Vascular Endothelial Growth Factor in Tumor Tissue and Blood Serum from Patients with Breast Cancer

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Enzyme immunoassay showed that the content of free vascular endothelial growth factor increases in tumor cytosols and blood serum from patients with breast cancer. A positive correlation was found between the contents of vascular endothelial growth factor in tumor cytosols and blood serum. After removal of the tumor the content of vascular endothelial growth factor decreased only in 49% patients. It should be emphasized that changes in these parameters did not depend on their initial values.

Key Words: *vascular endothelial growth factor; breast cancer; angiogenesis*

The problem of neoangiogenesis in malignant tumors attracted much recent attention. Undoubtedly, tumors cannot grow without the formation of a vascular network that supplies cells with oxygen and nutrient substances [9]. Studies of the molecular mechanisms underlying angiogenesis provided the basis for the transition from microscopic assay of vascular density in tumor tissue to evaluation of molecules regulating the formation and growth of new vessels. Vascular endothelial growth factor (VEGF) plays an important regulatory role in these processes [7]. For example, VEGF plays a key role in vascularization of breast cancer (BC) [2,4,14,15].

Recent retrospective clinical studies indicate that expression of VEGF in patients with BC determines the prognosis of this disease [6,10,12] and affects the sensitivity of tumors to hormonal and medicinal preparations [3,8,12]. Much attention is given to the synthesis and study of new preparations with antiangiogenic properties [11]. Evaluation of the intensity of VEGF-dependent angiogenesis can provide the basis for directed therapy with these preparations. There is no general opinion regarding the advantages and weaknesses of various methods or substrates (tumor tissue and blood) for clinical study of VEGF in patients with BC [3, 5,13]. Published data show that the blood contains free and bound VEGF [3]; however, information value of total VEGF content remains unknown.

Here we compared the contents of free and total VEGF in the serum and tumor tissue from patients with BC and malignant breast tumors and healthy donors.

MATERIALS AND METHODS

We examined 112 patients with various stages of BC. VEGF content was measured in serum, tumor cytosols, and histologically unchanged mammary gland tissue [1]. Blood serum was obtained routinely before the therapy and 2-14 days after removal of the tumor. Control groups included 16 conventionally healthy donors and 7 patients with breast fibroadenoma in studies of blood serum and mammary gland tissue, respectively.

The contents of free VEGF in the serum and cytosols were measured using standard commercial kits for direct enzyme immunoassay (CytElisa Human VEGF, Cytimmune Science Inc.). The total VEGF content was estimated with competitive enzyme immunoassay kits (Accucyte Human VEGF, Cytimmune Science Inc.). The measurements were performed on an

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TABLE 1. Serum Concentrations of Free and Total VEGF in Healthy Donors and Patients with BC before and after Surgery (*M*±*m*, Range)

Group	Free VEGF, pg/ml		Total VEGF*, ng/ml	
	before surgery	after surgery**	before surgery	after surgery
Control group (*n*=16)	2.8±0.6⁺	—	10.80±1.64	—
	0.97-6.36		6.34-18.24	
Patients with BC (*n*=52)	35.20±6.28	46.5±12.5	9.90±0.73	10.60±0.76
	0.99-209	2.53-474	3.97-16.55	4.08-18.24

Note. *Changes in total VEGF concentration in 22 patients; **repeated measurements of total VEGF concentration in 39 patients. ⁺$p<0.05$.

EL×800 automatic universal microplate reader (Bio-Tek Instruments, Inc.). VEGF concentration in cytosols was expressed in pg/mg cytosol protein. Protein content was measured by the method of Lowry.

The results were analyzed by Student's *t* test, Mann—Whitney test, Kruskal—Wallis median test, Wilcoxon pairwise test, Pearson correlation test (*r*), and Spearman rank correlation test (*R*).

RESULTS

The serum concentration of total VEGF little varied and was similar in patients with BC and healthy donors (Table 1, Fig. 1). However, the content of free VEGF in the serum from patients with BC varied in a wide range and considerably surpassed the normal ($p<0.05$). The content of total VEGF in the serum from patients with BC and healthy donors was 3 orders of magnitude higher than the concentration of free VEGF. No correlation was found between the concentrations of total and free VEGF in the serum from patients with BC.

The upper confidence limit for free VEGF concentration in the serum from healthy donors was 6.98 pg/ml. If we take this value as the upper limit of normal (according to published data), the concentration of free VEGF in the serum surpassed the normal in 81% patients with BC. It should be emphasized that free VEGF concentration in the serum from healthy donors did not surpass the lower quartile for patients with BC (9.94 pg/ml).

The concentrations of free and total VEGF in the serum from 39 and 22 patients with BC, respectively, were measured twice (before the therapy and 2-14 days after surgery). Table 1 shows that the mean concentrations of total and free VEGF in the serum from patients with BC remained unchanged after removal of the tumor: this parameter decreased in some BC patients, and increased in others.

Thus, the concentration of free VEGF decreased by 1.33-101 pg/ml in 19 of 39 patients (49%, median 8.5 pg/ml), increased by 1.0-265 pg/ml in other 19 patients (median 14 pg/ml), and remained unchanged in 1 patient. These changes in serum concentration of free VEGF after surgery did not depend on the initial content of this factor.

The total VEGF concentration decreased by 0.13-9.47 ng/ml in 10 of 22 patients (45%, median 1.52 ng/ml), and increased by 0.07-7.25 ng/ml in other 12 patients (median 2.77 ng/ml). We revealed no relationship between these changes and initial content of total VEGF.

The mean content of free VEGF in BC was 4-5 times higher than in fibroadenomas and histologically unchanged mammary gland tissues (the latter difference was significant, Table 2). Differences in median values were less significant due to asymmetric distribution of these parameters in patients with BC. Nonparametric tests also revealed significant differences in median values for VEGF content between histologically unchanged and tumor tissues.

In 24 of 38 patients VEGF concentration in tumors was higher than in normal tissues. In 23 patients

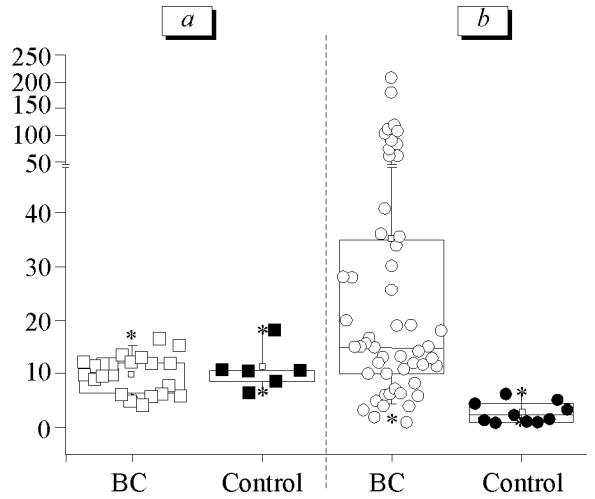

Fig. 1. Concentrations of total (ng/ml, *a*) and free vascular endothelial growth factor (pg/ml, *b*) in the serum from patients with breast cancer (BC) and healthy donors. Boxes corresponds to the range from the upper to the lower quartile. Whishers: 95% confidence interval.

TABLE 2. Free VEGF Concentration in Cytosols of Malignant Tumors, Fibroadenomas, and Histologically Unchanged Tissues of the Mammary Gland

Tissue	Free VEGF, pg/mg protein		
	range	*M*±*m*	median (quartiles)
Histologically unchanged tissue of the mammary gland (*n*=38)	7.47-116	42.50±5.17*	31.9** (15.6-64.2)
Fibroadenoma (*n*=7)	10.8-70.0	34.1±10.3	15.8** (11.4-60.2)
BC	0.57-2847	169±33.7	56.9 (21.1-173)

Note. *$p<0.05$ compared to BC (Student's t test); **$p<0.01$ compared to BC (Wilcoxon pairwise test).

(61%) VEGF concentration surpassed the normal by more than 30%. The maximum increase in VEGF concentration in tumor tissue was 2430% (median 370%). In 11 patients (30%) VEGF content in histologically unchanged mammary gland tissue increased by more than 10%. The maximum increase in VEGF concentration in histologically unchanged tissues was 69% (compared to tumor tissue, median 25%). A positive correlation was found between VEGF contents in histologically unchanged tissue and tumors (R=0.54, $p<0.001$).

In the practical aspect it is important to evaluate whether VEGF concentration in the blood reflects its production in the tumor tissue, because the correlation between these parameters allows noninvasive evaluation of the intensity of angiogenesis in tumor tissue. A positive correlation was found between the concentrations of free VEGF in tumor cytosols and serum from patients with BC (R=0.32, $p<0.05$, Fig. 2). However, we revealed no correlation between the concentrations of free VEGF in the tumor tissue and serum (R=0.12, $p>0.05$).

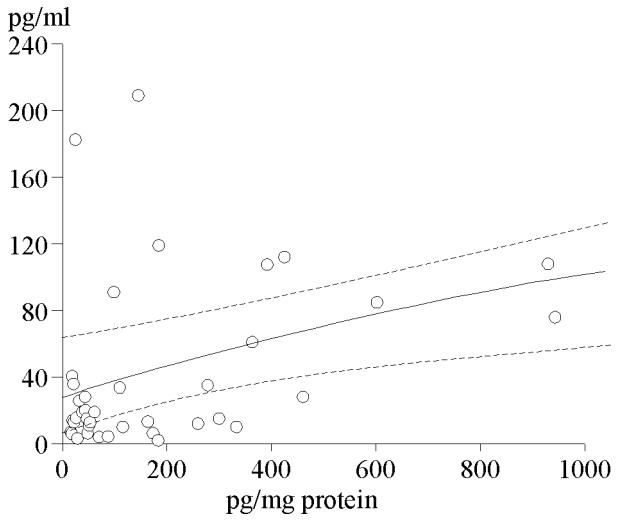

Fig. 2. Relationship between the concentrations of free vascular endothelial growth factor (VEGF) in tumor cytosols (abscissa) and serum (ordinate) from patients with breast cancer (R=0.32, $p<0.05$, Spearman rank correlation test).

It can be hypothesized that serum concentration of free VEGF reflects its production in tumor tissue. However, not in all patients the concentration of free VEGF in the serum decreased after removal of the tumor. No relationship was revealed between the degree and directionality of changes and free VEGF concentration in tumor tissue. The concentration of VEGF could decrease or increase at high and low contents of free VEGF in tumor tissue.

In more than 50% patients with BC the intensity of VEGF production in tumor tissue was higher than in histologically unchanged mammary gland tissue. The content of free VEGF in the serum surpassed the normal in more than 80% patients with BC. It should be emphasized that blood concentration of free VEGF in most patients did not return to normal 2-14 days after removal of primary tumors. Moreover, this parameter increased in 50% patients. The changes in these parameters did not depend on their initial values. The total VEGF concentration in the serum from patients with BC did not surpass the normal and remained practically unchanged after removal of the tumor. These results indicate that free VEGF concentration in the serum from patients with BC more adequately reflects the state of angiogenesis-regulating systems in tumor tissue than total VEGF content. The concentration of total VEGF is probably a sum of different constituents and does not reflect the intensity of angiogenesis in tumor tissue.

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