ONCOLOGY

Vascular Endothelial Growth Factor and Its Type 2 Receptor in Tumors and Serum of Patients with Renal Cancer

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It was found that renal cancer tissue is characterized by increased content of VEGF not depending on the parameters of normal tissue and expression of VEGFR2 receptor. At the same time, the content of VEGFR2 in the tumor is determined by the state of the surrounding tissue and increases in low number of patients with tumors invading the renal capsule. Serum concentrations of VEGF and VEGFR2 in patients with renal cancer did not significantly differ from the corresponding values in the control group. No significant correlations between the levels of VEGF and VEGFR2 in the blood and tumor tissue were found.

Key Words: *VEGF, VEGFR2, renal cancer; angiogenesis*

Molecular changes in the tumor tissue developing in renal caner (RC) are closely related to angiogenesis processes in these tumors. Acceptance of the role of vascular endothelial growth factor (VEGF or VEGF A) as the major regulator of tumor angiogenesis became an important step in understanding of the pathogenesis of RC.

VEGF induces the growth of endothelial cells, increases vascular permeability, and probably participates in the maintenance of viability of endothelial cells *in vivo* and *in vitro* [4,12]. There are data that VEGF not only exhibits proangiogenic activity, but also directly participates in the regulation of cell proliferation in some tumors [2,13]. Biological effects of VEGF are mediated by specific membrane receptors, representatives of the class of receptor tyrosine kinases. It is accepted that angiogenesis processes in the tumors involve two types of VEGF receptors, VEGFR1/Flt-1 and VEGFR2/Flk-1/KDR playing different biological roles [4]: VEGFR1 induces protease activity in endothelial cells and stimulates migration of macrophages into the tumor tissue, while VEGFR2 induces differentiation, proliferation, and migration of vascular endothelial cells. It remains unknown, whether the functions of two types of VEGF receptors in tumor cells are different. It was demonstrated that inhibition of VEGF/VEGFR-signal pathways stops tumor progression in experimental systems [12].

The preparations inhibiting tyrosine kinase activity of receptors for VEGF and some others proangiogenic factors are successfully used for the treatment of metastasizing RC [6,10-12], while the results of clinical and laboratory studies demonstrated the role of VEGF in the development of RC

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N. E. Kushlinsky, M. F. Trapeznikovaand unfavorable prognostic value of high tissue expression of this protein [3,5,8,9]. Expression of VEGF receptors in renal tumors is little studied and published data on clinical significance of the level of VEGF and its receptors in physiological fluids and tissues of patients with RC are contradictory.

In the present study we compared the content of VEGF and VEGFR2 in tumors, histologically unchanged adjacent tissues, and serum from patients with RC and in the serum from healthy individuals and analyzed the relationship between these parameters and major clinical and morphological characteristics of this pathology.

MATERIALS AND METHODS

The study included 37 primary patients with RC aging 33-78 years (median 56 years): 18 men (48.7%) and 19 women (51.4%). The distribution by the stages of the disease was as follows: 11 patients (29.7%) with T1aN0M0, 9 patients (24.3%) with T1bN0M0, 8 patients (21.6%) with T2N0M0, T1bN2M0, T3aN2M0, and T3bN2M0 were diagnosed in 2 patients (5.4%) each, T2N1M0, T3aN0M0, and T3bN0M0 were diagnosed in 1 patient (2.7%) each.

The content of VEGF was determined in the serum (prepared routinely before the start of specific treatment) and cytosols of tumors and histologically unchanged renal tissues (samples obtained during surgery) [2]. Control serum samples were obtained from 57 healthy individuals of the corresponding age (12 men and 45 women).

The content of VEGF and VEGFR2 was measured using Quantikine® Human VEGF Immunoassay and Quantikine® Human VEGFR2 Immunoassay kits (R&D systems) according to manufacturer's instructions. The concentration of factors in tissues was expressed in pg per 1 mg protein; protein content was measured by the method of Lowry.

The parameters were compared using Student *t* test, Mann—Whitney test, median Kruskal—Wallis test, paired Wilcoxon test, Pierson correlation test (*r*), and Spearman rank correlation test (*R*). The data were processed statistically using Statistica 6.0 software.

RESULTS

In all studied samples of RC and unchanged kidney tissue, measurable amounts of VEGF and VEGFR2 were detected; their levels greatly varied (Table. 1). The content of VEGF in tumors of 87% patients surpassed the corresponding parameter in histologically unchanged tissue by 2-53 times (median 8.6), the differences between the mean and median values of this parameter in the tumor and normal tissue were highly significant ($p<0.001$). The content of VEGFR2 in the tumor tissue was elevated in only 39% patients and the differences between the tumor and histologically unchanged kidney tissue did not attain statistical significance. No correlations were revealed between the levels of VEGF and VEGFR2 in RC and between these parameters in normal tissue. At the same time, a significant relationship was found between the level of VEGFR2 in the tumor and normal tissue ($R=0.41$, $p=0.02$). No correlation of this kind was found for VEGF.

Thus, the content of VEGF significantly increased in RC tissue. This parameter does not depend on parameters of normal tissue and expres-

TABLE 1. Content of VEGF and VEGFR2 in Tumors and Histologically Unchanged Tissue of 37 Patients with RC (pg/mg protein)

Parameter	Tumor (T)		Unchanged kidney (N)		T>N, %
	M±*m*	median, range	*M*±*m*	median, range	
VEGF	571±73*	525⁺ 1.6-1796	71.3±8.7	62.8 16.9-253	87ˣ
VEGFR2	765±127	568 53-2731	707±53	647 14.4-1405	39

Note. $p<0.001$ compared to unchanged tissue. * Mann—Whitney test, ⁺Student test, ˣWilcoxon test.

TABLE 2. Serum Content of VEGF and VEGFR2 in Patients with RC and Healthy Donors

Group	Number of patients	VEGF, pg/ml		VEGFR2, ng/ml	
		M±*m*	median, range	*M*±*m*	median, range
Control	57	329±38	286 48.2-911.0	12.2±0.5	11.3 6.8-23.1
RC patients	37	355±47	301 46.3-1322	11.9±0.5	11.5 6.5-22.8

TABLE 3. Content of VEGF and VEGFR2 in Tumors and Serum of RC Patients Depending on Clinical and Morphological Characteristics of the Disease

Parameter	*n*	VEGF		VEGFR2	
		tumor, pg/mg protein	serum, pg/ml	tumor, pg/mg protein	serum, pg/ml
Tumor size					
T1a	11	522 (11.6-923)	302 (59.1-442)	310 (52.6-2347)	11.2 (9.3-17.0)
T1b	14	407 (73.5-948)	261 (66.8-785)	672 (241-2351)	10.2 (7.4-16.3)
T2	9	672 (13.5-1795)	387 (68.6-908)	597 (59.7-2730)	10.8 (9.5-18.5)
T3	6	902 (283-1492)[1]	174 (46.3-1322)	461 (46.3-1322)	9.5 (6.5-22.8)
Metastases into lymph nodes					
N^-	28	523 (16.9-253)	305 (59.1-822)	621 (52.6-2730)	11.6 (6.5-18.5)
N^+	7	588 (22.3-164)	139 (46.3-1323)	474 (67.7-655)	10.6 (7.9-22.8)
Invasion into capsule					
no	24	407 (11.6-1492)	232 (46.3-785)	219 (59.7-672)	10.9 (6.5-15.5)
yes	13	778 (76.5-1795)	403 (107-1322)[2]	744 (52.6-2730)[3]	16.4 (9.7-22.8)[4]
Type of growth					
intrarenal	6	694 (353-1106)	375 (103-1322)	609 (67.7–2342)	12.6 (8.8-22.8)
mixed	23	584 (11.6-1795)	286 (46.3-908)	621 (59.7-270)	12.0 (6.5-18.5)
extrarenal	6	192 (63.7-653)	207 (66.8-420)	296 (59.6- 919)	10.9 (9.4-15.4)

Note. [1]$p<0.05$ compared to T1a and T1b, [2]$p<0.05$, [3]$p<0.01$, [4]$p<0.001$ compared to group without invasion (Mann—Whitney).

sion of VEGFR2 receptor. The content of VEGFR2 in the tumor is largely determined by the state of adjacent tissue and increases in only low number of patients. Despite detection of these relationships, no definite conclusions can be made on coordination of VEGF-dependent mechanisms in RC tissue similar to that observed in breast cancer [1,2]. However, VEGFR1 is probably a more important transmitter of VEGF signal in RC.

Serum concentrations of the studied proteins in RC patients did not significantly differ from those in the control group (Table 2). VEGF concentration surpassed the upper limit of normal (790 pg/ml) in only 4 patients, while VEGFR2 level was below the upper limit of normal (22.9 ng/ml) in all patients. A positive correlation between serum contents of VEGF and VEGFR2 (R=0.39, p=0.015) was found (no such correlations were revealed in tissues).

It is very important to study the relationship between the production of VEGFR2 and especially VEGF in the tumor tissue and the concentration of these factors in the peripheral blood, because the existence of this correlation creates the possibility of relatively noninvasive evaluation of the intensity of angiogenesis processes in the tumor. Serum concentration of VEGFR2 did not correlate with its content in the tumor tissue and only a weak correlation was observed between the levels of VEGF in the blood and tumor (r=0.32; p=0.058). These findings suggest that the content of VEGF and VEGFR2 in the peripheral blood of RC patients did not significantly differ from normal and cannot be used as diagnostic markers of angiogenesis activity in the tumor tissue.

To answer the question on possible prognostic value of these parameters we analyzed the relationship between the content of VEGF and VEGFR2 in tumors and serum and the major clinical and morphological factors of RC prognosis (Table 3). The following important relationships are worthy to note: increased content of VEGF in tumors correlated with their enlargement, lower levels of VEGFR2 in tumors and serum were observed in patients without tumor invasion into the renal capsule. The level of VEGF also increased in patients with invasion of the tumor into the renal capsule.

Thus, the content of VEGF in tissues is a promising marker of this pathology reflecting dissemination of the tumor process. The absence of significant elevation of this factor in the blood of RC patients reported by other authorities [14,15] can be explained by the fact that the majority of examined patients had stage I (54%) or stage II (22%) RC. Expression of VEGFR2 is enhanced only in patients with disseminated process, *i.e.* invasion of the tumor into the renal capsule, which agrees with the data on successful treatment with inhibitors of

tyrosine kinase activity of VEGF receptors in patients with disseminated RC [6,10].

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