Chapter 8
Vascular Permeability/Vascular Endothelial Growth Factor

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Keywords: endothelial cells, vascular permeability, tumor angiogenesis, metastasis, ascites, tyrosine kinase receptor

Abstract: The vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) family has more than seven members including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF, and *Trimeresurus flavoviridis* (*T. f.*) svVEGFs. Except for VEGF-E and *T.f.* svVEGFs, all members are encoded in the mammalian genome and involved in angiogenesis and/or lymphangiogenesis. Among these five gene products, VEGF-A (also known as VEGF and VPF) binds two receptor-type tyrosine kinases, VEGFR1 and VEGFR2, and transduces major signals for angiogenesis and vascular permeability. VEGF-A expression is efficiently induced by hypoxia, and regulates not only physiological but also most of the pathological angiogenesis, such as tumor angiogenesis. Since VEGF-A utilizes VEGFR2 as a direct stimulator for angiogenesis, this VEGF-VEGFR2 system represents an ideal pharmaceutical target for suppressing various diseases. Interestingly, VEGFR1 has also been shown to be deeply involved in various pathological processes in cancer as well as inflammatory diseases via a mechanism different from VEGFR2, suggesting that VEGF-VEGFR1 is another attractive target for suppressing human diseases. VEGF-C/D and their receptor VEGFR3 play a central role in lymphangiogenesis, and the blocking of this system significantly decreases lymph node metastasis in animal models of cancer. VEGF-E, a VEGFR2-specific ligand, induces angiogenesis with fewer side effects such as edema and inflammatory responses which are commonly observed on treatment with VEGF-A. Thus, VEGF-E is a useful candidate for proangiogenic therapy.

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Introduction

The blood vessel system is essential for the development and maintenance of the tissues in the body, supplying oxygen and nutrition in vertebrates. This system is also involved in a variety of diseases including cancer (Fig. 8.1) [1–3]. In 1983, Senger et al. isolated a protein with strong vascular permeability activity, and designated it vascular permeability factor (VPF) [4]. Some years later, Ferrara and Henzel purified a protein with growth-promoting activity for vascular endothelial cells (ECs) named VEGF [5]. Surprisingly, molecular cloning revealed that the two proteins are identical and encoded by a single gene which is now known as *VEGF* (or *VEGF-A*) [6,7].

Extensive studies on the VEGF family have to date revealed more than seven members, with VEGF-A essential not only for vasculogenesis, the formation of new blood vessels from endothelial progenitor cells in embryogenesis, but also for angiogenesis, the formation of new blood vessels from the pre-existing vasculature [8–11]. Furthermore, VEGF-A was demonstrated to be a key player for tumor angiogenesis [12–14], and anti-human VEGF-A neutralizing antibody in combination with chemotherapy has recently been approved by the U.S. Food and Drug Administration (FDA) for the treatment of late-stage colorectal cancer [15] and non-squamous lung cancer.

VEGF-C mostly binds VEGFR3, and this system is the first to be shown to directly regulate lymphangiogenesis [16–18]. Strong suppression of VEGF-C/VEGFR3 signaling induces dysfunction and loss of the lymphatic system, resulting in lymphedema and a poor lipid-absorbance [19]. Tumor cells that express *VEGF-C or D* have extensive potential to metastasize to the lymph nodes, strongly suggesting that VEGF-C/D-VEGFR3 signaling is an important target for decreasing lymph node metastasis in cancer patients [17,20].

VEGF and its receptor are considered fundamental regulators of angiogenesis/lymphangiogenesis in vertebrates, and also closely linked to vascular permeability (Fig. 8.2) [14,21]. Because of these biological activities, VEGF-related molecules have developed in various organisms including viruses, and

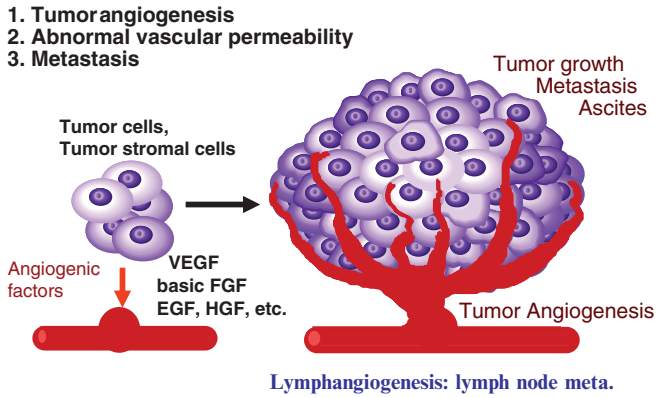

FIG. 8.1. Involvement of the vascular system in tumor progression. Tumor cells and tumor stromal cells such as macrophages, smooth muscle cells and fibroblasts secrete various angiogenic factors, and stimulate tumor angiogenesis as well as vascular permeability. Blood vessels in the tumor enhance tumor-growth and metastasis. Lymphangiogenesis significantly increases lymph-node-oriented metastasis.

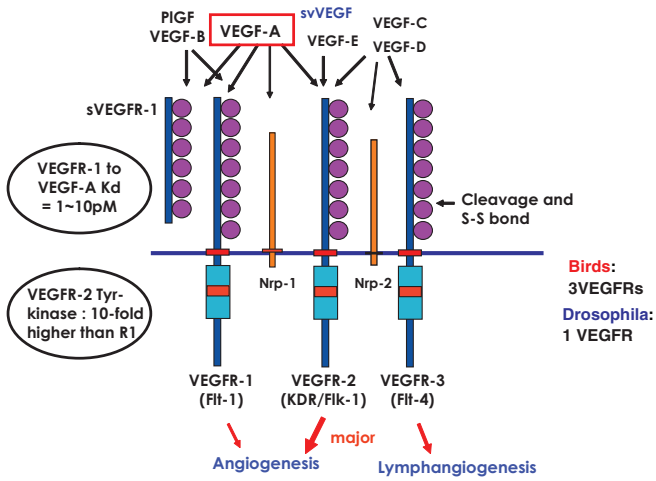

FIG. 8.2. VEGF and its receptor system. Major signals of angiogenesis are generated from VEGFR2. Although VEGFR1 has a weak tyrosine kinase activity, it also stimulates angiogenesis via recruitment of bone marrow-derived mononuclear cells. VEGFR1 plays an important role in inflammation and atherosclerosis. Soluble VEGFR1 is involved in placental regulation and avascularity in the cornea.

are utilized for specialized purposes. The VEGF-E family encoded by the Orf viral genome [22] has a similar structure to VEGF-A, but activates only VEGFR2, and efficiently induces angiogenesis in virally infected tissue in the skin [23–25]. This angiogenic response may facilitate viral replication and production in the host by supplying nutrition and oxygen. On the other hand, snake venom VEGF-like proteins such as the *T.f.* svVEGF (snake venom-derived VEGF in *T.f.* snake in southern Japan) are a family secreted from the venom tissue, and significantly increase vascular permeability [26–30]. This activity may be used to efficiently distribute the snake venom-derived toxins into the body of target animals.

During the past two decades, many angiogenic factors such as VEGF, fibroblast growth factor (FGF), Angiopoietin, hepatocyte growth factor (HGF), and epidermal growth factor (EGF) have been described [3,31,32]. To understand more deeply the molecular basis underlying the formation and regression of blood vessels in our body, further extensive studies on the characteristics of each factor as well as the interrelationship among these factors, particularly between VEGF and the others, appear to be important.

VEGF-A

Function of VEGF-A

VEGF-A promotes the differentiation of endothelial progenitor cells in the early embryo, and stimulates vascular endothelial cell growth, survival, tubular formation, and migration [8,13,14]. VEGF-A is a strong vascular permeability factor with a high specific activity [21]. VEGF-A stimulates the secretion of growth/survival factors from ECs towards surrounding cells such as smooth muscle cells and hepatocytes [33]. Furthermore, VEGF-A induces the expression of factors related to coagulation such as tPA and PAI-1, suggesting it to be an important regulator of blood coagulation [34]. VEGFR2 is also expressed to some extent on lymphatic endothelial cells; thus, VEGF-A could be one of the regulators in lymphangiogenesis [17,35]. Recent reports suggest that VEGFR is expressed on neuronal cells or oligodendrocytes, raising the possibility that under certain conditions, the VEGF-VEGFR system is a direct regulator of cell growth/survival in the nervous system [36–38].

Embryonic Lethality of *VEGF-A* (+/–) Heterozygotic Mice

VEGF-A shows endothelial cell-specific growth-promoting activity, implying a crucial role in blood vessel formation during embryogenesis. Carmeliet et al. [39] and Ferrara et al. [40] demonstrated that the knockout of *VEGF-A* in mice is embryonic lethal even among heterozygotes. They confirmed that these mice are not functionally null for the *VEGF-A* gene via a gene-silencing of the wild-type locus, and that these mice had multiple defects in angiogenesis, such as a disconnection of the heart with the aorta and a poor development of the dorsal aorta. These results strongly suggest that the concentration of VEGF-A in tissues is crucial for normal development of the closed circulatory system in embryos, and that half the normal level of VEGF-A is insufficient to complete morphogenesis in the vascular system. Heterozygotic lethality is extremely rare among mammals.

Isoform of *VEGF-A* Gene Products.

The human *VEGF-A* gene encodes at least 9 different products (isoforms) consisting of 121 to 206 amino acids due to alter-

native splicing [14, 41]. Three major isoforms, 121, 165 and 189-amino-acids long exist, which are well conserved from mammals to other vertebrates [29]. The most abundant isoform in vivo is the 165-amino-acid type, which is expressed in a variety of cells in the body.

VEGF-A belongs to the VEGF/PDGF (platelet-derived growth factor) super-gene family [6–8, 42], whose major characteristics are (1) growth factor with a homodimeric structure, (2) eight conserved cysteines in a monomer at the same positions, and (3) three intramolecular S-S bonds to form three loop structure within the monomeric peptide.

A major difference between the 165- and 189-amino acid isoforms from the 121-isoform is the presence of a basic stretch of residues that bind heparin and heparan-sulfate-containing acidic molecules. The affinity of heparin for the basic stretch in the 165-amino-acid isoform is weaker than that in the 189-isoform [14]. In addition, VEGF-A_{165} binds neuropilin-1 (NRP-1), a co-receptor for VEGF-A through this same basic stretch, which is expressed on the cell surface [43,44]. Isoform-specific mutant mice bearing only VEGF-A_{120} (VEGF-A120/120) or only VEGF-A_{188} (VEGF-A188/188) die in the embryonic stage due to multiple defects in angiogenesis, whereas VEGF-A164/164 mice are healthy, indicating that the 164-isoform (165-isoform in humans) of VEGF-A is essential and sufficient for the basic development and morphogenesis of the closed circulatory system [45,46]. Two major reasons for the importance of VEGF-A_{165} could be as follows: (1) because of the mild affinity of the basic stretch for heparin, VEGF-A_{165} has an appropriate balance between free and bound forms, resulting in a proper gradient in the concentration of this angiogenic factor surrounding the VEGF-A_{165}–secreting cells [47]; and (2) in association with NRP-1 through the basic stretch, VEGF-A_{165} binds with higher affinity than VEGF-A_{121} to the receptor, and efficiently activates the tyrosine kinase of VEGFR2 to transduce angiogenic signals. VEGF-A_{189} binds heparin in the extracellular matrix and NRP-1 on the cell surface. However, due to an extremely high affinity for these molecules, the VEGF-A_{189} isoform does not diffuse efficiently, making a narrow-range gradient of VEGF-A_{189} [47].

The isoform VEGF-A165b, which carries a carboxy terminal sequence different from VEGF-A_{165}, has less affinity for VEGFR; thus, it might be a negative regulator of angiogenesis under certain conditions.

Regulation of *VEGF-A* Gene Expression.

The *VEGF-A* gene is regulated at both the transcriptional and post-transcriptional levels. Growth factors operating via the transcription factors Fos/Jun complex and nuclear factor κB (NF-κB), and hormones such as estrogen, appear to be the major stimulators of *VEGF-A* gene expression under normoxic condition [48–50]. In addition, hypoxic stress blocks the function of von Hippel-Lindau (VHL), a component of the ubiquitin-ligase system, and stabilizes the transcription factor hypoxia-inducible factor complex (HIFα/HIFβ) important for *VEGF-A* gene induction [51–57]. The HIF complex binds at a hypoxia-responsive element (HRE) site in the *VEGF-A* gene and upregulates transcription of *VEGF-A*. Furthermore, hypoxic conditions increase the stability of *VEGF-A* mRNA post-transcriptionally, resulting in the production of more VEGF-A protein.

Involvement of VEGF-A in Pathological Angiogenesis and Vascular Permeability.

VEGF-A levels are increased in a variety of diseases such as cancer, rheumatoid arthritis, diabetic retinopathy, age-related macular degeneration, and atherosclerosis [58–61]. In tumor tissues, VEGF-A is secreted not only from the tumor cells themselves, but also from infiltrating macrophage-lineage cells and mesenchymal cells [62,63]. Blocking of the VEGF-A/VEGFR system with VEGF-neutralizing antibody, soluble VEGFR1 including 'VEGF-Trap', and a low-molecular weight chemical tyrosine kinase inhibitor in a tumor-implanted mouse system significantly decreased tumor growth and metastasis [12, 64–68]. Furthermore, clinical trials for the treatment of colorectal [15], breast, renal, and non-small cell lung cancer with anti-VEGF-A/VEGFR therapy showed a statistically significant increase in disease-free survival with minimal side effects [68a]. Based on these results, the FDA has recently granted approvals to angiogenic inhibitors with anti-VEGF-VEGFR activity (VEGF-A neutralizing antibody and VEGFR tyrosine kinase inhibitor) to treat colorectal, renal, and a part of lung cancer patients (Nonsquamous non-small cell lung cancer [68b]).

VEGF-A has potent vascular permeability activity. Senger et al. [4] and Luo et al. [69] demonstrated that VEGF-A is highly accumulated in ascites fluid of the ascites-tumor model, and that the blocking of VEGF-A strongly suppressed the volume of ascites, number of tumor cells, and hemorrhagic tendency [70]. Thus, the VEGF-VEGFR system is a good target for decreasing symptoms in patients bearing tumor-induced ascites.

Rheumatoid arthritis (RA) models in mice using a variety of inducers revealed that suppression of the VEGF-A/VEGFR system significantly decreased the clinical as well as pathological scores in arthritis, suggesting that anti-VEGF-A/VEGFR therapy could be beneficial for RA-patients [71–73].

Age-related macular degeneration is also related to an increase in VEGF levels in the eye. An aptamer, which is a short RNA molecule specifically blocking the VEGF-A_{165} isoform, and an anti-VEGF-A neutralizing antibody were shown to be effective at suppressing macular degeneration.

PlGF

The placenta growth factor (PlGF) is a member of the VEGF family, highly expressed in the placenta, and binds and activates

only VEGFR1 [11,74]. PlGF has a few isoforms with or without the basic stretch, and a longer form with the basic stretch that binds NRP-1 similar to VEGF-A_{165} and VEGF-A_{189}. Because of the weak tyrosine kinase activity of VEGFR1, PlGF has a limited effect on angiogenesis in vitro such as the proliferation of ECs. Carmeliet et al. [75] showed that *PlGF*-gene knockout mice are basically healthy and fertile, but under certain ischemic conditions, the *PlGF* (-/-) mice are impaired in angiogenesis, wound healing, and cancer. These results imply that PlGF might be another target for suppressing tumor growth.

VEGF-B

VEGF-B also binds and activates only VEGFR1 similar to PlGF. VEGF-B is expressed in a wide variety of tissues, but particularly in heart and skeletal muscle. *VEGF-B* gene knockout mice have no abnormalities in the embryonic stages, but after birth, have smaller hearts, demonstrating an insufficient recovery from an experimentally induced myocardial ischemia [76].

VEGF-C and VEGF-D

VEGF-C and -D are related in structure with approximately 30 and 100 amino-acid sequences in the amino- and carboxy-terminal regions, respectively [16–19]. These extra sequences are proteolytically removed, and the shortest form thus generated has the greatest ability to bind and activate VEGFR3, turning on the signaling cascade for lymphangiogenesis. This short form of VEGF-C also binds and activates VEGFR2 to some extent, suggesting angiogenic activity under certain conditions.

VEGF-D (-/-) mice exhibit no phenotype, but *VEGF-C* (-/-) mice die late in embryogenesis due to a defect of lymph vessel formation [19]. Furthermore, mice heterozygous at the *VEGF-C* gene locus often die during the perinatal stage. These mutant embryos show severe lymphedema and chylous ascites in the abdominal cavity, clearly indicating that a proper concentration of VEGF-C is essential for the development and normal function of lymph vessels. The *VEGF-C* gene is at first expressed in the mesenchymal cells near the budding of lymph vessels from the vein in embryos; thus, the VEGF-C and VEGFR3 system is used in a paracrine manner for the development of lymph vessels [19].

A high level of VEGF-C and –D in tumor cells is a prognostic factor for cancer patients [17,20]. Also, tumor cells exogenously expressing VEGF-C or VEGFD show a high degree of potential for lymph node metastases in mice. These results strongly suggest that blocking of the VEGF-C/D system, using inhibitors such as soluble VEGFR3-Fc, is important for suppressing lymph node metastasis. VEGF-C and -D are potential candidates in the treatment of lymph edema caused by a VEGFR3-inactivation mutation [77] or by postnatal lymph vessel deficiency.

VEGF-E (Orf-VEGF)

Orf virus, a parapox virus, infects sheep, goats, and sometimes humans, and induces a local and transient angiogenesis in skin. Lyttle et al. [22] identified a sequence in the viral genome which could encode a VEGF-related molecule. Ogawa et al. found that VEGF-E_{NZ7} protein encoded in the Orf-viral strain NZ7 binds and activates only VEGFR2, and strongly induces proliferation of vascular endothelial cells [23]. Essentially the same results were obtained in other VEGF-Es encoded in the strains NZ2 and D1701 [24,25]. Furthermore, Kiba et al. and Zheng et al. clearly showed that VEGF-E_{NZ7} and its chimeric forms, together with the human PlGF sequence, strongly induced angiogenesis in subcutaneous tissues in K14-promoter transgenic mice with less edema or inflammatory responses [78,79]. On the other hand, K14- and related promoter-driven *VEGF-A* transgenic mice have a variety of side effects such as severe edema, hemorrhage, and inflammation [80,81]. These results suggest that VEGF-E may be an attractive molecule to use for proangiogenic therapy in the clinic.

T.f. svVEGF

The venom of a snake named “Habu”, *Trimeresurus flavoviridis* (*T. f.*), targets blood vessels as well as muscle tissues in animals. Takahashi et al. [26] isolated a VEGF-like protein from this snake venom, referred to as *T.f.* svVEGF, which has weak endothelial cell proliferating activity but strong vascular permeability activity. Surprisingly, *T.f.* svVEGF significantly activates VEGFR1 but only weakly activates VEGFR2, and this coordinated activation of two VEGFRs appears to induce permeability-oriented signaling within the vascular ECs. Takahashi et al. also isolated the snake *VEGF-A* gene that encodes a protein highly homologous to human VEGF-A (amino acid identity: 71%). Snake *VEGF-A* mRNA is expressed essentially in all the tissues of this animal with three representative isoforms (121, 165, and 189 amino acids), whereas *T.f. svVEGF* mRNA is expressed specifically in the venom tissue. Similar VEGF-like proteins were reported in other snake venoms although the affinity of these proteins for VEGFRs is not well characterized yet [26,28]. Snake venom VEGF is unique among the VEGF family in terms of being an exocrine-type protein and of its permeability-oriented activity. In phylogenetical development, it is most likely that snakes possess the gene for a permeability-dominant, VEGF-like protein to enhance the efficacy of toxins by increasing the permeability of blood vessels in the targeted animals.

VEGF-receptor

Receptor for VEGF and Signaling Within Endothelial Cells

The VEGF family has three high-affinity receptors, VEGFR1 (Flt-1: Fms-like tyrosine kinase-1), VEGFR2 (KDR: Kinase-insert Domain-containing Receptor in humans; Flk-1: Fetal-liver kinase-1 in mice), and VEGFR3 (Flt-4) [16,82–87]. These receptors are structurally highly related to each other, and conserve the seven-Ig (Immuno-globulin) like domain-containing extracellular domain, the tyrosine kinase domain with about a 70-amino acid-long kinase-insert sequence, and the carboxy terminal tail. VEGFRs are distantly related to 5-Ig domain-containing tyrosine kinase receptors such as the PDGF receptor (PDGFR) [42]. Thus, the VEGFR and PDGFR families belong to a super-family of tyrosine kinase genes. Mammals and birds conserve three VEGFR systems, and reptiles and amphibians are also suggested to keep this set. However, the zebrafish has four genes for VEGFR, indicating a redundancy via gene duplication [88]. The ligand-receptor relationship among VEGFs-VEGFRs is shown in Fig. 8.2.

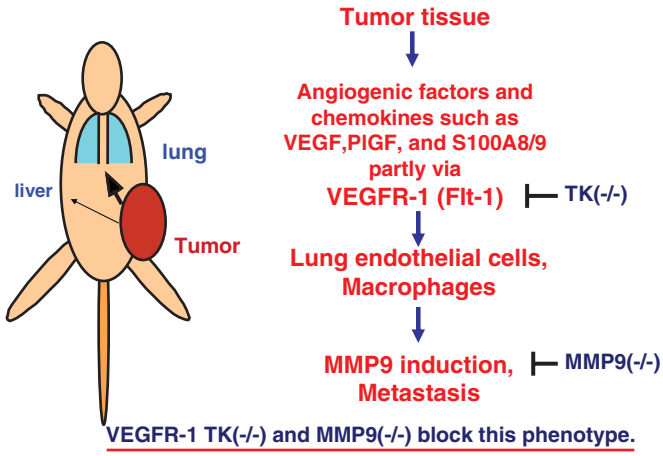

FIG. 8.3. VEGFR1 stimulates tumor metastasis via pre-metastatic induction of MMP9 and other factors in tissues such as lung. Primary tumors stimulate secretion of a variety of factors such as MMP9, SDF-1, S100A8 and S100A9 from lung, spleen and other tissues before metastasis, and enhance tumor metastasis (ref. 101, 102, 125). Lung is the highest tissue for metastasis. Blocking of VEGFR1 signaling by anti-VEGFR1 neutralizing antibody or VEGFR1 TK-deficiency in mice significantly suppresses the process and hence metastasis.

VEGFR1

VEGF-A, a key player for angiogenesis in vivo, binds VEGFR1 and VEGFR2. VEGFR1 has a higher affinity for VEGF-A with a Kd of 2~10 pM, which is about 10-fold that of VEGFR2, whereas the kinase activity of VEGFR1 is one order of magnitude weaker than that of VEGFR2 [89–91]. In addition, the *VEGFR1* gene encodes not only a full-length receptor but also a soluble form that carries the first 6-Ig domains without a transmembrane domain or tyrosine kinase domain [82,92]. This characteristic, that *VEGFR1* encodes both forms, is conserved not only in mammals but also in birds and frogs, indicating that it has been established at an early stage in the phylogenetic development of vertebrates [93].

During embryogenesis in mammals, the *VEGFR1* gene is essential for the normal development of blood vessels, and mutant mice without *VEGFR1* die due to the overgrowth and disorganization of blood vessels [94]. This negative role of VEGFR1 in vascular development is exerted by tight-binding at the extracellular domain of VEGFR1 since tyrosine kinase-domain-deficient (VEGFR1 TK-/-) mice are healthy and develop an almost normal circulatory system [95].

Interestingly, VEGFR1 is expressed in adulthood not only in vascular ECs but also in monocyte / macrophage lineage cells [96–98], playing an important role in the progression of various diseases such as cancer, rheumatoid arthritis, and atherosclerosis (Fig. 8.3) [71,99–104]. Furthermore, soluble VEGFR1 was found to be abnormally expressed in high amounts in preeclampsia patients, such that the levels of soluble VEGFR1 in the maternal serum were well correlated with the degree of preeclampsia, including hypertension and proteinurea [105–107]. The intravenous injection of soluble VEGFR1 into normal pregnant rats induced symptoms similar to preeclampsia in humans, strongly suggesting that an excess amount of soluble VEGFR1 abnormally traps the physiologically required VEGF-A in the body, particularly in the kidney, resulting in the dysfunction and apoptosis of vascular endothelial cells in the glomeruli.

Soluble VEGFR1 is also highly expressed in the cornea of mammals. Ambati et al. [108] showed that the avascularity of the cornea is maintained by soluble VEGFR1, and aniridia patients with vascularized cornea lost expression of soluble VEGFR1 in corneal epithelial cells.

VEGFR2

In the embryonic stage, *VEGFR2/flk-1*-gene minus mutant mice die due to a lack of vasculogenesis [109]. This indicates that VEGF-A-VEGFR2 is essential for the differentiation of hemangioblasts into ECs as well as for the proliferation and morphogenesis of ECs. Activation of VEGFR2 tyrosine kinase results in the autophosphorylation of several tyrosine (Y) residues in its intracellular domain, and Y951, Y1054, Y1059, Y1175, and Y1214 were highly phosphorylated [110,111]. Among them, Y1175 is important for triggering the downstream signaling from the receptor. Phorphorylation of Y1175 recruits PLCγ (phospholipaseCγ) and activates the PLCγ-PKC-Raf-MEK-MAP-kinase pathway resulting in DNA synthesis and angiogenesis (Fig. 8.4) [110,112]. Surprisingly, unlike other representative tyrosine kinase receptors such as EGFR, the activation of Ras (Ras-GTP formation) is

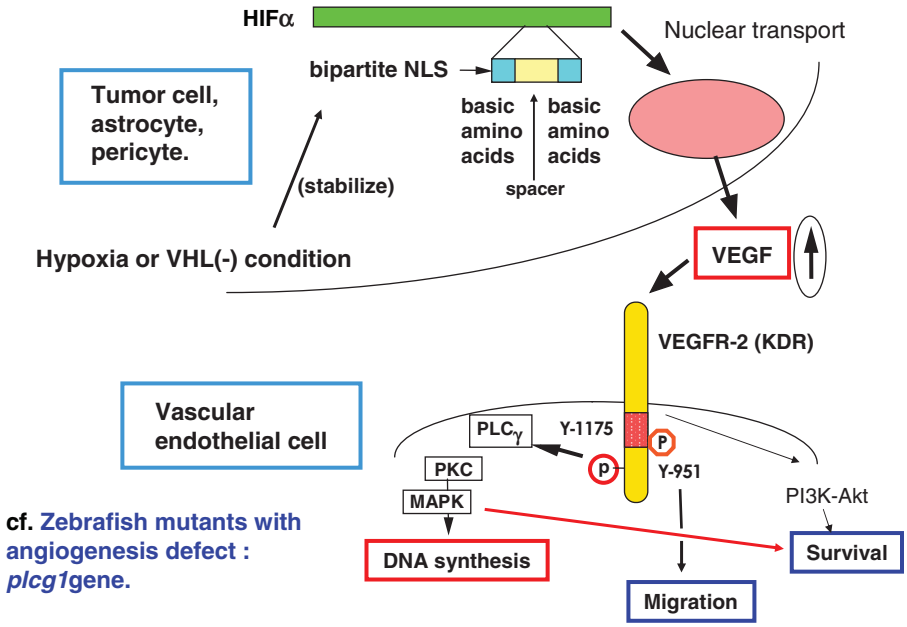

FIG. 8.4. Expression of *VEGF-A* gene and signal transduction from VEGFR2. Hypoxia is a crucial inducer for the *VEGF-A* gene expression in tumor tissue. VEGF-A activates VEGFR2 tyrosine kinase, and stimulates the PLCγ-PKC-Raf-MAP-kinase pathway toward angiogenesis. The TSAd pathway is a regulator for cell migration.

very weak downstream from VEGFR2. Although PDGFR is structurally related to VEGFR, it acquired a kinase-insert (KI) sequence different from that in VEGFR, and PDGFR-KI contains phosphoinositide 3-kinase (PI3K)-Akt activation motifs which connect to the Ras pathway. Therefore, the signaling system of VEGFR appears very unique, much milder than that of regular tyrosine kinase signaling, most likely for keeping the vascular structure stable during angiogenesis. For the migration signal from VEGFR2, the adaptor protein TSAd is reported to be involved in this process by its binding to the phosphorylated Y951 residue of VEGFR2 [113].

Phosphorylation of Y1175 is essential for the function of VEGFR2 in vasculogenesis, since a single tyrosine to phenylalanine mutation at Y1173 in murine VEGFR2 (corresponding to Y1175 in humans) results in embryonic death due to a lack of blood vessel formation [114]. Other crucial signaling molecules, such as VE-cadherin, integrins, and c-Src, were also reported to bind VEGFR2, regulating endothelial cell-cell interaction, cell-matrix adhesion, and vascular permeability [115–117]. Nitric oxide (NO) synthesizing system such as eNOS and iNOS appears to be partly related to the downstream signaling from VEGFR2 [118].

VEGFR2 is directly involved in many forms of pathological angiogenesis such as tumor angiogenesis and the formation of ascites. Thus, in addition to VEGF-A, VEGFR2 is also an important target in the pharmacological development of anti-cancer drugs. A VEGFR-tyrosine kinase inhibitor was recently approved by the FDA as a therapeutic agent to treat renal cell carcinoma patients [119], and many other tyrosine kinase inhibitors such as ZD4190 and PTK787/ZK 222584 are currently being evaluated in clinical trials [67,68,120].

VEGFR3

As described in detail in Chapter 43, the VEGF-C/D and VEGFR3 system is the major regulator for lymphangiogenesis in vertebrates. A deficiency of this system induces a severe defect of angiogenesis and lymphangiogenesis, and embryos die in the middle stages of pregnancy, E10.5 in mice [121]. Other signaling systems such as Angiopoietin-Tie2 cooperate with VEGF-C/D-VEGFR3 for the physiological development of lymph vessels (refer to Chapter 10 for a discussion on Angiopoietins) [122]. Lymphangiogenesis is highly related to lymph node metastasis in cancer, indicating that blocking of VEGF-C/D and/or suppression of VEGFR3 signaling is an effective way to decrease metastasis and malignancy in cancer.

Co-Receptor: Neuropilin-1 and Neuropilin–2.

Vascular and lymphatic endothelial cells express the membrane proteins neuropilin-1 and −2 (NRP-1, NRP-2), respectively. They function as a co-receptor for the VEGF family. Particularly, VEGF-A_{165} binds NRP-1 via the basic stretch, and this association increases significantly the affinity of VEGF-A for VEGFR2, stimulating its signaling of angiogenesis [44]. The association of VEGF-A_{165} with NRP-1 is essential for embryogenesis, since a lack of the VEGF-A_{165} isoform or a lack of NRP-1 results in similar embryonic lethality due to

poor development of the dorsal aorta, insufficient aorticopulmonary truncus, and a lack of remodeling in angiogenesis in the yolk sac [123]. NRP-2 also associates with VEGF-C/D, and is suggested to increase in the signaling of lymphangiogenesis [124].

Prospective

Recently, the VEGF-VEGFR system was reported to be involved in the neuronal system and some neuronal disorders in mice (see Chapter 42). VEGF and its receptor are basically used in tissues in a paracrine manner, where the cells adjacent to vascular endothelial cells such as smooth muscle cells and astrocytes secrete VEGF, and activate VEGFR on endothelial cells. On the other hand, endothelial cells secrete cytokine(s) other than VEGF to communicate to adjacent cells. However, such a paracrine mechanism might be disrupted in pathological situations, and an autocrine type activation or a reverse activation may occur. Thus, poor signaling from VEGFRs might directly induce severe cellular damage not only in vascular endothelial cells but also in other cell types such as neurons. Further studies are necessary to fully understand how deeply VEGF/VEGFR is involved in a variety of systems in the body, and how it is linked to various diseases.

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