REVIEWS

Role of Vascular Endothelial Growth Factor during Breast Cancer

N. E. Kushlinskii and E. S. Gershtein

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 604-612, June, 2002
Original article submitted February 18, 2002

We review the results of experimental and clinical observations on neoangiogenesis in patients with breast cancer. Vascular endothelial growth factor is an important positive regulator of this process. Experiments showed the possibility of using various direct and indirect antiangiogenic means in the therapy of breast cancer, but clinical efficiency of these methods was not proved. Expression of vascular endothelial growth factor can serve as a prognostic criterion in breast cancer. Antiangiogenic preparations should not be used as monotherapy, but as the treatment complementary to standard therapy.

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

General Concept on the Regulation of Angiogenesis

Angiogenesis is a complex process that proceeds in 4 stages: proteolysis of the basal vascular membrane and intercellular matrix, migration and fixation of endothelial cells, their proliferation, and formation of tubular structures [60]. Until recently the density of blood vessels in tumor tissue (microvascular density) evaluated by microscopy served as the major criterion of the intensity of neoangiogenesis. Studies of the molecular mechanisms of angiogenesis showed that the formation and propagation of new vessels in tumors depend on the dynamic balance between various regulatory angiogenic and antiangiogenic factors.

Vascular endothelial growth factor (VEGF), or vascular permeability factor, is the main positive regulator of angiogenesis. Unlike other factors, VEGF produces its mitogenic effect only on endothelial cells. VEGF is a homodimeric abundantly glycosylated protein with a molecular weight of 46-48 kDa. There are at least 5 isoforms of VEGF that display the same biological activity, but differ in biological availability [20]. This characteristic is determined by the size of molecules and regulated at the gene (alternative splicing of mRNA) and epigenome levels (proteolytic cleavage of synthesized molecules with the involvement of plasminogen activators). Molecules consisting of 121 and 165 amino acid residues are the main soluble and biologically active forms of VEGF. VEGF-165 is the major isoform of VEGF in tissues.

Three receptors for VEGF (receptor tyrosine kinases) are localized on the surface of endothelial cells. VEGF-1 receptor is the product of *flt-1* genes. VEGF-2 receptor receiving the name KDR is a human homologue of mouse *flk-1* genes. As differentiated from VEGF-1 and VEGF-2 receptors, VEGF-3 receptor (product of *flt-4* genes) interacts with the VEGF homologue (VEGF-C), but not with classic VEGF (VEGF-A). These receptors are transmembrane glycoproteins with a molecular weight of 170-235 kDa. For efficient binding, VEGF should interact with heparin-like components of the extracellular matrix (ECM).

Apart from mitogen-activated protein kinase cascade, which is common for most receptor kinases and

Laboratory of Clinical Biochemistry, N. N. Blokhin Russian Oncology Center, Russian Academy of Medical Sciences, Moscow

0007-4888/02/1336-0521$27.00

regulates expression of proliferation-associated genes, *c-ets-1* protooncogene encoding transcriptional factor Ets-1 is the most important gene controlled by VEGF in endothelial cells. *In situ* hybridization showed that *c-ets-1* is expressed in endothelial cells at the early stage of angiogenesis [76]. The product of this gene Ets-1 contributes to the expression of angiogenic phenotype in these cells. Ets-1 activates gene transcription and synthesis of proteins for proteases that cleave ECM, including urokinase plasminogen activator, stromelysin, collagenase 1, MMP-1, MMP-3, MMP-9, and β_2-integrin [65]. These effects are most pronounced 2 h after addition of VEGF and other angiogenic factors (acid and basic fibroblast growth factors and epidermal growth factor) and can be blocked with antisense oligonucleotides to *ets-1* [38]. Protease activation facilitates disintegration of endothelial cells and their invasion into the basal layer of vessels, potentiates generation of products formed after ECM degradation and promoting chemotaxis of endothelial cells, and initiates and mobilizes growth factors in ECM [56].

Role of VEGF in the Regulation of Angiogenesis during Breast Cancer

M. Toi *et al*. [70-72] showed that microvascular density and increase in this parameter evaluated by immunohistochemical staining for factor VIII-related antigen are higher in tumors with intensive staining for VEGF compared to those with weak staining for VEGF. VEGF is mainly localized in the cytoplasm of tumor cells. It was revealed that VEGF expression correlates with expression of other angiogenic factor, platelet-derived endothelial cell growth factor. VEGF concentration in highly vascularized tumors markedly surpasses that in low vascularized tumors [73,74]. No relationship was found between tissue levels of VEGF and other potentially angiogenic factors, including basic fibroblast growth factor and hepatocyte growth factor. Moreover, the concentrations of these factors do not correlate with microvascular density.

H. Yoshiji *et al*. [83] compared expression of VEGF, Flt-1 receptor, basic fibroblast growth factor, and platelet-derived growth factors-α and -β in breast cancer (BC) tissues and surrounding normal tissues of the mammary gland by means of immunohistochemical assay. Only expression of VEGF in tumor cells markedly surpassed that in normal cells. Studies with RNA hybridization technique showed that VEGF expression in tumor is higher than in normal tissues [2,3,29]. Moreover, VEGF expression increases in intraductal BC and positively correlates with activity of angiogenesis (as estimated by immunohistochemical staining for factor VIII-related antigen) [29].

H. Zhang *et al*. [85] first provided direct evidence for the effect of VEGF from BC cells on angiogenesis. The VEGF-121 gene was transfected into estrogen-dependent BC cells of line MCF-7. Expression and secretion of VEGF by transfected cells (V12) were confirmed by 3 independent methods: competitive radioreceptor analysis, *in vitro* stimulation of growth in human endothelial cells, and induction of angiogenesis in rabbit cornea. After transplantation of V12 cells to athymic nude mice they produced more vascularized tumors with the heterogeneous distribution of vessels compared to parent MCF-7 cells. V12 tumors grew more rapidly than MCF-7 tumors. It should be emphasized that the sensitivity of cells to tamoxifen and their hormone dependence remained unchanged.

Studies with antibodies against VEGF demonstrated its effects on the growth and metastasizing of BC. Experiments on mice with spontaneous BC characterized by high risk of lung metastases showed that polyclonal antibodies against VEGF inhibit tumor growth (by 44%) and decrease the incidence and size of lung metastases (by 73 and 84%, respectively) [77,78].

H. Lichtenbeld *et al*. [50] developed a method for estimating angiogenic activity of various mammary gland tissues *in vivo*. Samples of tumor and normal tissues from the mammary gland were placed in a chamber formed by the dorsal skin fold in athymic nude mice. The induction of angiogenesis was evaluated. BC samples and tissues of the mammary gland with hyperplasia and apocrine metaplasia markedly activated angiogenesis. In 66% patients with BC samples from histologically unchanged regions of mammary gland tissues stimulated angiogenesis. By contrast, mammary gland tissues obtained during cosmetic surgeries did not modulate angiogenesis. It should be emphasized that the induction of angiogenesis coincided with VEGF production by tumor cells or mammary gland cells.

The regulation of neoangiogenesis in BC and other tumors involves the paracrine system. In this system VEGF is produced by tumor cells. Receptors for VEGF that receive the signal are localized on vascular endothelial cells. L. Brown *et al*. revealed the existence of this paracrine system in BC [9]. Tissue samples from 68 patients with BC were assayed by *in situ* RNA hybridization. VEGF expression was high in cells of invasive, metastatic, and intraductal breast carcinomas. Vascular endothelial cells in these tumors expressed receptors for VEGF-1 and VEGF-2. Studies performed by A. Kranz *et al*. [43] produced similar results. Receptors for VEGF (KDR) were revealed also on epithelial cells of mammary ducts. There is evidence for the existence of VEGF receptors (Flt-1 and Flk-1) on BC cells. It should be emphasized that VEGF and Flk-1 receptor expression correlates with

the index of tumor cell proliferation estimated by expression of the Ki-67 antigen [80]. Published data show that tumor cells and stromal cells isolated from primary human breast carcinomas *in vitro* produce VEGF. The intensity of VEGF production in these cells is much higher than in cells from normal mammary gland [69]. The polymerase chain reaction (PCR) analysis showed that tumor cells mainly contain receptors for VEGF-2 (KDR/Flk-1), while stromal cells express only VEGF-1 receptors (Flt-1). These data indicate that in patients with BC, VEGF not only stimulates neoangiogenesis, but also acts as the autocrine/paracrine regulator of proliferation of tumor and/or stromal cells.

It was hypothesized that in patients with BC, VEGF interacts with Flt-1 receptors and stimulates migration of macrophages into the tumor tissue. Macrophages synthesize various angiogenic factors (*e. g.*, VEGF) and stimulate angiogenesis. A positive correlation was found between the index of tumor tissue infiltration by macrophages and intensity of VEGF expression [49].

VEGF secretion by BC cells is induced by various exogenous and endogenous factors. Cultured BC cells with various biological phenotypes markedly differ in the basal expression of VEGF mRNA and its sensitivity to stimuli. Hypoxia most efficiently induces VEGF expression in various cells, while steroid hormones do not modulate this process [67].

HIF-1 plays an important role in the stimulation of neoangiogenesis in hypoxia [8]. This transcriptional factor is induced during hypoxia. Considerable amounts of HIF-1 in BC tissues correlate with high index of proliferation and intensive expression of VEGF and estrogen receptors (ER). No relationship was found between expression of HIF-1 and VEGF in BC cells and expression of the apoptosis-inducing factor p53. However, the inhibitor of apoptosis Bcl-2 potentiated the stimulatory effect of hypoxia on VEGF synthesis in BC cells [5]. Hybridization assay showed that MCF-7 cells overexpressing Bcl-2, possessing high metastatic potential, and resistant to adriamycin are characterized by more intensive expression of mRNA for most angiogenic isoforms of VEGF (VEGF-121 and VEGF-165) compared to parent MCF-7 cells. *Bcl-2*-transfected cells *in vivo* formed tumors with a higher degree of vascularization and more intensive expression of VEGF than parent cells.

At the same time, VEGF that acts as the survival factor for endothelial cells not only stimulates their proliferation, but also inhibits apoptosis via the induction of Bcl-2 expression [59]. It should be emphasized that VEGF produced the same effect on BC cells. Therefore, VEGF acts as the antiapoptotic factor not only in endothelial, but also in tumor cells.

Various growth factors and signal molecules are involved in the regulation of VEGF expression in BC cells. L. Yen *et al.* [82] studied a panel of BC cell lines characterized by stable overexpression of a ligand-free receptor ErbB-2. Heregulin-β1 interacting with ErbB-3 and ErbB-4 receptors induced VEGF expression in most BC cells, but not in normal mammary gland cells. Basal secretion of VEGF was intensified in cells with high content of ErbB-2. In T47D cells with functionally inactivated ErbB-2, basal secretion of VEGF and its induction by heregulin were suppressed. The effects of heregulin on VEGF synthesis are realized via the classic signal pathway with phosphatidylinositol-3 kinase and protein kinase B (Akt) and induction of transcriptional factor HIF-1 that stimulates expression of the VEGF gene [6,47].

Several growth factors belonging to the family of platelet-derived growth factor-β probably regulate VEGF expression in BC cells. A positive correlation was found between the concentrations of platelet-derived growth factor-β_1 and VEGF in tumor tissues and plasma from patients with BC. In *in vitro* experiments platelet-derived growth factor-β_1 induced production of VEGF by cultured MDA-MB-231 cells [17]. Intensive expression of platelet-derived growth factor-β_2 and its receptors is typical of tumors with high microvascular density [13,14].

Hormonal regulation of VEGF synthesis in BC cells by sex steroids (particularly by estrogens) is still the controversial subject. Although estrogens induce VEGF-mediated angiogenesis in the endometrium [35], there is no evidence for the existence of this mechanism in BC. Experiments with cultured MCF-7 cells showed that 17β-estradiol (E_2) produces a biphasic increase in the synthesis of VEGF mRNA, which is accompanied by accumulation of the corresponding protein in the culture medium [62]. Anti-estrogen ICI 182.780 blocked this effect, which suggests that it is realized via ER. However, classic antiestrogens tamoxifen and toremifen producing the partial estrogenic effect did not abolish VEGF induction by E_2, but even initiated VEGF synthesis. Molecular and biological assays showed that ER are involved in the regulation of VEGF synthesis in BC cells. The VEGF gene included 2 sequences, which are homologous to estrogen-sensitive elements and specifically bind ER-α and ER-β [34]. The effects of estrogens and antiestrogens on VEGF synthesis probably depend on the type of BC cells. Published data show that *in vivo* growth of human BC KPL-1 cells was stimulated by ICI 182.780, but inhibited by E_2 [44]. E_2-propionate blocked angiogenesis and stimulated apoptosis in tumors containing KPL-1 cells. E_2 *in vitro* had no effect on VEGF synthesis and rate of cell proliferation. It should

be emphasized that VEGF expression in KPL-1 cells was induced by medroxyprogesterone acetate.

S. Hyder *et al*. [33,36] also demonstrated an inducing effect of progestins on VEGF synthesis in BC cells. Experiments with T47-D cells showed that progesterone dose-dependently increases VEGF content in the culture medium (by 3-4 times). Progesterone in a concentration of 10 nM produced the most pronounced effect. However, other steroid hormones (estrogens, androgens, and glucocorticoids) did not modulate VEGF production. Moreover, progestins had no effect on other BC cell lines, including hormone-dependent MCF-7 and ZR-75 cells and hormone-independent MDA-MB-231 cells. The effect of progesterone on T47-D cells was blocked by antiprogestin RU-486, which suggests that these changes are realized via the classic receptor mechanism. Interestingly, plasma VEGF level in women is low in the luteal phase of the menstrual cycle and inversely depends on plasma progesterone concentration [32]. The plasma obtained during this period was less potent than that taken in phase I of the menstrual cycle in stimulating VEGF production by MCF-7 cells.

Expression of VEGF-A isoforms in tumors and surrounding normal tissues of the mammary gland from 19 patients with BC was studied by PCR analysis. The intensity of VEGF expression in normal mammary gland of premenopausal patients far surpassed that in postmenopausal women. Moreover, expression of VEGF in normal tissues decreased with aging. However, VEGF expression in the tumor tissue did not depend on the age and menopausal status of patients [28]. These data suggest that angiogenesis in normal mammary gland is regulated by hormones, while during malignant transformation this control is lost.

Apart from best known and most abundant angiogenic factor VEGF-A, there are other factors belonging to the VEGF family (VEGF-B, VEGF-C, and VEGF-D). Functional activity of VEGF-C was extensively studied. This factor interacts with VEGF-3 receptors (Flt-4) on endothelial cells of lymphatic vessels and stimulates lymphangiogenesis. Experiments on athymic nude mice with a new marker of lymphatic endothelium LYVE-1 showed that overexpression of VEGF-C in BC cells contributes to the development of metastases in regional lymph nodes and lungs [68]. As differentiated from VEGF-A and VEGF-B present in BC tissues independently on the stage of the disease, VEGF-C is found only in tumors with lymph node metastases. VEGF-D was detected only in inflammatory BC tissues [44]. At the same time, VEGF-C receptor expression in invasive and intraductal breast carcinomas was higher than in normal mammary gland and fibroadenomas. However, VEGF-3 receptor expression increased in endothelial cells of blood vessels, but not in lymphatic vessels [75]. It was hypothesized that VEGF-C and VEGF-A act as angiogenic factors for blood vessels. It cannot be excluded that VEGF-C is involved in the regulation of lymphangiogenesis.

Thus, VEGF plays an important role in BC. This factor produces paracrine and autocrine effects on the endothelium of blood vessels, cells of tumors and tumor stroma, infiltrating macrophages, and cells of lymphatic vessels and stimulates the growth and spreading of tumors. These data indicate that VEGF can serve as a biological prognostic criterion of BC and the major target for antiangiogenic antitumor therapy.

Diagnostic Value of VEGF Assays in Patients with BC

Published data show that high content of VEGF is an independent predictor of poor prognosis in BC at the early stage and/or low sensitivity of patients with spread tumors to standard hormonal preparations and radio- or chemotherapy [2,12,19,21,24-27,29,51-53,55,57,61, 67,68,70,72,83]. It was important to evaluate whether various antiangiogenic preparations can be used for adjuvant therapy of patients with high VEGF concentration in tumor tissue. However, general methodical approaches and criteria that would allow us to reveal patients with high content of VEGF are still not elaborated.

Clinical value of high VEGF content in tissues from patients with BC attracts recent attention. In parallel with these assays, the following questions are studied: whether intensive expression of VEGF in tumor tissues affects the content of this protein in blood serum/plasma; and whether the concentration of circulating VEGF reflects its content in tumor tissues and activity of angiogenesis. In 1996-1997 the first experiments showed that blood VEGF level increases in patients with tumors. Examination of patients and donors (including 137 patients with BC) revealed that in 8.8% patients plasma VEGF content surpasses a threshold concentration of 180 pg/ml [81]. Plasma VEGF concentration correlated with the extent of tumor spread and VEGF expression in tumor tissues. VEGF-165 was the main isoform of VEGF in the plasma.

L. Dirix *et al.* [16] reported that VEGF level increases in 57% patients with untreated metastatic cancer independently on its localization. After therapy VEGF content increased in $^2/_3$ of the patients with progressive tumors and less than in 10% patients with favorable clinical course of the disease.

P. Salven *et al.* [64] examined patients with various tumors (including BC). They showed that in patients with disseminated cancer, plasma VEGF level is much higher than in healthy donors and patients

with local tumors. In 74% untreated patients with disseminated cancer, plasma VEGF concentration surpassed 200 pg/ml, but decreased after successful therapy. Studies of A. Kraft *et al*. [42] produced similar results. These authors reported that plasma VEGF concentration increases in 0-20 and 11-65% patients with local and metastatic tumors, respectively. It should be emphasized that in these observations VEGF level in healthy donors was much higher than that revealed previously. Studies of these authors and B. Zebrowski *et al*. [84] demonstrated that VEGF concentration in ascitic fluid from patients with tumors considerably surpasses that in patients with non-tumor ascites.

P. Salven *et al*. [63] showed that plasma VEGF level in patients with metastatic BC is much higher than in patients with benign tumors. VEGF concentration in patients with local BC surpassed that in patients with benign tumors (insignificantly). In patients with metastatic cancer treated with specific drugs VEGF content was lower than in patients receiving only symptomatic therapy. Interestingly, plasma VEGF level in patients with locally spread invasive ductal carcinomas was significantly higher than in patients with invasive lobular carcinoma. It should be emphasized that in the latter group this parameter was lower than in patients with benign tumors. These results are consistent with published data that in ductal BC tissues the concentrations of mRNA and VEGF are higher than in lobular carcinoma tissues [48]. Ductal and lobular carcinomas did not differ in microvascular density. Only in ductal carcinoma tissues a positive correlation was found between mRNA and VEGF contents and vascular density. It can be suggested that VEGF regulates angiogenesis primarily in ductal BC tissues.

The contents and expression of VEGF were compared in tumor tissues and plasma. G. Callagy *et al*. [10] assayed tissue expression of VEGF by immunohistochemical methods. The authors revealed that this parameter, but not plasma VEGF concentration, correlates with microvascular density and stage of BC. Therefore, tissue expression of VEGF is a more reliable prognostic factor than plasma VEGF concentration. No relationship was found between plasma level of VEGF and its expression in tumor tissues.

J. Adams *et al*. [1] immunohistochemically measured VEGF content in the serum and plasma and VEGF expression in tissues from 201 patients with local and spread BC and benign tumors of the mammary gland and healthy women. In patients with metastatic BC, VEGF content in the plasma and serum surpassed normal. Plasma VEGF concentration in patients with metastatic BC was higher than in patients with benign tumors and local BC. In patients with local BC only plasma VEGF level surpassed the control. These data suggest that plasma concentration of VEGF more adequately reflects its production in tumor tissues, since serum VEGF is primarily released from platelets. Surprisingly, VEGF concentration in the plasma and serum was highest in BC patients during remission receiving tamoxifen. No correlation was found between the concentration of circulating VEGF and any of the prognostic factors, including microvascular density and tissue expression of VEGF.

These data indicate that there is no evidence that the measurements of VEGF concentrations in the serum and plasma can be used instead of assays of tissue protein expression to study angiogenesis, make prognosis of the disease, and evaluate the efficiency of therapy.

VEGF-Dependent Angiogenesis as the Target for Antitumor Therapy during BC

Angiogenesis plays an important role in the growth and spreading of BC, and VEGF is a key factor involved in this process. Therefore, directional inhibition of FEGV expression and/or blockade of its effects hold much promise for the elaboration of new approaches to adjuvant therapy of this disease [30,31]. Substances nonspecifically blocking the interaction between various growth factors and tyrosine receptors (*e.g.*, less toxic suramin analogues) were proposed as antiangiogenic agents. These agents should be used in combination with compounds initiated under hypoxic conditions, since the inhibition of angiogenesis promotes their activation.

At present more than 20 angiogenic modulators undergo phase I clinical trials in USA [40].

J. Folkman [22] classified antiangiogenic preparations into direct and indirect inhibitors of angiogenesis. Various substances producing direct effect on endothelial cells, including angiostatin, endostatin, TNP-470, and combrestatin, and natural inhibitors of angiogenesis thrombospondins and pigment epithelium-derived factor are direct inhibitors of angiogenesis. The distinctive feature of these preparations is that they do not cause the resistance in endothelial cells and, therefore, may be used for a long time. Indirect inhibitors of angiogenesis are presented by preparations that modulate production of angiogenic factors by tumor cells or block their effects at various stages of the process. These preparations include monoclonal antibodies or antisense oligonucleotides to VEGF and its receptors. Since the effects of indirect inhibitors are directly associated with tumor cells or their ability to produce angiogenic factors, the probability of resistance to these preparations and traditional antitumor agents is similar.

The preparation with an unknown mechanism of the antiangiogenic influence, thalidomide, was first

introduced as a sedative in the 1970s and then forbidden due to the teratogenic side effect. Now this preparation undergoes phase II clinical trials as the antitumor drug [18]. Patients with BC did not display any objective reaction to thalidomide in a dose of 100 mg. However, partial response or stabilization was observed in 6 of 18 patients with renal cancer.

Previous experiments with the induction of angiogenesis in rabbit cornea by VEGF-producing MCF-7 cells showed that the thalidomide analogue linomide in a dose of 100 mg/kg efficiently inhibits this process [86]. Monoclonal anti-VEGF antibodies MV833 suppress the growth of human BC xenotransplants in athymic nude mice [4]. However, no correlation was found between the inhibitory effect of MV833 and amount of VEGF secreted in tumor tissues or expression of VEGF receptors. Experiments with transplantation of spheroids consisting of BC cell lines MCF-7, ZR-75, and SK-BR-3 into the subcutaneous dorsal chamber in athymic nude mice demonstrated that monoclonal anti-VEGF antibodies A4.6.1 in a daily dose of 200 μg markedly suppress angiogenic activity of these cells and promote the antitumor effect of doxorubicin [7].

ZD4190, low-molecular-weight specific inhibitor of tyrosine kinase in VEGF-1 and VEGF-2 receptors displayed considerable antitumor activity on BC heterografts [79]. However, peroral administration of this preparation in doses producing no direct antiproliferative effect on tumor cells markedly inhibited the growth of tumors with a size of 0.5 cm^3. Tyrosine kinase inhibitor ZD1839 (Iressa) acting as a selective inhibitor of tyrosine kinase of endothelial growth factor receptors also possessed antiangiogenic activity in relation to BC heterografts [11]. It is believed that this preparation inhibits VEGF synthesis induced by ligands of the receptor for endothelial growth factor, but does not produce the direct effect on VEGF receptors. Probably, humanized monoclonal antibodies against ErbB2/neu also indirectly modulate angiogenesis [39].

Preparations that produce functional disturbances in microtubules act as potent inhibitors of angiogenesis. 2-Methoxyestradiol and Taxol possessing these properties inhibit VEGF-induced angiogenesis by 54 and 37%, respectively [1]. 2-Methoxyestradiol suppresses the growth of human BC implanted to athymic nude mice by 60%. Experiments on mice with highly vascularized breast tumor Met-1 demonstrated antiangiogenic activity of Taxol [46]. Taxol in noncytotoxic daily doses of 3-6 mg produced an antiangiogenic effect, which was associated with inhibition of VEGF secretion.

These properties of Taxol were used to evaluate the efficiency of therapy in patients with metastatic BC [54]. Observations were performed on 14 patients receiving 3 courses of monotherapy with Taxol (175 mg/m^2 intravenously) for 21 days. Plasma VEGF concentration was measured by enzyme immunoassay before and after each course of the therapy. Partial response to the therapy, stabilization, and progression of the disease were observed in 3, 6, and 5 patients, respectively. Plasma VEGF level before the therapy was high in 8 of 14 patients. The mean concentration of VEGF decreased after treatment with Taxol in patients with partial response and stabilization, but remained unchanged in patients with progression of the disease. It should be emphasized that the number of patients, in which VEGF content returned to normal or decreased more than by 50%, was much higher in the group with partial response (5 of 9 patients) than in the group with progression of the disease (none of 5 patients). It was hypothesized that the stabilizing effect of Taxol in patients with invasive tumors is associated with inhibition of VEGF secretion and blockade of angiogenesis.

Experiments on immunodeficient mice showed that gene therapy can be used to suppress angiogenesis and inhibit BC growth [15]. The plasmid containing genes for the natural inhibitor of angiogenesis endostatin was introduced 2 times into mouse Mca-4 tumors at a 7-day interval. Fourteen days after the first treatment tumor weight decreased by 51% compared to the control. These changes were accompanied by an increase in the distance between tumor cells and adjacent vessels, decrease in the total density of vessels, and intensification of apoptosis in tumor cells containing and expressing the endostatin gene.

Another approach to antiangiogenic therapy of BC is the use of antisense cDNA for VEGF. S. A. Im *et al*. [37] transfected human BC cells MDA231-MB with the adenoviral vector containing cDNA for VEGF-165 (Ad5CMV-alphaVEGF). Transfection *in vitro* inhibited VEGF secretion, but had no effect on the growth of cells. *In vivo* injection of Ad5CMV-alphaVEGF in tumors formed by MDA231-MB cells in athymic nude mice was followed by the inhibition of their growth, suppression of VEGF expression in the tumor tissue, and decrease in microvascular density (compared to animals receiving the vector without anti-VEGF cDNA).

Experimental data indicate that various direct and indirect antiangiogenic methods can be used for the therapy of patients with breast cancer. However, no evidence for clinical efficiency of these techniques was provided. Most authors believe that indirect and, particularly, direct antiangiogenic preparations producing the cytostatic (not cytotoxic) effect in patients with large tumors should not be used as monotherapy, but are complementary to standard therapy.

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