ONCOLOGY

Relationship between the Expression of VEGF Signal Components and Matrix Metalloproteinases in Ovarian Tumors

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The content of vascular endothelium growth factor is significantly increased, while the level of matrix metalloproteinase-2 is 2-fold reduced in ovarian cancer tissue compared to benign tumors. A trend to an increase in the levels of matrix metalloproteinases 7 and 9 and reduction of vascular endothelial growth factor type 2 receptors in tumor tissue was also detected. A highly significant negative correlation between the levels of vascular endothelial growth factor and matrix metalloproteinase 2 and positive correlations between vascular endothelial growth factor and matrix metalloproteinase 7, vascular endothelial growth factor and matrix metalloproteinase 9, matrix metalloproteinase 2 and vascular endothelial growth factor type 2 receptors were revealed. In the tumors assayed after preoperative therapy, relative normalization of the studied parameters was observed: the level of vascular endothelial growth factor decreased significantly, while the levels of matrix metalloproteinase 2 and vascular endothelial growth factor type 2 receptors increased. The levels of the markers differed significantly in ovarian tumors of different histological types, and the levels of vascular endothelial growth factor type 2 receptors were higher in patients with stage III compared to stage I and the content of matrix metalloproteinase 7 was higher in stage III compared to stage II cancer.

Key Words: *vascular endothelial growth factor; vascular endothelial growth factor receptor; matrix metalloproteinase; ovarian tumors*

Difficulties of early diagnosis and high metastatic and invasive potential of ovarian cancer (OC) necessitate profound studies of the mechanisms of its dissemination and progress, and our knowledge of these mechanisms can become the basis for creation of new drugs with target modulation of the molecules playing the key role in invasion, distant metastatic growth, and ascites formation. Neoangiogenesis is a key factor of tumor growth and dissemination. Vascular endothelial growth factor (VEGF) plays the central role in the regulation of this process. VEGF is endothelial cell growth inductor, it increases vascular permeability and presumably participates in the maintenance of endothelial cell survival *in vivo* and *in vitro*. Biological effects of VEGF are mediated through specific membrane receptors belonging to the receptor tyrosine kinase class: VEGFR-1/Flt-1 and VEGFR-2/Flk-1/KDR.

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VEGFR-1 induces protease activity in endothelial cells and stimulates migration of macrophages to tumor tissue, while VEGFR-2 induces differentiation, proliferation, and migration of vascular endothelial cells [2].

In addition to its proangiogenic activity, VEGF is directly involved in the regulation of tumor cell proliferation [6,11]. The effects of VEGF on tumor progress are realized via cooperation with matrix metalloproteinases (MMP). MMP-dependent hydrolysis of the basement membrane and extracellular matrix adjacent to the tumor is one of the main molecular mechanisms of invasion and metastasizing [3,9].

Experimental studies showed that VEGF release from OC cells induced by MMP-9 collagenase, promotes the formation of ascites from human adenocarcinoma xenografts in mice [4,15]. On the other hand, experiments have shown inhibition of MMP-9 expression and of OC invasion under the effect of bevacizumab, an anti-VEGF drug (Avastin) [3]. Hence, it seems that VEGF stimulates OC invasion by stimulating the production and functional activity of MMP-9. A similar relationship was demonstrated for two other MMP, collagenase MMP-2 and matrilysine MMP-7 [4,14]. The induction of MMP under the effect of VEGF and the stimulatory effect of this growth factor on OC cell invasion and migration is mediated primarily by VEGFR-2 [11]. Different MMP and their tissue inhibitors with antiangiogenic effects play an important individual role in the regulation of tumor angiogenesis [9].

The use of antiangiogenic drugs, including natural and synthetic inhibitors of MMP, is assumed to be one of the most promising trends of molecular target antitumor therapy, while VEGF signal system components and MMP are regarded as possible biological markers of prognosis and drug sensitivity of tumors, including OC [7,8,10,12,13].

We compared the levels of VEGF, VEGFR-1, VEGFR-2, and some MMP family representatives (MMP-2, -7, -9) in malignant, benign, and borderline ovarian tumors and analyzed the relationships between these parameters and the main clinical morphological features of OC.

MATERIALS AND METHODS

The study was carried out in 50 patients with OC (33 primary patients and 17 patients after preoperative therapy), 9 patients with borderline, and 22 with benign tumors of the ovaries. Material for analysis was collected during surgeries; tissue fragments (200-500 mg) were delivered on ice to laboratory and stored at -70°C until processing. Tissue specimens for ELISA were lyzed (1:3) 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 leupeptine, as described previously [1]. The resultant lysates were centrifugated for 30 min at 20,000 rpm and 4°C on an OptimaTM TLX centrifuge (Beckman).

The concentrations of the studied proteins in the supernatant were measured using standard direct ELISA kits Human VEGF Immunoassay, Human VEGFR1 Immunoassay, Human VEGFR2 Immunoassay, Human/Mouse/Rat MMP-2 (total), Human MMP-7 (total), and Human MMP-9 (total) (Quantikine^{R}, R&D Systems) according to manufacturer's instructions. The measurements were carried out on an EL_{X}800 automated universal microplate reader (Bio-Tek Instruments Inc.). Marker levels in tissues were converted per mg total protein measured by Lowry's method.

The data were processed using Statistica 7.0 software. The parameters were compared and the relationships between them analyzed by nonparametric Mann—Whitney and Kruskal—Wallis tests and Spearman's rank correlation test (R). The differences and correlations were considered significant at $p<0.05$.

RESULTS

Measurable quantities of VEGFR-1 were found in all the studied ovarian tumors; VEGF and VEGFR-2 were found in 97% primary cancer cases; VEGF was found in all borderline and in just 60% benign tumors, VEGFR-2 in all benign and in 91% borderline ovarian tumors. The level of VEGF significantly increased with transition from benign to borderline and malignant tumors ($p<0.0001$), the levels of VEGFR-1 virtually did not differ in the three tumor types, while the level of VEGFR-2 was low in OC tissue and borderline tumors in comparison with benign tumors of the ovaries, but these differences did not reach the level of statistical significance (Table 1).

In tumors of OC patients operated after chemotherapy, the content of VEGF was significantly lower, while the levels of VEGFR-2 were higher than in tumors of primary patients and approached the corresponding values in benign tumors (Table 1).

The levels of VEGFR-1 in tumors of primary patients and patients who received a course of therapy did not differ. A significant positive correlation between VEGFR-1 and VEGFR-2 (R=0.44; p=0.002) and a negative relationship between VEGF and VEGFR-2 (R=-0.33; p=0.007) in the total group were revealed. No regularities of this kind were detected in the subgroups, for example, in primary patients with cancer.

Measurable levels of MMP-2 were found in all analyzed ovarian tumors. MMP-9 was found in 94% primary OC specimens, 90% benign, and 82% bor-

TABLE 1. Levels of VEGF, VEGFR-1, and VEGFR-2 in Ovarian Tumors

Tissue	VEGF, pg/mg protein	VEGFR-1, ng/mg protein	VEGFR-2, pg/mg protein
Benign tumors (*n*=22)	9.6 (0-492)	651 (248-1260)	155 (40-735)
Borderline tumors (*n*=9)	207⁺ (45.2-2459.0)	748 (121-950)	99.7 (0-413)
Primary cancer (*n*=33)	457⁺ (0-1422)	684 (317-1340)	114 (0-691)
Cancer after chemotherapy (*n*=17)	18.9* (0-1069)	652 (301-1088)	179* (73-395)

Note. *$p<0.05$ compared to primary cancer; ⁺$p<0.0001$ compared to benign tumors. Here and in Tables 2, 3: medians and ranges of values (in parentheses) are presented.

derline tumors. MMP-7 was found in all borderline tumors, 90% benign, and 91% primary OC specimens.

The levels of MMP-2 in tissues of primary OC were significantly lower, while the levels of MMP-7 and MMP-9 were elevated in comparison with benign tumors (Table 2). The levels of MMP-2 in borderline tumors were the same as in OC tissue, while MMP-9 levels were similar to those in benign tumors. The levels of MMP-7 in borderline tumors were higher than in tumors of other types. The levels of MMP-2 in tumors analyzed after a course of therapy increased significantly in comparison with the levels in primary cancer, MMP-7 levels did not change, and MMP-9 levels slightly reduced (Table 2).

Hence, the regularities detected for MMP-2 were opposite to those found for other MMP and for VEGF. These findings are in line with experimental data indicating reduced expression of MMP-2 in cancer cells compared to nontumorogenic cells of ovarian surface epithelium [5] and clinical observations indicating a favorable prognostic significance of high MMP-2 level and low MMP-9 and VEGF levels in OC tissue [12,13].

The only significant positive correlation between MMP-7 and MMP-9 levels was found for benign ovarian tumors (R=0.47; p=0.02); no other correlations between the levels of various MMP expression in ovarian tumors were found. On the other hand, highly significant negative correlations between VEGF and MMP-2 levels in the total group of patients and in patients with primary cancer were found (R=-0.47; p=0.00002 and R=-0.40; p=0.02, respectively), while VEGFR-2 content in primary cancer tissue positively correlated with MMP-2 level (R=0.39; p=0.03). Positive correlations between VEGF and MMP-7, VEGF and MMP-9 (R=0.29; p=0.03 for both cases), and between MMP-2 and VEGFR-2 (R=0.43; p=0.0006) were found in the total group. Variety and different directions of relationships between the levels of expression of VEGF signal components and representatives of the MMP family, found in patients with different ovarian tumors, indicate an intricate mutual regulation of these two systems and its changes with transition from benign to malignant tumors.

In order to evaluate clinical significance of the studied markers in the tumors of OC patients, the correlations of these parameters with the main clinical morphological features of the disease were analyzed. The levels of VEGFR-2 were significantly higher in tumors of patients with stage III compared to stage I disease; the content of MMP-7 was higher in stage III compared to stage II (Table 3; $p<0.05$ in both cases). Despite the fact that the majority of tumors were serous adenocarcinomas, while other groups were small,

TABLE 2. Levels (ng/mg protein) of MMP-2, -7, and -9 in Ovarian Tumors

Tissue	MMP-2	MMP-7	MMP-9
Benign tumors (*n*=22)	25.0 (5.8-106.0)	1.02 (0-14.7)	11.4 (0-90)
Borderline tumors (*n*=9)	14.3 (4.8-45.6)	7.7 (0.7-10.1)	14.5 (0-174)
Primary cancer (*n*=33)	13.1* (1.1-86.7)	3 (0-19.7)	23 (0-450)
Cancer after chemotherapy (*n*=17)	32.7⁺ (13.4-114.0)	3.4 (0-14.0)	19.1 (1.2-43.2)

Note. *$p<0.05$ compared to benign tumors; ⁺$p<0.01$ compared to primary cancer.

TABLE 3. Relationships between the Levels of VEGF, VEGFR-1, and VEGFR-2 and MMP in Tumors of OC Patients and Clinical Morphological Characteristics of the Disease

Stage or type		VEGF, pg/mg protein	VEGFR-1, ng/mg protein	VEGFR-2, pg/mg protein	MMP-2, ng/mg protein	MMP-7, ng/mg protein	MMP-9, ng/mg protein
Stage	I (*n*=11)	367	782	73.6*	11.1	3.72	26.8
		(0-1422)	(317-1340)	(0-188)	(1.2-40.5)	(0.02-19.7)	(0-450)
	II (*n*=4)	358	550	124	30.1	1.08^{+}	68.1
		(0-1029)	(445-1103)	(107-315)	(2.2-46.0)	(0-2.6)	(0-262)
	III (*n*=17)	467	674	125*	13.1	6.1^{+}	18.6
		(0-868)	(444-952)	(26.5-691.0)	(1.1-86.7)	(0.6-13.8)	(0-157)
	IV (*n*=1)	354	689	206	22.9	8.6	164
Histological type	serous (*n*=18)	388^{x}	684^{o}	156^{x}	16.2$^{xx\#}$	5.9	18.9
		(0-868)	(445-952)	(52.7-691.0)	(2.9-86.7)	(0.57-13.80)	(0-164)
	mucinous (*n*=3)	1313^{x}	510	26.5^{x}	1.2^{xx}	3.7	37.9
		(704-1421)	(510-510)	(0-73.6)	(1.1-11.9)	(1.3-19.7)	(9.2-133.0)
	endometrioid (*n*=4)	314	857^{o}	77.1	7.1	7.9	115.7
		(0-1105)	(796-1340)	(4.1-188.0)	(5.0-40.5)	(2.1-11.3)	(9.8-450.0)
	clear-cell (*n*=2)	666	519	92.8	2.8$^{\#}$	4.0	44.7
		(545-788)	(519-519)	(50.4-135.0)	(2.2-3.3)	(1.6-6.4)	(2.8-86.5)

Note. $^{*}p_{(I\text{-}III)}<0.05$, $^{+}p_{(II\text{-}III)}<0.05$; $^{x}p<0.01$, $^{xx}p<0.05$ between serous and mucinous cancer; $^{o}p<0.05$ between serous and endometrioid cancer; $^{\#}p<0.05$ between serous and clear-cell cancer.

the levels of markers differed significantly in OC of different histological types (Table 3). The levels of VEGF were higher, while the levels of VEGFR-2 and MMP-2 were lower in mucinous compared to serous cancer; MMP-2 level was reduced and VEGFR-1 level elevated in endometrioid compared to serous cancer. No significant relationships between the studied parameters and primary tumor size and differentiation degree, presence of distant metastases and/or ascites were detected.

Hence, VEGF content in OC tissue was significantly higher in comparison with its level in benign tumors, while the levels of MMP-2 collagenase were lower. A trend to an increase of MMP-7 and MMP-9 levels and to VEGFR-2 reduction in OC tissue was observed. All parameters in fact normalized in tumors analyzed after preoperative therapy: VEGF level decreased significantly, while the levels of MMP-2 and VEGFR-2 increased. The absence of clear-cut correlations between tissue concentrations of these markers and the main clinical morphological characteristics of OC does not rule out their potential significance as independent factors for prognosis of relapse-free and total survival, which can be proven after a longer follow-up of a larger group of patients. High level of VEGF in OC tissue confirms good prospects of adding antiangiogenic drugs, including natural and synthetic MMP inhibitors, to protocols for the treatment of this grave disease.

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