ONCOLOGY

Matrix Metalloproteinases 2, 7, and 9 in Tumors and Sera of Patients with Breast Cancer

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Enzyme immunoassay showed that the content of matrix metalloproteinases (MMP) 2 and 7 in tumors was higher than in the adjacent histologically intact tissue in 91 and 76% patients with breast cancer, respectively, while MMP-9 levels in the tumor and intact tissue were virtually the same. Serum concentrations of MMP-2 and MMP-7 did not correlate with their levels in the tumors, were within the normal range, and virtually did not decrease after removal of the primary tumor. Serum levels of MMP-9 in patients were significantly lower than in the control and increased after surgery in 85% patients. No clear-cut relationship between the studied parameters and clinical morphological prognostic factors of breast cancer was detected.

Key Words: *matrix metalloproteinase; breast cancer; histology; enzyme immunoassay*

Destruction of the adjacent basal membrane and extracellular matrix by tumor-associated proteases playing also an important role in metastatic growth and neoangiogenesis is one of the main mechanisms of malignant tumor invasion. Several classes of proteases are involved in invasion and metastatic growth, including the multigenous family of matrix metalloproteinases (MMP) or matrixins called so for their capacity to specifically hydrolyze all main extracellular matrix proteins and consisting of more than 20 zinc-dependent endopeptidases secreted or bound to the cell surface [9]. The MMP substrates, in addition to the majority of extracellular matrix components, are other proteases, chemotaxic molecules, latent growth factors, soluble and membrane-associated proteins binding growth factors [7]. Activities of MMP in the extracellular space are specifically inhibited by tissue inhibitors (TIMP), structurally related proteins. Three of these proteins (TIMP-1, -2, and -4) are secreted in a soluble form, while one (TIMP-3) is bound to the extracellular matrix. Tissue inhibitors play an important independent role in the regulation of tumor and normal cell growth and differentiation and are characterized by antiangiogenic effects [4]. The MMP also have certain antiangiogenic activity, as they are involved in the formation of antiangiogenic peptides from larger proteins [6,10].

The expression and activities of many MMP increase in tumors of different origin; this activation is regulated by the paracrine mechanism with participation of growth factors and cytokines secreted by macrophages and lymphocytes, infiltrating the tumor, and by tumor stroma cells [9,15]. Therefore, MMPs and TIMPs are regarded as prospective biological markers for prognosis and drug sensitivity of malignant tumors, while the use of natural and synthetic inhibitors of MMP is assumed to be a promising approach

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to antitumor therapy. Experimental studies revealed the involvement of MMP in the regulation of invasive, migration, and even proliferative activities of breast cancer and demonstrated the possibility of suppression of tumor dissemination by synthetic MMP inhibitors [6,11-14]. The data on the impact of MMP for prognosis and clinical course of breast cancer are still scanty and opinions on the subject vary [5,8,13,15].

We compared the levels of MMP-2, -7, and -9 in tumors and histologically intact tissue of patients with breast cancer and in the sera of these patients before and after surgery and analyzed the relationship of these parameters with the main clinical morphological characteristics of the disease.

MATERIALS AND METHODS

The study was carried out in 45 patients with breast cancer, stages I-III, aged 26-73 years (median 54 years). Control group consisted of 8 healthy women. Ten patients presented with stage I, 12 and 12 with stages IIA and IIB, 6 with IIIA, and 5 patients had disseminated process of stages IIIB-IIIC. By their histological structure, 36 tumors were ductal infiltrative cancer, 5 lobular infiltrative cancer; other types of mammary carcinomas were represented by solitary cases. The tumors were moderately differentiated in the majority (33) patients, low differentiated in 7, and highly differentiated in 5. The expression of receptors (estrogens, progesterone, HER2/neu) was studied by immunohistochemical methods in all tumors.

The levels of MMP-2, -7, -9 were measured in tumor extracts, specimens of histologically intact mammary tissue, and in the sera collected by the standard methods before and 5-15 days after surgery. Tissue specimens for EIA were lyzed in 1:3 proportion in 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 leupeptin. The resultant lysates were centrifuged (30 min, 20,000 rpm, 4°C; Optima™ TLX centrifuge, Beckman). The measurements were carried out using standard kits for direct EIA: Human/Mouse/Rat MMP-2 (total), Human MMP-7 (total), Human MMP-9 (total) (QuantikineT, R&D Systems) according to instructions. The measurements were carried out on an EL_X800 automated universal reader for microplates (Bio-Tek Instruments, Inc.). The concentrations in tissues were expressed in nanograms per mg total protein, measured by Lowry's method.

The values were compared and relationships analyzed using the Mann–Whitney and Kruskal–Wallis tests and Spearman rank correlation test (R). The differences and correlations were considered significant at $p<0.05$.

RESULTS

Measurable levels of MMP-2 and -7 were found in all the studied specimens of tumors and histologically intact mammary tissue; MMP-9 was detected in 30 of 40 (75%) tumors and in 63% specimens of intact mammary tissue. The levels of MMP-2 and -7 in the tumors were elevated significantly in comparison with histologically intact mammary tissue in 91 and 76% patients, respectively (Table 1). In addition, the level of MMP-9 in the tumor was higher than in intact tissue in 61% patients, but the differences in the group in general did not reach the level of statistical significance (Table 1). The MMP-9 (but not MMP-2 and -7) levels in the tumor and intact mammary gland tissue correlated (R=0.36; $p<0.05$). In addition, positive correlations were found for tumor levels of MMP-2 and -7 and of MMP-7 and -9 (R=0.49 and R=0.48, respectively; $p<0.05$).

Hence, the expression of MMP-2 and -7 in the tumors increased significantly, which was in line with previous reports demonstrating an important role of these proteins in breast cancer pathogenesis [7,12,15]. No significant elevation of MMP-9 expression was found. However, activation of this proteinase in mammary tumors was previously detected by other methods [5,15].

TABLE 1. Levels of MMP (ng/mg protein) in Breast Tumors and Histologically Intact Mammary Gland Tissue

Parameter	N	Tumor (T)		Mucosa (N)		T>N, %
		median	range	median	range	
MMP-2	45	40.1*	3.6-111	10.5	2.3-67.3	91
MMP-7	25	0.7**	0.04-15.0	0.2	0.03-2.2	76
MMP-9	40	8.2	0-294	2.7	0-225	61

Note. *$p<0.0001$, **$p<0.01$ compared to intact tissue (paired Wilcoxon's test).

TABLE 2. Serum Levels of MMP (ng/ml) in Normal Subjects and Patients with Breast Cancer before Treatment and 5-15 Days after Surgery

Parameter	Control group (*N*=8)	*N*	Breast cancer patients		Reduction, %
			before surgery	after surgery	
MMP-2	256 (161-303)	45 (151-389)	228 (153-361)	245	52
MMP-7	3.9 (2.8-4.5)	25 (1.9-9.4)	4.05 (2.8-11.0)	4.13	52
MMP-9	398 (279-496)	40 (87-446)	229* (136-847)	284**	15

Note. +Percent of patients in whom the marker level decreased after tumor removal. *$p<0.05$ compared to the control (Mann–Whitney's test); **$p<0.0001$ compared to the parameter before surgery (Wilcoxon's paired test).

Analysis of the sera showed no appreciable elevation of MMP-2 and -7 concentrations in breast cancer patients in comparison with the controls, while serum MMP-9 levels in patients were significantly lower than in the controls (Table 2). No significant correlations between tissue and serum concentrations were detected for any of the studied parameters. This could indicate nontumor origin of MMP circulating in the peripheral blood of patients with breast cancer. The fact that MMP-2 and -7 levels decreased after removal of the primary tumor in just half of the patients and that this reduction was not statistically significant (just 0.2-51.0% for MMP-2 and 2.5-39.0% for MMP-7; Table 2) supported this hypothesis. The reduced level of MMP-9 increased by 0.8-75.0% after surgery ($p<0.0001$) in 85% patients, but remained below the control (Table 2).

In order to evaluate clinical significance of measurements of these markers in the tumors and peripheral blood of patients, we analyzed the relationships between these parameters and the main clinical morphological characteristics of the disease: stage, size, histological structure, differentiation degree, receptor status of the primary tumor (Table 3), and the presence of metastases in regional lymph nodes. The level of MMP-9 in the tumor increased significantly with decreasing the degree of tumor differentiation. This indirectly indicated an unfavorable prognostic role of high level of this proteinase in mammary tumor. Elevation of MMP-9 and MMP-7 levels was also found in tumors with prognostically unfavorable receptor status (negative by estrogen receptors, $p<0.05$, and/or HER2/neu positive). The level of MMP-2 in fact did not depend on the estrogen and HER2/neu receptor status, but was higher in progesterone receptor-positive tumors ($p<0.05$) than in tumors negative by this marker. The main regularities detected for serum levels were significant elevation of MMP-2 level ($p<0.05$) and reduction of MMP-9 level in stage III breast cancer in comparison with stages I-II ($p<0.05$). Serum level of MMP-9 was also significantly lower in low differentiated cancer and in primary tumors larger than 4 cm ($p<0.05$ in both cases).

Hence, production of MMP-2 and -7 in tumors was significantly elevated in comparison with histologically intact mammary tissue in the majority of breast cancer patients. Serum levels of these proteinases virtually did not differ from those in the controls and in fact did not change after tumor removal. The expression of MMP-9 was also elevated in the tumors of an appreciable part of the patients, but this increase was less pronounced in comparison with two other proteinases and did not reach the level of statistical significance. Paradoxically, serum MMP-9 concentration was reduced greater in comparison with the normal level, this decrease being more pronounced in some prognostically unfavorable subgroups, while after surgery the level of MMP-9 normalized (increased); this fact deserves further studies. It is noteworthy that we observed a similar reduction of MMP-9 level in gastric cancer [3], while in ovarian cancer [2] and colorectal cancer [1] the level of this marker in the peripheral blood was elevated. Hence, our data suggest regarding all three studied MMPs as possible markers of breast cancer and targets for molecular therapy; none of them is a prospective serological marker of this disease.

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TABLE 3. Levels of MMP in Tumors (ng/mg protein) and Sera of Patients with Breast Cancer and Some Clinical Morphological Characteristics of the Disease

Parameter		MMP-2		MMP-7		MMP-9	
		tumor	serum	tumor	serum	tumor	serum
Stage	I	45.2 (21.3-61.4)	229 (185-294)	1.6 (0.3-1.8)	3.8 (3.5-4.5)	0 (0-8.1)	217 (167-314)
	II	35.0 (22.1-52.8)	235 (193-229)	0.6 (0.2-1.4)	4.7 (3.5-4.0)	8.6 (2.2-18.6)	254 (221-314)
	III	40.1 (27.9-64.0)	280 (242-316)	0.8 (0.6-1.3)	4.6 (4.1-5.1)	13.6 (0-25.1)	210 (140-265)
Tumor size	<2 cm	31.7 (21.3-46.9)	265 (201-315)	1.1 (0.6-4.1)	3.8 (3.5-5.2)	0 (0-52.6)	304 (182-446)
	2.0-3.9 cm	42.4 (22.4-64.0)	242 (222-265)	0.6 (0.3-1.0)	4.6 (3.7-4.1)	8.8 (1.8-18.6)	225 (167-253)
	≥4 cm	47.2 (36.5-69.2)	227 (193-265)	1.8 (1.4-2.2)	3.9 (3.5-5.2)	11.1 (0-14.1)	182 (145-223)
Malignancy degree	highly differentiated	37.8 (19.6-65.1)	221 (207-235)	1.0 (0.1-1.8)	3.8 (3.5-4.0)	0.9 (0-5.2)	223 (167-279)
	moderately differentiated	41.9 (26.3-56.4)	248 (193-315)	0.7 (0.4-1.6)	4.6 (3.7-5.2)	8.8 (0.6-19.6)	250 (187-314)
	low differentiated	36.9 (24.9-66.2)	241 (201-280)	1.0 (0.6-1.4)	3.9 (3.5-4.3)	13.9 (5.2-114)	167 (126-241)
Receptor status	ER^+	36.9 (22.4-36.9)	252 (193-294)	1.8 (1.3-4.1)	3.8 (3.5-5.2)	24.0 (18.6-80)	213 (176-304)
	ER^-	42.4 (26.4-61.4)	243 (223-309)	0.6 (0.3-1.4)	4.6 (3.7-4.1)	3.9 (0-11.5)	236 (145-275)
	PR^+	31.7 (22.4-45.6)	242 (193-315)	1.1 (0.6-1.8)	4.3 (3.5-5.2)	18.4 (0.3-28.6)	219 (167-314)
	PR^-	46.2 (32.8-68.1)	248 (222-265)	0.7 (0.3-1.4)	4.7 (3.9-4.5)	4.7 (0-11.9)	238 (165-262)
	$HER2/neu^+$	41.3 (26.3-56.4)	246 (201-308)	0.7 (0.4-1.6)	4.6 (3.9-4.2)	7.6 (0-19.6)	232 (182-253)
	$HER2/neu^-$	40.1 (22.4-64.0)	241 (196-294)	2.6 (1.0-4.1)	4.1 (3.6-5.1)	12.8 (5.8-16.4)	217 (167-304)

Note. Medians and quartiles of the values are presented; statistically significant differences are described in the paper. ER: estrogen receptors; PR: progesterone receptors.

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