Matrix Metalloproteinases 2, 7, and 9 and Tissue Inhibitor of Metalloproteinases-1 in Tumors and Serum of Patients with Ovarian Neoplasms

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The content of matrix metalloproteinases 7 and 9 was significantly increased, while the content of metalloproteinase 2 was reduced in ovarian cancer tissue compared to benign tumors. In blood serum from patients with ovarian cancer, the concentrations of matrix metalloproteinases 7 and 9 and their type 1 tissue inhibitor were significantly elevated, while the concentration of matrix metalloproteinase 2 was reduced compared to the corresponding parameters in healthy women. After chemotherapy, tissue and serum concentrations of metalloproteinases and their inhibitor in patients practically returned to normal. A significant positive correlation between serum levels of matrix metalloproteinases 7 and 9 and tissue inhibitor of metalloproteinases-1 in patients with ovarian cancer and the size of primary tumor (ultrasound examination) and a positive correlation between these parameters and the concentration of classical ovarian cancer marker CA-125 were demonstrated.

Key Words: *matrix metalloproteinases 2, 7, and 9; and tissue inhibitor of metalloproteinases-1; ovarian tumors*

Ovarian cancer is one of the most invasive malignant tumors; in most patients the disease is diagnosed at late stages, when the tumor is already disseminated along the peritoneum. Difficulties of early diagnostics and high metastatic and invasive potential of ovarian cancer determine the necessity of profound study of the mechanisms of tumor dissemination; understanding of these mechanisms can provide the basis for the creation of new preparations directly modulating metastasizing and invasion processes.

It is known that destruction of the basal membrane and extracellular matrix by tumor-associated proteases is an important mechanism of these processes. Several classes of proteases are involved into tumor invasion and metastasizing, including a multigenic family of matrix metalloproteinases (MMP) consisting of more than 20 secreted or cell surface-bound zinc-dependent endopeptidases specifically hydrolyzing all main proteins of the extracellular matrix. Apart from extracellular matrix components, MMP can cleave other proteases, chemotactic molecules, latent forms of growth factors, and soluble and membrane-associated proteins binding growth factors [4]. MMP activity in the extracellular space is specifically inhibited by tissue inhibitors (TIMP), structurally related proteins; three of them (TIMP-1, TIMP-2, and TIMP-4) are secreted in a soluble form and one (TIMP-3) is bound to the extracellular matrix [9].

Previous studies demonstrated enhanced expression of many MMP in tumors of different histogenesis. It should be noted that activation is mediated by the paracrine mechanism with participation of growth factors and cytokines secreted by stromal tumor cells or macrophages and lymphocytes infiltrating the tumor. MMP also participate in the regulation of tumor an-

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giogenesis. An important role of TIMP exhibiting antiangiogenic activity in the regulation of growth and differentiation of tumor and normal cells was recently reported [9,15]. In light of this, different MMP and TIMP are now considered as possible markers of the prognosis and drug sensitivity of malignant tumors, in particular, ovarian cancer [5,7,8,11-14], while the use of natural and synthetic inhibitors of MMP is a perspective approach to antitumor therapy [4].

Here we compared the contents of MMP-2, MMP-7, MMP-9, and TIMP-1 in tumor tissue and blood serum of patients with ovarian cancer, benign neoplasms, and borderline ovarian tumors and analyzed the relationship between these parameters and the main clinical and morphological peculiarities of ovarian cancer.

MATERIALS AND METHODS

The study included 50 patients with ovarian cancer (28 women with first diagnosed tumor and 22 women after preoperation therapy), 7 patients with borderline tumors, and 20 patients with benign ovarian neoplasms; the control group included 15 healthy women. All groups were comparable by the age; the medians were 34, 38, 46, and 40 years, respectively.

The content of the studied proteins was determined in the serum (prepared routinely before the start of specific treatment) and tumor lysates prepared as described previously [1]. The material was obtained during surgery: tissue samples (200-500 mg) were transferred to the laboratory on ice and stored at -70°C until assay. The markers were measured using standard kits for direct enzyme immunoassay (EIA): Human/Mouse/Rat MMP-2 (total), Human MMP-7 (total), Human MMP-9 (total, Quantikine®, R&D Systems), and Human TIMP-1 (BioSource) according to manufacturer's instructions. The measurements were performed on an EL_X800 automatic universal microplate reader (Bio-Tek Instruments Inc.). Serum and tissue concentrations of the studies proteins were expressed in ng/ml and ng/mg total protein, respectively (total protein content was measured by the method of Lowry).

The data were processed statistically using Statistica 7.0 software. The parameters were compared and the relationships between them were analyzed by nonparametric methods: Mann–Whitney and Kruskal–Wallis tests, Spearman rank correlation test (R), and Pearson direct correlation test (r). The differences and correlations were significant at $p<0.05$.

RESULTS

Measurable amounts of MMP-2 and TIMP-1 were detected in all studied ovarian tumors. MMP-9 was found in 85% samples of primary ovarian cancer, in 94% benign neoplasms, and in 67% borderline tumors; MMP-7 was detected in all primary ovarian cancer samples, in 95% benign neoplasms, and in 89% borderline tumors (Table 1). The content of MMP-7 and MMP-9 was significantly increased in primary ovarian cancer tissue compared to that in benign neoplasms. In borderline ovarian tumors, the level of MMP-7 did not differ from that in cancer tissue, while MMP-9 level corresponded to the values observed in benign neoplasms (Table 1). The levels of TIMP-1 were similar in benign and malignant ovarian tumors, in borderline tumors this parameter was reduced, but the differences did not attain the level of statistical significance. In tumors analyzed after preoperation treatment, the levels of MMP-7, MMP-9, and TIMP-1 were considerably lower than in primary tumors, but the differences were significant only for MMP-9 (Table 1).

TABLE 1. Content of MMP-2, MMP-7, MMP-9, and TIMP-1 (ng/mg protein) in Tumor Tissue of Patients with Ovarian Neoplasms (Median and Parameter Range are Presented)

Group	MMP-2	MMP-7	MMP-9	TIMP-1
Benign tumors	28.4	0.74	11.4	34.6
	(8.6-105.7)	(0-11.8)	(0-90)	(10.8-96.3)
Borderline tumors	18.4	5.4	9.4	15.0
	(4.8-46.0)	(0-10.1)	(0-174)	(14.4-64.2)
Cancer (without treatment)	15.4*	5.9*	56.2*	39.3
	(2.9-86.7)	(0.18-13.2)	(0-450)	(5.2-106)
Cancer (after chemotherapy)	43.6^{++}	4.7	12.5^{+}	19.1
	(13.4-114)	(0-14)	(1.2-262)	(10.4-59)

Note. *$p<0.05$ compared to the corresponding parameter in patients with benign tumors; $^{+}p<0.05$, $^{++}p<0.001$ compared to the corresponding parameter in untreated patients with cancer.

The regulations observed for MMP-2 were opposite to those for other markers (Table 1). The level of MMP-2 was significantly lower in primary malignant tumors compared to benign neoplasms, the maximum level of MMP-2 was found in tumors analyzed after chemotherapy. These peculiarities agree with experimental data on reduced expression of MMP-2 in cancer cells compared to non-tumor cells of the surface ovarian epithelium [2] and with clinical observations on favorable prognostic value of high MMP-2 and low MMP-9 levels in ovarian cancer tissue [3,7,12].

Analysis of blood serum revealed increased concentrations of MMP-7 and TIMP-1 in patients with ovarian cancer (both primary and receiving chemotherapy) compared the corresponding parameters in the control group (Table 2), which agrees with previous reports [6,10,11]. In 74% patients with primary ovarian cancer and in 84% patients examined after pre-operation chemotherapy, the level of MMP-7 was above the upper 95% boundary of normal (4.67 ng/ml). In patients with benign and borderline ovarian tumors, the level of MMP-7 did not differ from the normal. The level of TIMP-1 surpassed the upper 95% boundary of normal (504 ng/ml) in 40% patients with primary cancer and 30% patients receiving chemotherapy. In patients with benign and borderline tumors, the level of TIMP-1>504 ng/ml was observed in only 11 and in 30% cases, respectively. Serum concentration of MMP-9 surpassed the control only in patients with primary ovarian cancer, in 29% cases this parameter surpassed the upper 95% boundary of normal (504 ng/ml). In patients receiving chemotherapy, serum level of MMP-9 was significantly lower than that in untreated patients and considerably below the control (Table 2). Similarly to tissue levels, serum level of MMP-2 in patients with ovarian cancer was lower than in patients with benign and borderline tumors and significantly lower than in the control group (Table 2). Serum level of MMP-2 in patients examined after chemotherapy was higher than in untreated patients ($p>0.05$) and did not differ significantly from the corresponding value in the control group. A reliable positive correlation between serum and tissue concentrations was revealed only for MMP-9 ($R=0.31$; $p=0.021$).

To evaluate clinical significance of the studied markers in tumors and peripheral blood of patients with ovarian cancer, we evaluate the relationships between these parameters and the main clinical and morphological peculiarities of the tumors: stage, size, histological structure, and differentiation degree of the primary tumor and the presence of distant metastases and/or ascitis. We revealed a positive correlation between serum levels of MMP-7 ($R=0.59$; $p<0.001$), MMP-9 ($R=0.56$; $p<0.05$), TIMP-1 ($R=0.52$; $p<0.01$) in patients with primary ovarian cancer and the size of the primary tumor (data of ultrasound examination). No other significant correlations were revealed. However, serum levels of MMP-7, MMP-9, and TIPM-1 significantly correlated with the levels of classical ovarian cancer marker CA-125 ($R=0.41$, 0.58 and 0.48, respectively, $p<0.05$).

Thus, significantly elevated content of MMP-7 and MMP-9 and reduced content of MMP-2 in ovarian cancer tissue compared to benign tumors were demonstrated by enzyme immunoassay. In tumors examined after preoperation chemotherapy these parameters returned to normal: the levels of MMP-7 and MMP-9 decreased and the level of MMP-2 increased. Reduced

TABLE 2. Serum Levels of MMP-2, MMP-7, MMP-9, and TIMP-1 (ng/ml) in Patients with Ovarian Neoplasms (Median and Parameter Range Are Presented)

Group	MMP-2	MMP-7	MMP-9	TIMP-1
Control	281	4.4	305	322
	(161-370)	(2.78-4.67)	(3.56-647)	(133-507)
Benign tumors	231**	4.3	303	396
	(174-333)	(1.7-10.4)	(141-623)	(283-527)
Borderline tumors	239	4.7	385	466
	(181-484)	(2.5-10.7)	(172-469)	(455-608)
Cancer (without treatment)	202*	11.5**+x	411*	474***
	(105-445)	(2.4-50.6)	(123-922)	(68.6-654)
Cancer (after chemotherapy)	241	7.2*	181o	476**+
	(155-377)	(2.9-40.0)	(58.6-900)	(292-669)

Note. *$p<0.05$, **$p<0.01$, ***$p<0.0001$ compared to the control group; $p<0.05$ compared to: +benign tumors, xborderline tumors, ocancer without treatment.

level of TIMP-1 was also revealed in patients receiving chemotherapy compared to untreated patients. The absence of a clear-cut relationship between tissue concentrations of the studied markers and the main clinical and morphological characteristics of ovarian cancer does not exclude their potential role as independent factors of prognosis of relapse-free and total survival, which can be proved after increasing the time of observation and the number of patients.

The regularities revealed for the tumor tissue were also noted in the serum of these patients; however, significant positive correlation between the serum and tissue concentrations was revealed only for MMP-9. Analysis of the relationships between serum levels of the studied MMP and TIMP-1 and clinical and morphological factors and concentration of CA-125 suggests that MMP-7 is the most promising serological marker for differential diagnostics of malignant, borderline, and benign ovarian neoplasms, while MMP-9 and TIMP-1 can be useful for monitoring of the effect of preoperation chemotherapy.

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