III-IV colorectal cancer (8 men and 12 women aged 42-77 years, median 63 years). Control group consisted of 10 donors of the same age. The studied proteins were measured in extracts from tumors and sites of histologically intact colorectal mucosa located at a distance of 3 cm from the tumor edge, and in plasma obtained by the standard method using EDTA before and 5-27 days after surgery.

Tissue samples for enzyme immunoassay were lyzed in a buffer of the following composition: 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, and 1 μg/ml leupeptine (1:3 tissue buffer ratio). The lysates were centrifuged for 30 min at 20,000 rpm and 4°C (Optima TM TLX centrifuge, Beckman). The measurements were carried out using standard kits for direct enzyme immunoassay: Human MMP-7 (total), Human MMP-9 (total), Human TIMP-4 (Quantikine®, R&D Systems), and Human TIMP-1 (BioSource) in accordance manufacturer's instruction. The measurements were carried out on an EL_X800 automated universal microplate reader (BioTek Instruments, Inc.). The concentrations of the studied factors in tissues were estimated per mg total protein, measured by the method of Lowry.

The results were compared and the relationships were analyzed using Student's *t*, Mann—Whitney, paired Wilcoxon, Pierson correlation (*r*), and Spearman rank correlation (*R*) tests. The data were processed using Statistica 6.0 software.

RESULTS

Both metalloproteinases and their inhibitors were detected in all studied specimens of tumors and histologically intact colorectal mucosa, their levels varying within a wide range (Table 1). The content of MMP-7 in tumors of 14 (70%) patients was higher than in histologically intact tissue by 38-6200%, the differences in the mean and median values of the parameter in tumor and normal tissue were significant ($p<0.01$). A significant increase in TIMP-1 level was detected in tumor tissue ($p<0.05$). The content of this protein in tumors of 17 (85%) examined patients was elevated by 22-407% compared to intact adjacent colorectal mucosa.

An elevated content of MMP-9 (by 19-703%) in comparison with the adjacent tissue was found in 60% patients, but the mean and median values of this parameter virtually did not differ (Table 1). In contrast to three other parameters, TIMP-4 level in tumors of 55% patients with colorectal cancer was lower than in the adjacent mucosa by 2-73%, the mean and median values being virtually the same (Table 1). No correlations between the levels of each protein in the tumor and adjacent tissue and between the levels of different MMP and TIMP in the tumor were detected.

Thus, the expression of MMP-7 and TIMP-1 increased significantly in tumors of patients with colorectal cancer, which agrees with the important role of these proteins in the pathogenesis of this disease [3,4,8]. No pronounced changes in the expression of MMP-9 and TIMP-4 were detected, though some reports indicate high expression of MMP-9 in

TABLE 1. Content of MMP-7, MMP-9, TIMP-1, and TIMP-4 in Tumors and Histologically Intact Mucosa of Patients with Colorectal Cancer

Parameter	Tumor (T)		Mucosa (N)		T>N, %
	M±*m*	median (range)	*M*±*m*	median (range)	
MMP-7, ng/mg protein	4.1±0.9*	2.3^{+} (0.33-11.7)	1.6±0.6	0.05 (0.04-9.9)	70
MMP-9, ng/mg protein	62.0±5.7	62.8 (12.9-102.0)	51.7±5.5	48.7 (6.5-103.0)	60
TIMP-1, ng/mg protein	44.5±4.3**	45.3^{++} (10-103)	25.9±3.2	22.1 (8.3-53.6)	85
TIMP-4 pg/mg protein	57.5±11.5	45.0 (16.8-255.0)	58.6±4.6	56.6 (23.5-99.0)	45

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

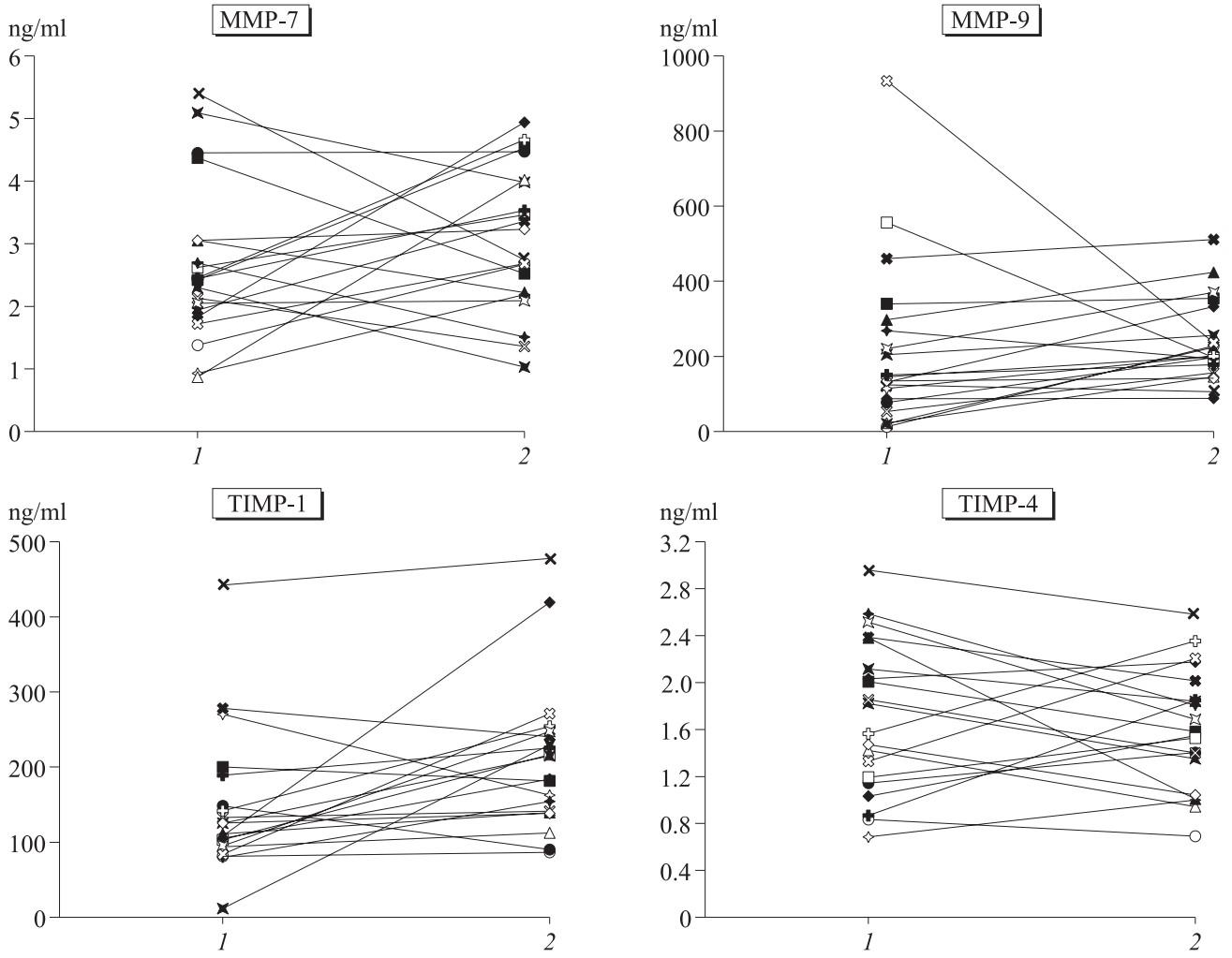

Fig. 1. Dynamics of MMP-7, MMP-9, TIMP-1, and TIMP-4 concentrations in the plasma of 20 patients with colorectal cancer. *1*) parameter before operation; *2*) 5-27 days after operation.

colorectal tumors [6] (data were obtained by other methods). The expression of recently discovered TIMP-4 in malignant tumors is little studied [5].

We compared plasma concentrations of the studied proteins in patients with colorectal cancer before treatment and after removal of the primary tumor and in controls (Table 2). A significant increase in the median values in patients with colorectal cancer in comparison with controls was detected only for MMP-9; in 64% patients the content of MMP-9 was above the upper threshold value, the content of TIMP-1 and TIMP-4 in 35 and 15%

TABLE 2. Plasma Levels (ng/ml) of MMP-7, MMP-9, TIMP-1, and TIMP-4 in Donors and Patients with Colorectal Cancer before and 5-27 Days after Surgery

Parameter	Control group	Patients with colorectal cancer		
		before operation	after operation	reduction, %[x]
MMP-7	2.4 (1.1-4.6)	2.4 (0.87-5.4)	3.0 (1.0-4.9)	35
MMP-9	32.9 (13.2-105)	140* (12.2-934)	200*[+] (88.3-511)	20
TIMP-1	123 (111-138)	118 (11.7-442.0)	199[++] (86.6-478.0)	20
TIMP-4	1.0 (0.52-2.5)	1.7 (0.7-3.0)	1.6 (0.7-2.6)	60

Note. *$p<0.01$ compared to control group (Mann--Whitney's test); [+]$p<0.05$, [++]$p<0.01$ compared to the corresponding value before surgery (paired Wilcoxon's test). [x]Reduction in comparison with preoperative level.

patients, respectively, and the content of MMP-7 in only 10% patients. The postoperative plasma level of MMP-9 in patients with colorectal cancer also significantly surpassed the normal.

It is important to what measure changes in the production of matrix metalloproteinases and their inhibitors in tumor tissue correlate with their concentrations in the peripheral blood, because the presence this correlation makes it possible to evaluate the metastatic and invasive potential of the tumor without surgical intervention. Unfortunately, no significant correlations between tissue and blood concentrations were detected for any of the studied parameters. No significant reduction of their plasma concentration after removal of the primary tumor was detected (Table 2), the levels of MMP-9 and TIMP-1 even increased after the intervention ($p<0.05$ and $p<0.01$, respectively), which is in line with previous data [4,15]. On the whole, changes in plasma protein concentrations were oppositely directed (Fig. 1) and did not depend on their initial levels in the tumor.

Hence, the production of MMP-7 and TIMP-1 in the tumor significantly increased in comparison with the adjacent histologically unchanged mucosa in the majority of patients with colorectal cancer, plasma levels of these proteins slightly surpassed the upper threshold level in just 10 and 35% patients, respectively. The expression of MMP-9 in the tumor was also elevated in many patients, but this increase was less pronounced, did not reach the level of statistical significance, and did not correlate with the increase in plasma concentration of this protein. No significant changes in TIMP-4 content in tumors and plasma of patients with colorectal cancer were detected. It is paradoxical that plasma levels of these proteins did not significantly decreas, while the concentrations of TIMP-1 and MMP-9 slightly increased after removal of the tumor node. This fact deserves further investigation. Hence, our findings indicate that of the four studied parameters only MMP-7 and TIMP-I can be regarded as possible biological markers of colorectal cancer and as targets for molecular targeted therapy.

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