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REVIEW

Molecular Mechanisms of Tumor Angiogenesis

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Abstract—The maintenance of growth of malignant tumors is closely related with the development of the vascular network supplying the tumor with blood. The vascularization of tumor tissue is similar to physiological angiogenesis, but in tumors it has some specific features. During the last 25 years a vast number of biomolecules have been found and described which are involved in the regulation of tumor angiogenesis. This review considers the action mechanisms and specific features of expression of the main angiogenic growth factors, such as the vascular endothelium growth factor (VEGF), angiopoietins (Ang-1, Ang-2), and the basic fibroblast growth factor (bFGF). The roles of cytokines, growth factors, proteolytic enzymes, and cell adhesion molecules in the regulation of the key steps of blood vessel generation in the tumor are considered. The significance of angiogenesis in the treatment of oncological diseases and possible approaches for inhibition of the regulatory signals of angiogenic factors are discussed.

Key words: tumor angiogenesis, vascularization, extracellular matrix, growth factors, endothelial cell proliferation, endothelial cell migration, proteolytic enzymes, cell adhesion molecules

The initial step in malignant transformation is associated with the appearance in the tissue of cells with an increased index of proliferative activity as compared to normal cells. An increase in the number of transformed cells results in tissue hyperplasia. At this step the transformed cells lose their tissue-specific features, and this results in production of atypical poorly differentiated cells which have no analogs among the cells of normal tissues. One of the main events which determines the transition of a heterogeneous population of transformed cells to a continuously progressing population which gives rise to solid tumors is a development in the tumor tissue of blood capillaries (vascularization) [1].

The close relationship between tumor progression and its vascularization was first hypothesized by Folkman in the 1970s [2]. Experimental studies on tumor development have clearly shown that at the tumor size more than 1-2 mm^3 its growth can continue only with the active invoking of new blood capillaries, and changes in the blood supply of the tumor dramatically affect its growth and metastasizing [3]. Therefore, tumor angiogenesis, i.e., the generation of new capillaries from the preexisting blood vessels, has been actively studied during the subsequent years.

Tumor angiogenesis is dependent on biochemical processes mediating the formation and development of the blood capillary network which supplies the tumor. Tumor angiogenesis is similar to physiological angiogenesis but has some specific features. Tumor angiogenesis includes two main phases, the activation phase and the formation phase [4]. During the first phase the growth of blood capillaries is initiated due to stimulation with angiogenic growth factors of endothelial cells which cover the internal wall of a blood vessel, due to destabilization of the preexisting vessel caused by retraction of adventitial cells and pericytes, and to local degradation of the basal membrane of the preexisting vessel. Afterwards, the endothelial cells migrate into the extracellular matrix that is accompanied by an active proliferation of these cells and by formation of a "vascular bud" from which capillaries are generated. During the formation phase the

Abbreviations: VEGF) vascular endothelium growth factor; bFGF, aFGF) basic and acidic fibroblast growth factors, respectively; Ang-1, Ang-2) angiopoietins; uPA) urokinase; tPA) tissue activator of plasminogen; IL-1, IL-6, IL-8, IL-12) interleukin-1, -6, -8, -12; TNF-α) tumor necrosis factor α; IGF) insulin-like growth factor; TGF-α, -β) transforming growth factor α, β; PDGF) platelet growth factor; TIMP) tissue inhibitors of metalloproteinases; MMP) matrix metalloproteinases; ECM) extracellular matrix; IFN-γ) interferon γ; HGF) hepatocyte growth factor; PLGF) placental growth factor; EGF) epidermal growth factor; PD-ECGF) platelet-derived endothelial cell growth factor; ICAM, VCAM) adhesion molecules of the immunoglobulin superfamily.

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migrating endothelial cells are structurally organized to produce capillary-like structures, mature capillaries are generated, and the blood flow is initiated.

Specific features of the structure of tumor blood vessels at different stages of their formation are determined by expression levels and by interactions of various angiogenic factors [5]. The realization of autocrine and paracrine mechanisms regulating the levels of angiogenic factors secreted by endothelial cells and the tumor stroma cells results in formation of "local vascularization foci" where the tumor tissue is actively developing blood capillaries [6].

Angiogenesis has great physiological and medical importance in the appearance and development of oncological diseases, and this is the reason for a constant interest in closer studies of biological effects of the known angiogenic factors and also the search for new effectors of angiogenesis and for approaches to purposefully influence this process.

ROLE OF ANGIOGENIC GROWTH FACTORS IN STIMULATION, PROLIFERATION AND MIGRATION OF ENDOTHELIAL CELLS

The vascularization of malignancies is accompanied by an increased secretion by the tumor cells of a number of angiogenic factors which either directly interact with the endothelial cells and stimulate their proliferative and migrational activities, or act indirectly through activation of other cells involved in angiogenesis.

During the 25 years of studies on tumor angiogenesis many effectors have been found and characterized that influence the formation of the blood vessel network in tumors. The angiogenesis can be regulated by compounds of various structures and biological activities produced by both the tumor cells and the cells involved in tumorigenesis: lymphocytes, macrophages, mast cells, endothelial cells, etc. (Table 1).

In searching for approaches to selectively regulate tumor angiogenesis, special attention is now given to studies on specific angiogenic factors that have receptors located only on endothelial cells. The most promising and intensively studied angiogenic factors include VEGF, angiopoietins Ang-1 and Ang-2, and the basic fibroblast growth factor (bFGF) which plays an important role in the stimulation and development of the tumor angiogenesis but is not a specific mitogen for endothelial cells.

Vascular endothelium growth factor (VEGF). VEGF was first isolated from the supernatant fluid of a tumor homogenate as a protein responsible for increased permeability of peritumoral vessels. Therefore, it was initially called the vascular permeability factor [7]. Later this factor was found to be a specific mitogen for endothelial cells, and in this connection it was given its actual name of vascular endothelium growth factor (VEGF) [8].

The VEGF gene was cloned in the early 1980s, and this stimulated intensive studies on physicochemical and biological features of this growth factor. VEGF is a homodimeric glycoprotein with molecular weight of 34-46 kD, with monomers bound by two disulfide bonds [9]. Five isoforms of the human VEGF are known (VEGF-121, VEGF-145, VEGF-165, VEGF-189, VEGF-206) which are different in the number of amino acids in the peptide chain and are products of the alternative splicing of mRNA of the single gene. VEGF-165 (later VEGF) is the predominant isoform which is secreted by the majority of normal and tumor cells [10]. Unlike VEGF-121 and VEGF-165 which are mainly detected as solubilized forms, the high-molecular-weight VEGF isoforms (VEGF-185, VEGF-206) are found as strong complexes with heparin on the external surface of the vascular endothelium [11].

Three genes homologous to the gene of VEGF have been recently identified. Based on homology of the primary structure and similarity of biological functions, the products of these three genes (placental growth factor (PLGF) [12], VEGF-B [13], VEGF-C [14], and VEGF-D [15]) are combined in the family of VEGF-proteins (VEGF, VEGF-B, VEGF-C, VEGF-D, and PLGF). The mitogenic effect of VEGF-proteins on endothelium is lower than the mitogenic effect of VEGF, but the proteins of this family also play an important role in the regulation of angiogenesis and can exist as physiologically active both homodimers and heterodimers with VEGF [14].

Biological effects of the VEGF family proteins are mediated through four receptors of high affinity (flt-1, flk-1/KDR, flt-4, and neuropillin-1 (NP-1)). The receptors flt-1, flk-1/KDR, and flt-4 are classical tyrosine kinase receptors with the extracellular domain containing seven immunoglobulin-like regions [16-18] (Fig. 1). The flt-1 and flk-1/KDR are expressed on the surface of the activated cells of blood vessels [19], whereas the flt-4 receptor is found on the surface of various cells, in particular, on the endothelium of lymphatic capillaries [18]. The interaction of VEGF with a corresponding receptor results in dimerization of the latter followed by autophosphorylation of tyrosine residues and by start-up of the cascade of intracellular events responsible for the physiological response of the cell to the external stimulus. The interaction of VEGF with the flk-1/KDR receptor mediates the differentiation of endothelial cells, whereas binding of VEGF to the flt-1 receptor stimulates the proliferative activity of endothelium and regulates the intercellular interactions during the generation of blood capillaries [20]. The activation of the flt-1 and flk-1/KDR receptors concurrently with their expression on the surface of endothelial cells results in secretion by the endothelial cells of plasminogen activators (uPA, tPA), plasmin, and collagenase, and this is responsible for an invasive phenotype of the peritumoral endothelium and

Table 1. Angiogenic factors

Angiogenic factors	Involvement of angiogenic factors in regulation of the main stages of angiogenesis					Cells which produce angiogenic factors in the tumor vascularization focus
	proliferation of endothelium	migration of endothelium	differentiation of endothelium	remodeling of ECM	formation of capillaries	
1	2	3	4	5	6	7
Peptide growth factors						
VEGF	+	+	+	+	+	tumor cells, endothelial cells, macrophages
aFGF, bFGF	+	+	+	+	+	tumor cells, endothelial cells, macrophages, fibroblasts
PLGF	±	+	?	—	—	tumor cells, endothelial cells
Ang-1	—	+	+	+	+	tumor cells, endothelial cells
Ang-2	—	—	—	+	—	tumor cells, endothelial cells
PDGF	+	+	+	—	+	macrophages, platelets
EGF	+	+	+	—	—	tumor cells, endothelial cells
IGF-I	+	+	+	—	—	tumor cells, endothelial cells
HGF	+	+	+	+	—	tumor cells, endothelial cells
TGF-α	+	+	+	—	—	tumor cells, endothelial cells, macrophages
TGF-β	suppresses	—	+	+	+	tumor cells, endothelial cells, macrophages, T-lymphocytes
Mediators of inflammatory reaction						
TNF-α	suppresses	—	+	+	—	tumor cells, macrophages
IL-8	+	+	?	—	—	tumor cells, endothelial cells, neutrophils, mononuclear cells
IL-1	±	±	—	—	?	endothelial cells, macrophages
Hormones						
estrogen	+	+	+	—	—	tumor cells

Table 1. (Contd.)

1	2	3	4	5	6	7
proliferin	?	+	?	—	—	tumor cells
Enzymes						
metalloproteinases	—	+	—	+	—	tumor cells, macrophages
urokinase	—	+	—	+	—	endothelial cells
thymidine phosphorylase	+	+	—	+	—	tumor cells, platelets
Cell adhesion molecules						
VCAM-1	—	+	?	—	+	endothelial cells
E-selectin	—	+	+	—	+	endothelial cells
$\alpha_v\beta_3$-integrins	+	+	?	—	+	endothelial cells

the migration of endothelial cells [21, 22]. The binding of VEGF-B to flt-1 also stimulates the migration of endothelial cells [23]. The interaction of VEGF-C and VEGF-D with the flt-4 receptor stimulates the lymphangiogenesis [24]. The neuropillin-1 (NP-1) receptor of VEGF is located on the surface of neuronal cells and belongs to the family of collapsin-semaphorin proteins [25]. The physiological role of the VEGF reception by neuropillin-1 is not yet clear. NP-1 binding to VEGF increases the migration of endothelium induced by VEGF [26]. Thus, the interaction of the VEGF family proteins with their receptors has a combined effect on angiogenesis providing the start-up and regulation of some important physiological processes.

Tumor endothelium is characterized by an increased expression of the VEGF receptors flt-1 and flk-1/KDR on the cell surface [20]. In most tumors VEGF regulates the angiogenic activity of the endothelial cells mainly by a paracrine mechanism, i.e., through secretion by the tumor of VEGF which is bound to its receptors on the surface of adjacent endothelial cells. In contrast, in angiosarcomas the expression of VEGF is regulated by an autocrine mechanism which is represented by both the expression of the growth factor itself and its receptors (flt-1, flk-1/KDR) on the surface of endothelial tumor cells [27].

It was shown in some studies of effects of artificial microenvironment on vascularization of implanted tumors that the modeling of stress conditions caused by gradients of temperature, pH, and interstitial pressure inside the tumors induced the expression of VEGF by the tumor cells [28-30]. One of the main factors inducing the expression of VEGF by tumor cells is hypoxia, which often occurs during the growth of prevascularized tumors [31]. The secretion of VEGF by the tumor in response to hypoxia is stimulated by the expression of the hypoxia-induced factor (HIF-1) which activates the transcription of the gene encoding VEGF [31]. The maintenance of the increased expression of the VEGF-encoding gene in tumor cells is associated with its regulation by a number of oncogenes and protooncogenes (raf [32], fos [33], src [34], etc.) activated by the tumor. An activation of the ras-oncogene in tumor cells plays an important role in the regulation of VEGF expression [35]. The Ras-induced transformation of various cell strains is shown to mediate increased expression of VEGF. In addition to the involvement of oncogenes in the stimulation of VEGF expression, an inactivation of the tumor suppressor p53 in the tumor cells also stimulates the expression of the VEGF gene, which is normally inhibited by this suppressor [36]. Cytokines (IL-1, IL-6, TNF-α, etc.) and growth factors (EGF, IGF, TGF-α, TGF-β, etc.) are also extracellular regulators of the VEGF expression, and their regulating effects are realized by binding to the corresponding receptors on the tumor cell surface [37-39].

VEGF is directly involved in the induction of the tumor angiogenesis, it activates the endothelium of peritumoral vessels, and it stimulates the proliferation, migra-

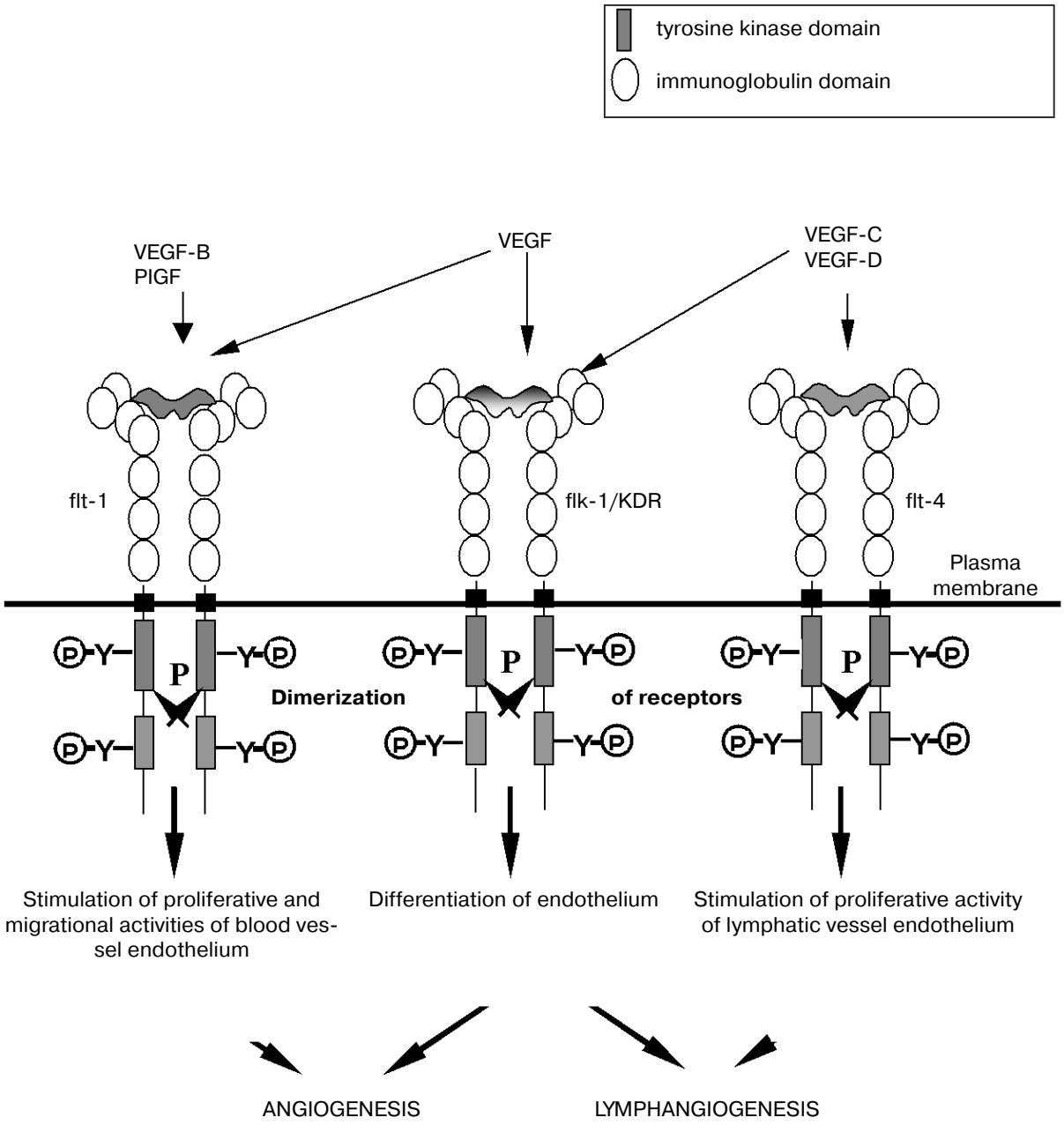

Fig. 1. Role of the VEGF family members and of their receptors in angiogenesis and lymphangiogenesis. The binding of the ligand VEGF results in dimerization of the receptors with subsequent autotransphosphorylation and activation of the signal-transmitting pathway (placental growth factor (PLGF)).

tion, and differentiation of endothelium cells. VEGF was recently found to induce the expression of some anti-apoptotic factors (bcl-2, A-1) and of survivins in proliferating endothelial cells, therefore, VEGF is suggested to be a factor of "viability maintenance" of endothelium during vascular remodeling of the tumor tissue [40, 41]. In its turn, VEGF expression in the tumor tissue stimulates the secretion by macrophages and by other cells involved in the tumorigenesis of growth factors (bFGF, TGF-β1, PDGF, etc.) which play an important role in formation of new blood capillaries [42]. And the angiogenic effect of VEGF significantly increases in the presence of growth factors nonspecific for the endothelium. In particular, a synergism of bFGF and VEGF is shown in the *in vitro* stimulation of proliferation and migration of endothelial cells [43].

The increased expression of VEGF in the majority of strongly vascularized human tumors (hemangioblastoma, lung carcinoma, melanoma, glioma, etc. [44-47]) closely correlates with the intensity of the tumorigenesis and the biological aggressiveness of the tumor. VEGF hyperexpression in the tumor sharply increases the permeability of the adjacent blood vessels, and this promotes the penetration of the tumor cells into the bloodstream, their dissemination in the body, and generation of multiple metastasizing foci [48]. On the other hand, an increased permeability of the tumor blood vessels provides accumulation of fibrin in the tumor tissue and makes prerequisites for formation of the tumor stroma [6]. Therapeutic approaches in oncology based on inhibition with monoclonal antibodies of the angiogenic effect of VEGF and of the expression of VEGF and its receptor seem promising to sharply slow down or arrest the vascularization and to suppress the tumor growth.

Angiopoietins. In addition to VEGF, which is a central factor in angiogenesis stimulation in normal and tumor tissues, a recently found family of other specific angiogenic growth factors, angiopoietins, is a subject of current interest. Unlike VEGF, the identification of angiopoietins was preceded by the finding in 1992 of their receptors Tie-1 and Tie-2, which are located on the endothelial cell surface and play the key role in embryonal vasculo- and angiogenesis [49]. Both receptors are transmembrane proteins that display tyrosine kinase activity and contain amino acid sequences homologous to certain regions of molecules of immunoglobulins and of the epidermal growth factor [50]. Later angiopoietin-1, the first ligand of the Tie-2 receptor, was identified [51]. At present, four proteins (Ang-1, Ang-2, Ang-3, and Ang-4) are combined into the angiopoietin family based on homology of their amino acid sequences [52].

Ang-1 is a glycoprotein with molecular weight of ~70 kD and consisting of 498 amino acids. The amino acid sequence of Ang-1 includes fragments homologous to myosin (a fragment of the amino acid sequence between residues 100 and 280) and to the carboxy-terminal fragment of fibrinogen (amino acids 280-498) [51]. Ang-1 is suggested to be a multimeric protein stabilized by intramolecular disulfide bonds [51]. In the adult organism Ang-1 is mainly located on the surface of blood vessel endothelium where it exists in complex with the receptor Tie-2 [53].

An important role of Ang-1 in angiogenesis is clearly shown by experiments on inactivation of the gene which encodes Ang-1; this inactivation is associated with a decrease in the number of large vessels, the thinning of walls of mature blood vessels, and disintegration of the blood vessel network [54]. Ang-1 is directly involved in maturation and stabilization of blood vessels by attracting pericytes and smooth muscle cells into the perivascular space of the blood vessel wall formed by a layer of "young" endothelial cells [54, 55]. Many authors consider Ang-1 to be a kind of "survival factor" of the endothelial cells which are involved in the formation of new blood capillaries because it prevents their elimination by apoptosis. An antiapoptotic effect of Ang-1 [56] on proliferating endothelial cells is shown to be due to induction of expression of the apoptosis inhibitor survivin. At present, cellular regulatory mechanisms mediating the biological effect of Ang-1 are insufficiently studied.

The discovery in 1997 by Maisonpierre of another member of this family, angiopoietin-2 (Ang-2), was a significant stage for studies on the role of angiopoietins in the regulation of angiogenesis [57]. Ang-2 is a glycoprotein consisting of 496 amino acids which has 60% structural homology to Ang-1 [57]. Unlike Ang-1, Ang-2 is mainly expressed in organs and tissues with intense vascular remodeling: ovaries, uterus, and placenta [57]. The Ang-1 receptor Tie-2 is also a receptor for Ang-2. However, the Ang-2 binding to Tie-2 fails to activate the receptor; therefore, Ang-2 is suggested to be a competitive inhibitor of the signal transmitted by Ang-1 [57].

Angiopoietins play the key role in the vascular remodeling of tissues, and their angiogenic effects are closely related with VEGF (Fig. 2). During embryogenesis VEGF is involved in the regulation of proliferation and differentiation of endothelial cells and also in the formation of young immature vessels. For the further maturation of blood vessels an important role is played by the interaction of Ang-1 with Tie-2 that mediates the attraction of cambial cells of the connective tissue into the vascular adventitium. In the resting endothelium of mature blood vessels Ang-1 is bound to Tie-2. In the vascularization focus caused by a physiological or pathological process the expression of Ang-2 is increased as compared to that of Ang-1 [57]. The interaction of Ang-2 with Tie-2 inhibits the signal transmission from Ang-1 and destabilizes the preexisting blood vessels due to removal of mesenchymal cells which are coating the external layer of the vessel [58]. On the increased expression of VEGF and other angiogenic factors this facilitates the formation of a "vascular bud" and promotes an activation of the vascular remodeling of the tissue. In the absence of stimulation of endothelial cells with VEGF but on the increased expression of Ang-2, the connective tissue bed of blood vessels is thinned, finally resulting in their complete regression (Fig. 2). Under physiological conditions, this mechanism seems to maintain a certain density of the blood vessel network which is responsible for the adequate blood supply of organs and tissues.

Holash et al. [59] studied the morphology of walls of the blood vessels infiltrating the strongly vascularized tumor glioma and found disorders: the adventitial cells constituting the vascular walls were absent and the endothelial cells were partly eliminated by apoptosis. On the assumption that Ang-2 was involved in these events, the authors studied the expression of Ang-2 and of Ang-1 during the tumor progress. The active expression of Ang-2 by endothelial cells of the blood vessels infiltrating the tumor was found even at

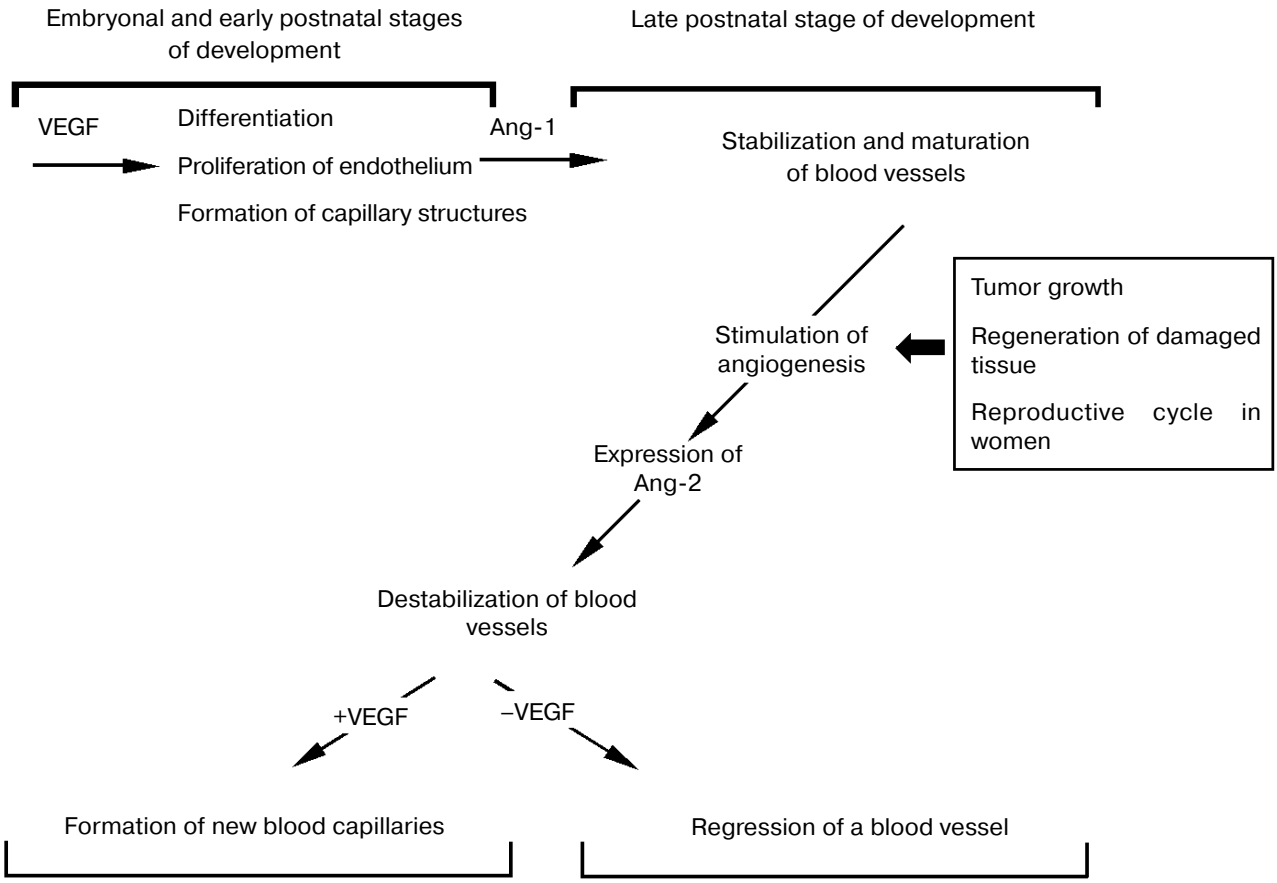

Fig. 2. Interaction of angiopoietins (Ang-1, Ang-2) and the vascular endothelium growth factor (VEGF) during angiogenesis.

early stages of the tumor development. Binding to Tie-2 also actively expressed on the tumor endothelium surface, Ang-2 inhibited the receptor and, consequently, the transmission of the "survival" signal from Ang-1 in the endothelial cells, and this resulted in the observed regression of the blood vessels adjacent to the tumor. The authors suggested that the blood vessel regression at early stages of the tumor development should be due to the switching of natural protective mechanisms of the body in response to the tumor signals which had to induce an engagement of blood vessels. The destruction of blood vessels should lead to disorders in the blood supply of the tumor and development of hypoxia in the tumor tissue. Under conditions of hypoxia, the tumor cells should start an active expression of VEGF, which is a powerful inhibitor of vascularization. The combined effect of VEGF produced by the tumor cells and Ang-2 secreted by the endothelium should dramatically stimulate the vascularization of the tumor and its rapid progress. Thus, a hypothetical mechanism of the organism's counteraction to the tumorigenesis by destruction of the tumor vessels is insufficiently effective and is overcome by the tumor.

Unlike the increased expression of Ang-2 during the whole tumorigenesis, Ang-1 expression in the tumor is relatively poor [60]. This is manifested by a poor development of the walls of the tumor blood vessels which lack pericytes and smooth muscle cells; the vessels are strongly fenestrated and highly permeable for the tumor cells. The content of angiopoietins in the tumor tissue can be considered a marker of the angiogenesis intensity in the tumor. Therapeutic strategies based on inhibition of the VEGF expression concurrently with an increase in the Ang-2 secretion are promising for effective suppression of the tumor vascularization in the treatment of oncological diseases.

Basic fibroblast growth factor (bFGF). The fibroblast growth factor was first isolated from extracts of bovine brain and pituitary body as two polypeptides: bFGF (18 kD) and aFGF (16 kD) which had a 55% homology of the amino acid sequences. But bFGF and aFGF are strongly different in acidic-basic properties—their p*I* values are 9.6 and 5.5, respectively [61]. Both polypeptides are ligands of the same cellular receptors and display a wide spectrum of biological activity: they are involved in the regulation of proliferation, differentiation, and migration of various cells of mesodermal and neuroectodermal origin [62]. The biological effect of bFGF on different cells is 30-100-fold higher than the effect of aFGF.

A significantly higher activity of bFGF is a reason for the greater interest of researchers in this factor; therefore, the literature mainly presents data on its biological functions. At present, bFGF and aFGF are considered members of a large family of protein growth factors which includes 19 relative proteins combined on the basis of homology of their amino acid sequences [63]. In the body bFGF is mainly located in cells and tissues of mesodermal origin, including brain, pituitary body, eye retina, kidney, adrenal, placenta, prostate, thymus, connective tissue cells, endothelial cells, etc.

The role of bFGF in angiogenesis was first shown in cultures of endothelial and smooth muscle cells [64]. bFGF increased the cell migrational ability due to a reversible decrease in their adhesion, an increase in the mobility of cell membranes, and the bFGF-mediated reorganization of the intracellular contractile apparatus. It was also shown that bFGF stimulated the proliferative activity of endothelium and played an important role in the remodeling of the extracellular matrix [64-67]. During the remodeling of the extracellular matrix bFGF stimulates proteolysis of the matrix components concurrently with the induction of synthesis by endothelial cells of the main connective tissue constituents: collagen, fibronectin, and proteoglycans [68]. During the organism's embryogenesis and post-embryogenesis the inactivation of the gene encoding bFGF fails to induce significant physiological changes associated with structural and functional disorders of the blood supply, but an increased expression of bFGF in different organs and tissues closely correlates with manifestations of the angiogenic activity in these organs; therefore, bFGF is suggested to be one of the most important regulators of angiogenesis *in vivo* [69].

Five isoforms of bFGF have been described (with molecular weights of 18, 22, 22.5, 24, and 34 kD) that are expressed by endothelial cells and produced by the alternative splicing of the same gene [70]. Physiological functions of different bFGF isoforms are closely associated with their intracellular and tissue locations. Thus, the high-molecular-weight isoforms of bFGF (22, 22.5, 24, and 34 kD) are characterized by the presence in their structure of the amino acid sequence which is responsible for their translocation into the nucleus [71]. On locating in the nucleus, these isoforms are involved in the regulation of endothelial cell proliferation via stimulation of the ribosomal gene transcription [72]. The low-molecular-weight bFGF isoform (18 kD) (further bFGF) lacks the secretory signal sequence, but, nevertheless, is somehow secreted into the extracellular space where it is deposited as a complex with heparin sulfate proteoglycans (HSPG) which are present on the surface of the endothelium cell membrane [73, 74].

The biological effect of bFGF is mediated through four structurally similar receptors (FGFR1-4) which are widely distributed on the surface of various cells [75]. All receptors are transmembrane proteins consisting of three domains: extracellular, transmembrane, and intracellular. The extracellular ligand-binding domain has three immunoglobulin-like amino acid sequences. The transmembrane domain is an α-helical region of the chain which penetrates the membrane. The intracellular domain displays the tyrosine kinase activity and an ability for autophosphorylation. The physiological role of the receptors was clearly demonstrated in experiments on their successive inactivation. The inactivation of genes encoding the FGFR1 and FGFR2 receptors resulted in embryonal death in the early stages of development (before gastrulation), and this makes difficult the study of their role during the late stages of development and in angiogenesis [76]. However, by a recent transfection of the dominant negative gene of FGFR1 into mouse embryo cells this receptor was shown to be necessary for the development and growth maintenance of blood vessels during embryogenesis [77]. A shutdown of the gene encoding FGFR3 resulted in serious disorders in skeletal development. There are no data on the inactivation of FGFR4 [78].

There is still no unambiguous concept on the spectrum of FGF receptors expressed by endothelial cells during different stages of angiogenesis. However, the angiogenic stimulation of endothelial cells is found to be associated with an active expression of FGFR1 on the cell surface (Fig. 3) [79]. The interaction of bFGF with the FGFR1 receptor occurs by at least two pathways. The first pathway is the releasing of bFGF from the complex with heparin sulfate proteoglycans by splitting the complex by tissue proteinases and the bFGF binding to FGFR1. The ligand–receptor interactions in this case have low affinity. The second pathway is the formation of bFGF dimers which bind to the FGFR1 receptor and induce the dimerization and activation of the latter [80]. A key role in this reception mechanism is played by heparin sulfate proteoglycans which bind bFGF on the endothelium cell surface, cause a local increase in the concentration of the growth factor, and promote its dimerization [81].

Ligand–receptor interactions trigger the cascade of intracellular events resulting in a biological response. And bFGF concurrently regulates a number of processes. Thus, bFGF increases the expression of urokinase and of its receptor on the endothelium surface, induces the expression of adhesion proteins on the cell membrane, and stimulates the synthesis of the extracellular matrix components [66, 82, 83]. The role of bFGF in angiogenesis seems to be mainly associated with the regulation of intercellular interactions resulting in the generation of blood capillaries.

As the main factor inducing the growth of the tumor vessels bFGF was initially isolated from chondrosarcoma, and this was the beginning of studies on the role of this factor in tumor angiogenesis [84]. The expression of bFGF by tumor cells was shown to mainly depend on the location of these cells in the body [85]. Thus, the injection of the renal carcinoma SN12 cells into the kidney or subcutaneously resulted in both cases in the development

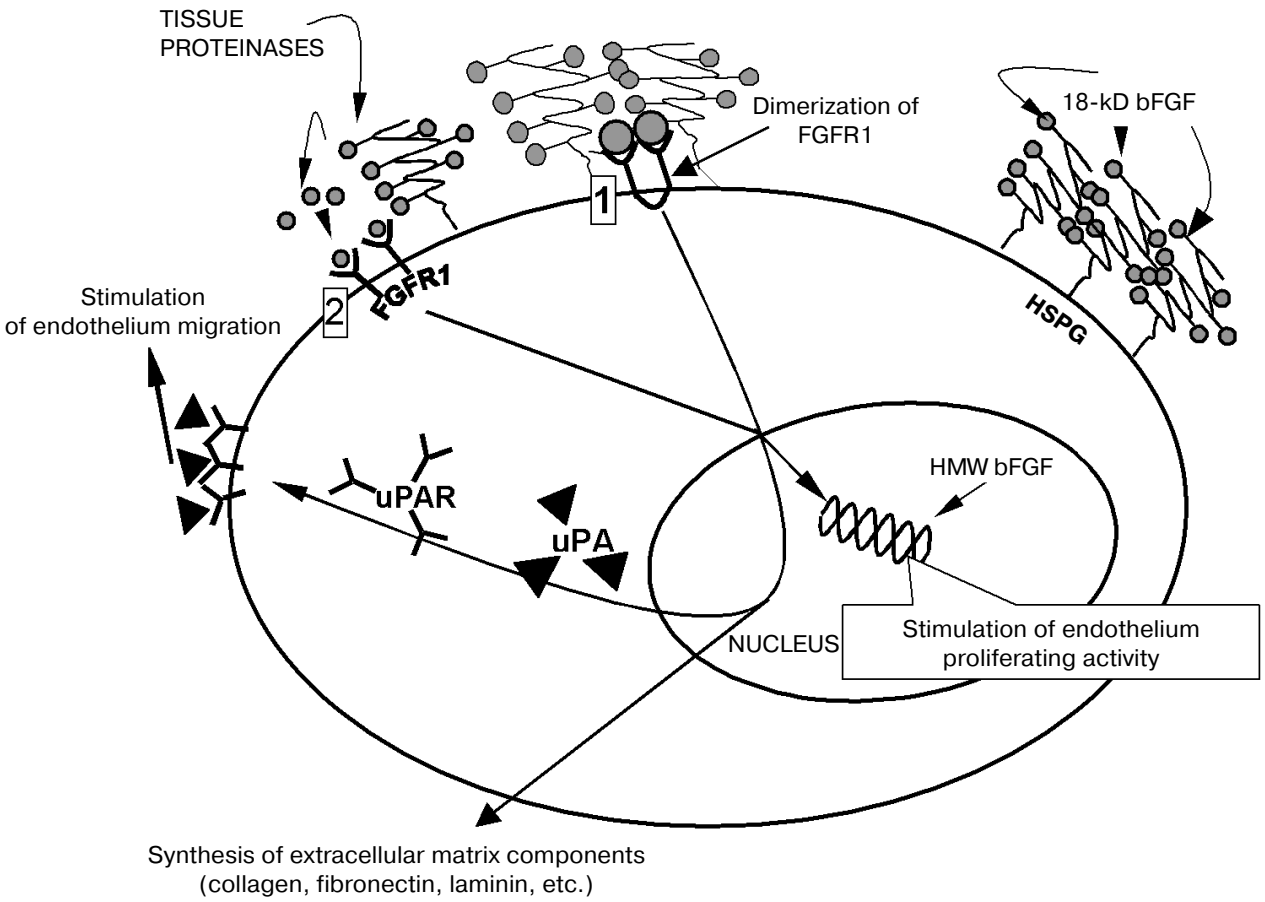

Fig. 3. Regulation of angiogenic activity of endothelial cells by the basic fibroblast growth factor (bFGF). In the resting endothelium the low-molecular-weight bFGF isoform (18 kD) is located on the endothelium cell surface as complexes with heparin sulfate proteoglycans (HSPG). High-molecular-weight isoforms of bFGF (HMW bFGF) are located in the nucleus and regulate the transcription of ribosomal genes. Activation of endothelial cells by angiogenic factors results in expression of the bFGF receptor, FGFR1, on the cell surface. Interactions of bFGF with FGFR1 of high (*1*) and low (*2*) affinity induce the triggering of the corresponding signal pathway and cause biological effects of bFGF.

of tumors, but these tumors were markedly different in the level of bFGF secretion. The level of bFGF secretion by the tumor growing in the kidney was 10-20 times higher than the level of secretion by the cells of the subcutaneous tumor. And the level of bFGF secretion by the cells in the renal tissue correlated with their high vascularization. The interrelation between the bFGF expression by the tumor and the degree of its vascularization is also recorded in a number of strongly vascularized tumors (hemangioma, Kaposi's sarcoma, carcinoma, and glioma) [86-88]. The type of bFGF expression markedly depends on the density of the tumor tissue. And there is an inverse correlation between an increase in the density of the tumor cell population and the level of the bFGF expression [89]. Thus, in the majority of solid tumors a high content of bFGF is mainly recorded in the tumor periphery and also in the "leader regions" of the tumor which determine the dominant direction of its invasion. At present, in clinical oncology an increased content of bFGF found in the patient is a prognostic parameter of a severe tumorigenesis [90]. Thus, bFGF is one of the most important factors involved in both the regulation of the physiological angiogenesis and induction and development of tumor vascularization. Therapeutic strategies based on inhibition of different steps of the regulatory effect of bFGF are now actively developed.

ROLE OF PROTEOLYTIC ENZYMES IN DEGRADATION OF BASAL MEMBRANE OF PRECURSOR BLOOD VESSEL AND IN REORGANIZATION OF EXTRACELLULAR MATRIX

Along with the stimulation of endothelial cells by angiogenic factors, the secretion of proteolytic enzymes is induced by both the tumor and endothelial cells, and this induction results in a local degradation of the basal mem-

brane of the precursor blood vessel and a weakening of intercellular interactions in the blood vessel wall. To generate new blood vessels, the basal membrane of the blood vessel has to be degraded previously. This membrane is a porous semipermeable coat consisting of laminin, collagen (IV and V types), fibronectin, and other glycoproteins. The urokinase type plasminogen activator (uPA), plasmin, and matrix metalloproteinases (MMP) are the most important enzymes involved in the proteolysis of the basal membrane.

Urokinase is a monomeric serine proteinase which catalyzes the conversion of plasminogen to plasmin. The enzyme is secreted by endothelial cells as an inactive proenzyme (pro-uPA) which binds to the urokinase receptor on the endothelial cell surface. Catalytic amounts of plasmin in the environment induce the proteolysis of pro-uPA with production of urokinase. Urokinase promotes the conversion of plasminogen to plasmin. Thus, the interaction of pro-uPA with the receptor hyperexpressed on the surface of activated endothelial cells increases the local enzymatic activity on the cell membrane surface that results in an increase in the plasmin level [91]. Plasmin catalyzes the degradation of connective tissue proteins (fibrin, fibronectin, laminin) and activates matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9) which degrade the extracellular matrix [92, 93]. Thus, the expression of urokinase by endothelial cells via successive proteolytic activation of a number of enzymes results in the degradation of the extracellular matrix, and this promotes the invasion of endothelium into the adjacent tissues.

Metalloproteinases are enzymes that are produced by various cells and play an important role in destruction of the extracellular matrix. The metalloproteinase family includes 16 enzymes which are expressed into the interstitial space as biologically latent forms [94]. The proenzymes are activated by proteinases. The metalloproteinase molecule consists of four domains: the predomain which is a signal peptide required for the enzyme secretion, the prodomain which is cleaved off on the enzyme activation, the catalytic domain which contains a zinc ion, and the regulatory domain which has a binding site for inhibitors of metalloproteinases (TIMP) [95]. Metalloproteinases have wide substrate specificity to connective tissue proteins. Their substrates include collagen (MMP-1, MMP-8, MMP-13), gelatin (MMP-9, MMP-7, MMP-3,

Table 2. Main members of the family of matrix metalloproteinases and their substrates

Enzyme	MMP	Main substrates
Group 1		
matrilysin	MMP-7	nonfibrillar collagen, gelatin, laminin, fibronectin, pro-MMP-1, pro-MMP-9
Group 2		
collagenase 1	MMP-1	fibrillar collagen (types I, II, III, VII, X), pro-MMP-2, pro-MMP-9
collagenase 2	MMP-8	fibrillar collagen (types I, II, III, VII, X)
collagenase 3	MMP-13	fibrillar collagen (types I, II, III)
stromalysin 1	MMP-3	nonfibrillar collagen, gelatin, laminin, fibronectin, pro-MMP-1, pro-MMP-9, pro-MMP-13
stromalysin 2	MMP-10	nonfibrillar collagen, elastin, laminin, fibronectin, pro-MMP-1
stromalysin 3	MMP-11	α-1 proteinase, nonfibrillar collagen, laminin, fibronectin
metalloelastase	MMP-12	elastin
enamelysin	MMP-20	amelogenin
Group 3		
gelatinase A	MMP-2	gelatin, collagen (types I, IV, V), laminin, fibronectin, pro-MMP-9, pro-MMP-13
gelatinase B	MMP-9	gelatin, collagen (types IV, V)
Group 4		
MT-1–MMP	MMP-14	pro-MMP-2, pro-MMP-13, gelatin, fibrillar collagen, laminin, fibronectin
MT-2–MMP	MMP-15	pro-MMP-2, gelatin, fibrillar collagen, laminin, fibronectin
MT-3–MMP	MMP-16	pro-MMP-2
MT-4–MMP	MMP-17	unknown

MMP-10, MMP-15), elastin (MMP-12), fibronectin (MMP-7, MMP-3, MMP-10, MMP-11, MMP-14, MMP-15), amelogenin (MMP-20), etc. (Table 2).

Urokinase, plasmin, and metalloproteinases can degrade the main components of the extracellular matrix, and this determines their leading role in its reorganization. The activities of these enzymes are regulated on the levels of their synthesis and secretion and of activation inside the extracellular matrix, and also by endogenous inhibitors: plasminogen activator inhibitors (PAI-1, PAI-2) [96], tissue inhibitors of metalloproteinases (TIMP) [97], and the plasmin inhibitor α-antiplasmin [98]. The complicated regulatory mechanisms provide effective control of the local proteolytic activity and prevent proteolysis of adjacent tissues.

The proteolytic activity is high in the vascularization focus caused by tumor development. The stimulation of the tumor endothelium by angiogenic factors produced by the tumor cells along with the expression of the angiogenic factors by the endothelial cells themselves result in the hyperexpression of urokinase and activation of plasminogen [99]. In their turn, the connective tissue cells involved in the tumorigenesis secrete enzymes which activate the latent forms of metalloproteinases and provide a high activity of metalloproteinases [100]. However, the tumor cells suppress the secretion of endogenous inhibitors of the proteolytic enzymes, such as PAI, TIMP, and α-antiplasmin [100]. On this background a focus of the local proteolytic activity is generated that results in partial proteolysis of the extracellular matrix, and this promotes the invasion of both the endothelial and tumor cells into adjacent tissues.

ROLE OF CELL ADHESION MOLECULES IN REGULATION OF INTERCELLULAR AND CELL–MATRIX INTERACTIONS IN ANGIOGENESIS

The proliferation and migration of endothelial cells resulting in formation of blood capillaries are mediated by the endothelium interaction with connective tissue cells and the extracellular matrix. In addition to soluble mediators of intercellular and cell–matrix interactions which include polypeptide growth factors, cytokines, and proteolytic enzymes an important role in angiogenesis is played by cell adhesion molecules expressed on the endothelial cell surface [101].

At present, four families of adhesive receptors are identified: integrins, selectins, the immunoglobulin superfamily, and cadherins.

Integrins are heterodimeric transmembrane proteins with molecules consisting of noncovalently bound α- and β-subunits, and the presence of them both is necessary for the protein interaction with cytoskeleton actin filaments and the extracellular matrix [102]. Integrins are multifunctional proteins which regulate the adhesion and migration of cells, their proliferative and apoptotic activities, and also the expression of several cell genes. An important role in tumor angiogenesis is played by $\alpha_v\beta_3$-integrins which are specifically presented on the surface of the activated endothelium of peritumoral blood vessels [103]. As receptors of a number of the extracellular matrix proteins (fibronectin, laminin, vitronectin, fibrinogen), $\alpha_v\beta_3$-integrins mediate the adhesion of endothelial cells to the matrix that provides their viability, the direction of cell migration, and the formation of the basal membrane around the newly generated capillaries [104]. On the stimulation of endothelium with tumor cells the expression increases of $\alpha_v\beta_3$-integrins which bind matrix metalloproteinases (MMP-2) on the endothelium surface and thus locate the proteolytic activity on the endothelial cell surface [105]. This results in degradation of the basal membrane of the precursor vessel and in invasion of the endothelial cells into the interstitial space. Integrins are also important mediators of the signal transmission of some cytokines and angiogenic growth factors [106]. Antibodies against $\alpha_v\beta_3$-integrins specifically inhibit the angiogenesis induced by bFGF and TNF-α, whereas antibodies against $\alpha_v\beta_5$-integrins inhibit the angiogenesis induced by VEGF [106]. In addition to integrins, other cell adhesion molecules also mediate angiogenesis. Thus, cadherins mediate the Ca^{2+}-dependent adhesive interactions between endothelial cells and are important components of adhesive contacts responsible for organization of the cell cytoskeleton [107]. The inactivation of genes encoding cadherins causes apoptosis of the endothelial cells, and this suggests an important role of cadherins in the maintenance of cell viability. Cadherins have been shown to be coreceptors of VEGF, which is the main angiogenic factor and plays the key role in angiogenesis [108]. Members of the immunoglobulin superfamily (ICAM-1, ICAM-2, VCAM-1) are expressed on the surface of the endothelium activated by cytokines (TNF-α, IL-1, IFN-γ) and are involved in the endothelium interactions with T-cells that provide the accumulation of mononuclear cells in the vascular zone [104]. ICAM-1 and VCAM-1 induce chemotaxis of the endothelium and angiogenesis [109]. P- and E-selectins cause the adhesion of leukocytes on the cytokine-activated endothelium and are involved in the endothelial cell migration and in the formation of blood capillaries [110]. Intercellular and cell–matrix interactions mediated by the cell adhesion molecules play an important role in the vascular remodeling of tissues. Interaction mechanisms of various adhesive molecules are now intensively studied.

ROLE OF NONSPECIFIC REGULATORS IN DEVELOPMENT AND MAINTENANCE OF TUMOR ANGIOGENESIS

The development in tumor of blood vessels is a rapid process which starts with appearance of multiple vascu-

larization foci. To initiate and maintain the tumor angiogenesis, a high level of angiogenic activity is required that can be provided via secretion by the tumor and other cells involved in the tumorigenesis (lymphocytes, macrophages, mast cells, etc.) of specific to endothelium angiogenic factors (VEGF, Ang-1) and of some cytokines and growth factors with a wide spectrum of biological effects. An increased expression by some tumors of the epidermal growth factor (EGF) [111], the insulin-like growth factor (IGF) [112], and the hepatocyte growth factor (HGF) [113] is shown to increase the proliferative activity of the endothelium of adjacent blood vessels. The platelet growth factor (PDGF) [114] and the fibroblast growth factor (aFGF, bFGF) [115] produced by the tumor induce the migration of endothelium and the secretion of urokinase by the endothelial cells, and this causes proteolysis of the extracellular matrix. The transforming growth factor (TGF-β1) which is expressed by the tumor and inhibits normal angiogenesis is a powerful stimulator of the tumor angiogenesis via a hemotactic effect on monocytes, macrophages, and lymphocytes providing their attraction into the vascularization zone [116]. The colony-stimulating factors (G-CSF, GM-CSF) secreted by the tumor activate granulocytes and macrophages which display the angiogenic activity by secretion of some angiogenic factors [117]. Thus, the activated macrophages produce a number of cytokines with angiogenic properties: the tumor necrosis factor (TNF-α), interleukins (IL-1, IL-8, IL-12), and the transforming growth factor (TGF-α) [118]. The multifunctional cytokine TNF-α has a wide spectrum of the angiogenic effects: the stimulation of the endothelium migration due to activation of expression and secretion of urokinase by endothelial cells; the involvement in formation of blood capillaries due to induction of secretion of cell adhesion proteins (ICAM-1); the induction of secretion of angiogenic growth factors (bFGF) by endothelial cells [119, 120]. The importance of the combined angiogenic effects of TGF-α and interleukins has been shown: interleukins (IL-8, IL-12) stimulate the proliferation and migration of endothelial cells [121] and TGF-α is involved in formation of capillary structures and in organization of the tumor vascular network [122]. The endothelial growth factor released by platelets (PD-ECGF) stimulates the growth, migration, and chemotaxis of endothelial cells [123].

An important role in the regulation of angiogenesis also belongs to low-molecular-weight (<10 kD) C-X-C polypeptides of the chemokine family which are produced by the majority of somatic cells of the body [124]. Members of this family regulate immunoinflammatory reactions. C-X-C chemokines are powerful chemoattractants for endothelial cells and for cells of the immune system. At present there is no clear concept on mechanisms of the angiogenic effect of chemokines. However, a stable correlation is found between the angiogenic effects of chemokines and their structural features. The presence in the structure of chemokines GRO-α, GRO-β, and GRO-γ of the amino acid sequence glutamine-leucine-arginine determines their angiogenic effects. On the contrary, the absence of this sequence in chemokines PF4, IP-10, and MIG determines their angiostatic properties [125]. The expression levels of angiogenic and angiostatic chemokines are regulated by angiogenic growth factors, which is a manifestation of a complicated organization of the angiogenesis regulatory system.

The type of expression and interaction of the tumor-produced angiogenic factors determines the structural features of the tumor blood vessels. Rapidly proliferating endothelial cells of blood vessels produce in the tumors large and broad blood vessels with a slow blood flow. The development of vessels in the tumor is characterized by poorly developed walls: they lack pericytes and adventitial cells, the basal membrane is discontinuous, and there are large gaps between the endothelial cells [126]. This results in a high permeability of the tumor vessels that promotes the entrance of the tumor cells into the blood circulation and their dissemination into various organs and tissues of the body (metastases). Despite an intense angiogenesis in the tumor tissue, solid tumors are relatively poor in blood vessels. The blood vessel network has the highest density in the tumor periphery, whereas the development of blood vessels inside the tumor is limited by large necrotic zones with a strong hypoxia and a high intratissue pressure [127]. There are general regularities of the development in tumor of blood capillaries, but the progress of angiogenesis in each tumor depends on both individual specific features of the tumor microenvironment determining its "angiogenic profile" and biochemical and morphological features of the microcirculatory beds in the organs where the pathologic process is in progress [128]. Thus, the vascularization of each tumor is particularly individual and essentially determines the degree of its malignant nature.

The development of the system of the tumor blood supply is a key step in the malignant transformation. The interaction between the tumor vascularization and their malignant transformation has been shown for various tumors. In this connection, the level of secretion of angiogenic factors which play an important role in the induction and development of tumor angiogenesis is a significant characteristic of tumorigenesis in the body, and therapeutic strategies directed to suppress tumor angiogenesis by affecting its physiological regulators are very promising for antitumor therapy.

REFERENCES

1. Hanahan, D., and Folkman, J. (1996) *Cell*, **86**, 353-364.
2. Folkman, J. (1972) *Ann. Surg.*, **8**, 409-416.
3. Folkman, J. (1990) *J. Natl. Cancer Inst.*, **82**, 4-6.

4. Kurz, H. (2000) *J. Neuro-Oncology*, **50**, 17-35.
5. Pries, A. R., Secomb, T. W., and Gaehtgens, P. (1998) *Amer. J. Physiol.*, **275**, H349-H360.
6. Folkman, J., Waston, K., and Ingber, D. (1989) *Nature*, **339**, 58-61.
7. Dvorak, H. F., Dvorak, A. M., and Churchill, W. H. (1979) *J. Natl. Cancer. Inst.*, **62**, 1459-1472.
8. Ferrara, N., and Henzel, W. J. (1989) *Biochem. Biophys. Res. Commun.*, **161**, 851-858.
9. Achen, M. G., and Stacker, S. A. (1998) *Int. J. Exp. Path.*, **79**, 255-265.
10. Park, J. E., Keller, G. A., and Ferrara, N. (1993) *Mol. Biol. Cell*, **4**, 1317-1326.
11. Houck, K. A., Leung, D. W., Rowland, A. M., Winer, J., and Ferrara, N. (1992) *J. Biol. Chem.*, **267**, 26031-26037.
12. Maglione, D., Guerriero, V., Viglietto, G., Delli-Bovi, P., and Persico, M. G. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 9267-9271.
13. Joukov, V., Pajusola, K., Kaipainen, A., Chilov, D., Lahtinen, I., Kukk, E., Saksela, O., Kalkkinen, N., and Alitalo, K. (1996) *EMBO J.*, **15**, 290-298.
14. Olofsson, B., Pajusola, K., Kaipainen, A., VonEuler, G., Joukov, V., Saksela, O., Orpana, A., Petterson, R., Alitalo, K., and Eriksson, U. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 2576-2581.
15. Lyttle, D. J., Fraser, K. M., Flemings, S. B., Mercer, A. A., and Robinson, A. J. (1994) *J. Virol.*, **68**, 84-92.
16. Devries, C., Escobedo, J. A., Ueno, H., Houck, K. A., Ferrara, N., and Williams, L. T. (1992) *Science*, **255**, 989-991.
17. Terman, B. I., Vermazen, M. D., Carrion, M. E., Dimitrov, D., Armellino, D. C., Gospodarowicz, D., and Bohlen, P. (1992) *Biochem. Biophys. Res. Commun.*, **34**, 1578-1586.
18. Pajusola, K., Aprelikova, O., Korhonen, J., Kaipainen, A., Pertovaara, V., Alitalo, R., and Alitalo, K. (1992) *Cancer Res.*, **52**, 5738-5743.
19. Quinn, T., Peters, K. G., Devries, C., Ferrara, N., and Williams, L. T. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 7533-7537.
20. McMahon, G. (2000) *The Oncologist*, **5**, 3-10.
21. Pepper, M. S., Ferrara, N., Orci, L., and Montesano, R. (1991) *Biochem. Biophys. Res. Commun.*, **181**, 902-908.
22. Unemori, E. N., Ferrara, N., Bauer, E. A., and Amento, E. P. (1992) *J. Cell. Physiol.*, **153**, 557-562.
23. Huang, K., Andersson, C., Roomans, G. H., Ito, N., and Claeeson-Welsh, L. (2001) *Int. J. Biochem. Cell Biol.*, **33**, 315-324.
24. Kaipainen, A., Korhonen, J., Mustonen, T., van Hinsbergh, V. M., Fang, G. H., Dumont, D., Breitman, M., and Alitalo, K. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 3566-3570.
25. Kolodkin, A. L., Levengood, D. V., Rowe, E. G., Tai, Y. T., Giger, R. J., and Ginty, D. D. (1997) *Cell*, **90**, 753-762.
26. Soker, S., Takashima, S., Miao, H. Q., Neufeld, G., and Klagsbrun, M. (1998) *Cell*, **92**, 735-745.
27. Hashimoto, M., Ohsawa, M., Ohnishi, A., Naka, N., Hirota, S., Kitamura, Y., and Aozara, K. (1995) *Lab. Invest.*, **73**, 859-863.
28. Dewhirst, M. W., Tso, C. Y., Oliver, R., Gustafson, C. S., Secomb, T. W., and Gross, J. F. (1989) *Int. J. Radiat. Oncol. Biol. Phys.*, **17**, 91-99.
29. Vaupel, P., Kallinowski, F., and Okunieff, P. (1989) *Cancer Res.*, **49**, 6449-6465.
30. Sutherland, R. M. (1988) *Science*, **240**, 177-183.
31. Schweiki, D., Itin, A., Soffer, D., and Keshet, E. (1992) *Nature*, **359**, 843-845.
32. Grugel, S., Finkenzeller, G., Weindel, K., Barleon, B., and Marme, D. (1995) *J. Biol. Chem.*, **270**, 25915-25919.
33. Saez, E., Rutberg, S., Mueller, E., Oppenheim, H., Smoluk, J., Yuspa, S., and Spiegelman, B. (1995) *Cell*, **82**, 721-732.
34. Mukhopadhyay, D., Tsiokas, L., and Sukhatme, V. P. (1995) *Cancer Res.*, **55**, 6161-6165.
35. Kerbel, R. S., Viloria-Petit, A., Okada, F., and Rak, J. (1998) *Mol. Med.*, **4**, 286-295.
36. Kieser, A., Weich, H., Brandner, G., Marme, D., and Kolch, W. (1994) *Oncogene*, **9**, 963-969.
37. Cohen, T., Nahari, D., Cerem, L. W., Neufeld, G., and Levi, B. Z. (1996) *J. Biol. Chem.*, **271**, 736-741.
38. Warren, R. S., Yuan, H., Matli, M. R., Ferrara, N., and Donner, D. B. (1996) *J. Biol. Chem.*, **271**, 29483-29488.
39. Goldman, C. K., Kim, J., Wong, W. L., King, V., Brock, T., and Gillespie, G. Y. (1993) *Mol. Biol. Cell*, **4**, 121-133.
40. Gerber, H. P., Dixit, V., and Ferrara, N. (1998) *J. Biol. Chem.*, **167**, 30336-30343.
41. Liu, W., Ahmad, S. A., Reinmuth, N., Shaheen, R. M., Jung, Y. D., Fan, F., and Ellis, L. M. (2000) *Apoptosis*, **4**, 323-328.
42. Senger, D. R., van de Water, L., Brown, L. F., Nagy, J. A., Yeo, K.-T., and Yeo, T.-K. (1993) *Cancer Metastasis Rev.*, **12**, 303-324.
43. Pepper, M. S., Ferrara, N., Orci, L., and Montesano, R. (1992) *Biochem. Biophys. Res. Commun.*, **189**, 824-831.
44. Wizigmann-Voss, S., Breier, G., Risau, W., and Plate, K. (1994) *Cancer Res.*, **55**, 1358-1364.
45. Mattern, J., Koomagi, R., and Volm, M. (1996) *Br. J. Cancer*, **73**, 931-934.
46. Phillips, H. S., Armanini, M., Stavrou, D., Ferrara, N., and Westphal, M. (1993) *Int. J. Oncol.*, **2**, 913-919.
47. Yuan, F., Chen, Y., Dellian, M., Ferrara, N., and Jain, R. K. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 14765-14770.
48. Bohle, A. S., and Kalthoff, H. (1999) *Langenbeck's Arch. Surg.*, **384**, 133-140.
49. Dumont, D. J., Yamaguchi, T. P., Conlon, R. A., Rossant, J., and Breitman, M. L. (1992) *Oncogene*, **7**, 1471-1480.
50. Partanen, J., Armstrong, E., Makela, T. P., Korhonen, J., Sandberg, M., Renkonen, R., Knuutila, S., Huebner, K., and Alitalo, K. (1992) *Mol. Cell. Biol.*, **12**, 1698-1707.
51. Davis, S., Gale, N. W., Aldrich, T. H., Maisonpierre, P. C., Lhotak, V., Pawson, T., Goldfarb, M., and Yancopoulos, G. D. (1994) *Science*, **266**, 816-819.
52. Nishimura, M., Takashi, M., Yashima, R., Yokoi, N., Yano, H., Sato, Y., and Seino, S. (1999) *FEBS Lett.*, **448**, 254-256.
53. Davis, S., and Yancopoulos, G. D. (1999) *Current Topics in Microbiology and Immunology* (Claesson-Welsh, L., ed.) Vol. 237, Springer-Verlag, Berlin-Heidelberg, pp. 174-184.
54. Suri, C., Jones, P. F., Patan, S., Bartunkova, S., Maisonpierre, P. C., Davis, S., Sato, T. N., and Yancopoulos, G. D. (1996) *Cell*, **87**, 1171-1180.
55. Hirchi, K. K., and D'Amore, P. A. (1996) *Cardiovasc. Res.*, **32**, 687-698.

56. Papapetropoulos, A., Fulton, D., and Mahbonbi, K. (2000) *J. Biol. Chem.*, **275**, 9102-9105.
57. Maisonpierre, P. C., Suri, C., Jones, P. F., Bartunkova, S., Wiegand, S. J., Radziejewsky, C., Compton, D., McClain, J., Aldrich, T. H., Papadopoulos, N., Daly, T. J., Davis, S., and Sato, T. N. (1997) *Science*, **277**, 55-60.
58. Jones, N., and Dumont, D. J. (2000) *Cancer Metastasis Rev.*, **19**, 13-17.
59. Holash, J., Wiegand, S. J., and Yancopoulos, G. D. (1999) *Oncogene*, **18**, 5356-5362.
60. Enholm, B., Paavonen, K., Ristimaki, A., Kumar, V., Gunji, Y., Klefstrom, J., and Alitalo, K. (1997) *Oncogene*, **14**, 2475-2483.
61. Gospodarowicz, D. (1975) *J. Biol. Chem.*, **250**, 2515-2520.
62. Basilico, C., and Moscatelli, D. (1992) *Adv. Cancer Res.*, **59**, 115-165.
63. Dow, J. K., and White, R. W. (2000) *Urology*, **55**, 800-806.
64. Gospodarovicz, D., and Neufeld, G. (1987) *Mesenchymal–Epithelial Interactions in Neural Development* (Wolff, J. R., et al., eds.) Springer-Verlag, Berlin-Heidelberg, pp. 192-211.
65. Presta, M., Maier, J. A. M., Rusnati, M., and Ragnotti, G. (1989) *J. Cell. Physiol.*, **141**, 517-526.
66. Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J. A., Fiddes, J. C., and Gospodarowicz, D. (1987) *Nature*, **325**, 257-259.
67. Pepper, M. S., and Meda, P. (1992) *J. Cell. Physiol.*, **153**, 196-205.
68. Pepper, M. S., Sappino, A. P., Stocklin, R., Montesano, R., Orci, L., and Vassalli, J. D. (1993) *J. Cell. Biol.*, **122**, 673-684.
69. Coltrini, D., Gualandris, A., Nelli, E. E., Parolini, S., Molinari-Tossati, M. P., Quarto, N., Ziche, M., Giavazzi, R., and Presta, M. (1995) *Cancer Res.*, **55**, 4729-4738.
70. Arnaud, E., Touriol, C., Boutonnet, M. C., Gensac, S., Vagner, H., and Prats, A. C. (1999) *Mol. Cell. Biol.*, **19**, 505-514.
71. Quarto, N., Finger, F. P., and Rifkin, D. B. (1991) *J. Cell. Physiol.*, **147**, 311-318.
72. Arese, M., Chen, Y., Florkiewicz, R. Z., Gualandris, A., Shen, B., and Rifkin, D. (1999) *Mol. Cell. Biol.*, **10**, 1429-1444.
73. Mignatti, P., Morimoto, T., and Gifkin, D. B. (1992) *J. Cell. Physiol.*, **151**, 81-93.
74. Rusnati, M., and Presta, M. (1996) *Int. J. Clin. Lab. Res.*, **26**, 15-23.
75. Cross, M. J., and Claesson-Welsh, L. (2001) *Trends Pharm. Sci.*, **22**, 201-207.
76. Deng, C. X. (1994) *Genes Dev.*, **8**, 3045-3057.
77. Hanneken, A., Maher, P. A., and Baird, A. (1995) *J. Cell Biol.*, **128**, 1221-1228.
78. Colvin, J. S. (1996) *Nat. Genet.*, **12**, 390-397.
79. Klint, P., and Claesson-Welsh, L. (1999) *Front. Biosci.*, **4**, D165-D177.
80. Plotnikov, A. V. (1999) *Cell*, **98**, 641-650.
81. Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P., and Ornitz, D. M. (1991) *Cell*, **64**, 841-848.
82. Mignatti, P., Mazzieri, R., and Rifkin, D. B. (1991) *J. Cell Biol.*, **113**, 1193-1201.
83. Klein, S., Giancotti, F. G., Presta, M., Albelda, S. M., Buck, C. A., and Rifkin, D. B. (1993) *Mol. Biol. Cell*, **4**, 973-982.
84. Baird, A., Mormede, P., and Bohlen, P. (1986) *J. Cell. Biochem.*, **30**, 79-85.
85. Bielenberg, D. R., and Fiedler, I. J. (1999) in *Antiangiogenic Agents in Cancer Therapy* (Teicher, B. A., ed.) Humana Press Inc., Totowa, N. J., pp. 77-91.
86. Halaban, R., Kwon, B. S., Chost, S., Delli-Bovi, P., and Baird, A. (1993) *Oncogene Res.*, **3**, 177-186.
87. Sturzl, M., Brandsteller, H., and Roth, W. K. (1992) *AIDS Res. Human Retroviruses*, **8**, 1753-1763.
88. Takahashi, J. A., Mori, H., Fukumoto, M., Igarashi, K., Jaye, M., Oda, Y., Kikuchi, H., and Hatanaka, M. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5710-5714.
89. Singh, R. K., Llansa, N., Bucana, C. D., Sanchez, R., Koura, A., and Fidler, I. J. (1996) *Cell Growth Differentiation*, **7**, 397-404.
90. Nguen, M., Watanabe, H., Budson, A. E., Richie, J. P., Hayes, D. F., and Folkman, J. (1994) *J. Natl. Cancer Inst.*, **86**, 356-361.
91. Mignatti, P., and Rifkin, D. B. (1993) *Physiol. Rev.*, **73**, 161-195.
92. Mazar, A. P., Henkin, J., and Goldfarb, R. H. (1999) *Angiogenesis*, **3**, 15-34.
93. Murphy, G., Stanton, H., Cowell, S., Butler, G., Knauper, V., Atkinson, S., and Gavrilovic, J. (1999) *Acta Pathol. Microbiol. Immunol. Scand.*, **107**, 38-44.
94. Yip, D., Ahmad, A., Karapetis, C. S., Hawkins, C. A., and Harper, P. G. (1999) *Invest. New Drugs*, **17**, 387-399.
95. Westermarck, J., and Kahari, V. M. (1999) *FASEB J.*, **13**, 781-792.
96. Declerk, Y. A., and Laug, W. E. (1996) *Enzyme Proteins*, **49**, 72-84.
97. Jonson, M. D., Kim, H. R., and Chesler, L. (1994) *J. Cell. Physiol.*, **160**, 194-202.
98. Blasi, F. (1997) *Immunol. Today*, **18**, 415-417.
99. Yamamoto, M., Sawaya, R., and Mohanam, S. (1994) *Cancer Res.*, **54**, 5016-5020.
100. Werb, Z., Thiennu, H. Vu., Rinkerberger, J. L., and Coussens, L. M. (1999) *APMIS*, **107**, 11-18.
101. Bischof, J. (1997) *J. Clin. Invest.*, **100**, S37-S39.
102. Hynes, R. O. (1992) *Cell*, **69**, 11-25.
103. Pasqualini, R., Koivunen, E., and Ruoslahti, E. (1997) *Nature Biotech.*, **15**, 542-546.
104. Brooks, P. C. (1996) *Cancer Metastasis Rev.*, **15**, 187-194.
105. Brooks, P. C., Stromblad, S., Sanders, L. C., Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., Quigley, J. P., and Cheresh, D. A. (1996) *Cell*, **85**, 683-693.
106. Friedlander, M., Brooks, P. C., Shaffer, R. W., Kincaid, C. M., Varner, J. A., and Cheresh, D. A. (1995) *Science*, **270**, 1500-1502.
107. Blaschuk, O. W., and Rowlands, T. M. (2000) *Cancer Metastasis Rev.*, **19**, 1-5.
108. Carmeliet, P., Lampugnani, M. G., and Moons, L. (1999) *Cell*, **98**, 147-157.
109. Koch, A. E., Halloran, M. M., Haskell, C. J., Shah, M. R., and Polverini, P. J. (1995) *Nature*, **376**, 517-519.
110. Labow, M. A., Norton, C. R., Rumberger, J. M., Lombard-Gillooly, K. M., Shuster, D. J., and Hubbard, J. (1994) *Immunity*, **1**, 709-720.
111. Alroy, I., and Yarden, Y. (1997) *FEBS Lett.*, **410**, 83-86.
112. Bar, R. S., Boes, M., Dake, B. L., Booth, B. A., Henley, S. A., and Sandra, *A.* (1988) *Am. J. Med.*, **85**, 59-70.

113. Grant, D. S., Kleiman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., and Kinsella, J. L. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 1937-1941.
114. Heldin, C. H., and Westermark, B. (1999) *Physiol. Rev.*, **79**, 1283-1316.
115. Stefanik, D. F., Rizkalla, L. R., Soi, A., Goldblatt, S. A., and Rizkalla, W. M. (1991) *Cancer Res.*, **51**, 5760-5765.
116. Pepper, M. S. (1997) *Cytokine Growth Fact. Rev.*, **8**, 21-43.
117. Balkwill, F. R. (1994) *Eur. Cytokine Network*, **5**, 379-385.
118. Mantovani, A., Bottazzi, B., Colotta, F., Sozzani, S., and Ruco, L. (1992) *Immunol. Today*, **13**, 265-270.
119. Hinsberg, V. M., Berg, E. A., Fiers, W., and Dooijewaard, G. (1990) *Blood*, **75**, 1991-1998.
120. Okamura, K., Sato, Y., Matsudi, T., Haeanaka, R., Ono, M., Kohno, K., and Kuwano, M. (1991) *J. Biol. Chem.*, **266**, 19162-19165.
121. Sunderkotter, C., Steinbrink, K., and Goebeler, M. (1994) *J. Leukocyte Biol.*, **55**, 410-422.
122. Nickell, K. A., Halper, J., and Moses, H. L. (1983) *Cancer Res.*, **43**, 1966-1971.
123. Brown, N. S., and Bicknell, R. (1998) *Biochem. J.*, **334**, 1-8.
124. Keane, M. P., and Strieter, R. M. (1999) *Chem. Immunol.*, **72**, 86-101.
125. Moore, B. B., Keane, M. P., Addison, C. L., and Arenberg, D. A. (1998) *J. Inv. Med.*, **46**, 114-120.
126. Less, J. R., Skalak, T. C., Sevick, E. M., and Jain, R. K. (1991) *Cancer Res.*, **51**, 265-273.
127. Less, J. R., Posner, M. C., Skalak, T. C., Wolmark, N., and Jain, R. K. (1997) *Microcirculation*, **4**, 25-33.
128. Jung, Y. D., Ahmad, S. A., Akagi, Y., Takahashi, Y., Liu, W., Reinmuth, N., Shaheen, R. M., Fan, F., and Ellis, L. M. (2000) *Cancer Metastasis Rev.*, **19**, 147-157.