Vascular Endothelial Growth Factor Signaling Pathways: Therapeutic Perspective

Marcin Kowanetz and Napoleone Ferrara

Abstract

The establishment of a vascular supply is one of the earliest and most important events occurring during embryonic development. Growth and maturation of a functional vascular network are complex and still incompletely understood processes involving orchestrated activation of vascular progenitors in the early stages of embryonic development followed by vasculogenesis and angiogenesis. These processes require a tightly regulated activation of several growth factors and their receptors. The role of vascular endothelial growth factors (VEGF) and their receptors has been studied extensively due to their prominent role during blood vessel formation. Mice deficient in various VEGF ligands or receptors show serious defects in vascular formation and maturation. Moreover, members of the VEGF family are involved in other significant biological processes, including lymphangiogenesis, vascular permeability, and hematopoiesis. Importantly, VEGF is released by tumor cells and induces tumor neovascularization. It is now well established that the VEGF axis represents an important target for antitumor therapy. Aberrant VEGF signaling is also a feature of several other pathologic conditions, such as age-related macular degeneration and rheumatoid arthritis.

Components of the Vascular Endothelial Growth Factor Signaling Pathway

In mammals, the vascular endothelial growth factor (VEGF) family consists of five members: VEGF-A (thereafter called VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor. In addition, alternative exon splicing results in generation of four main VEGF isoforms denoted VEGF121, VEGF165, VEGF189, and VEGF206 (1, 2). Moreover, VEGF165 can be cleaved by plasmin and various metalloproteinases at the COOH terminus, generating VEGF110 or VEGF113, two bioactive NH$_2$-terminal fragments (3).

Members of the VEGF family show different affinities for one of the three VEGF tyrosine kinase receptors: VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3. Moreover, several coreceptors, such as heparan sulfate proteoglycans and neuropilins, have been implicated in promoting activation of VEGFRs (reviewed in refs. 4, 5). VEGFR-1 is able to bind VEGF, VEGF-B, and placental growth factor; VEGFR-2 is activated primarily by VEGF but proteolytically cleaved forms of VEGF-C and VEGF-D may also activate this receptor; VEGFR-3 is activated only by VEGF-C and VEGF-D. Both VEGFR-1 and VEGFR-2 are expressed in vascular endothelial cells. In addition, VEGFR-1 is also expressed by monocytes and macrophages, hematopoietic stem cells, and certain nonendothelial cell types (6). VEGFR-2 is also expressed in hematopoietic stem cells (6).

Interestingly, subsets of liquid and solid tumor cells were found to express VEGFR-1 and VEGFR-2 (7). VEGFR-3 regulates lymphangiogenesis, and its expression in the adult seems to be largely restricted to lymphatic endothelial cells (6).

Gene targeting studies in mice have shown the importance of VEGFs and VEGFRs in the development of the vascular system. VEGF seems particularly important because loss of even a single Vegf allele results in embryonic lethality at days 11 to 12 (8, 9). Vegf2$^{-/-}$ mice die at embryonic days 8.5 to 9.5 due to defect in the development of hematopoietic and endothelial cells resulting in impaired vasculogenesis (10). Phenotypic analysis of animals lacking other VEGF-related ligands and receptors has revealed their role at different stages of vascular and lymphatic development (reviewed in ref. 11).

As for other receptor tyrosine kinases, VEGFR activation is dependent on availability of the ligand. Ligand binding to the receptor leads to receptor homodimerization or heterodimerization. However, the significance and signaling properties of VEGFR heterodimers have not been extensively investigated (reviewed in ref. 12). Dimerization of receptors leads to their activation and subsequent autophosphorylation on certain tyrosine residues, which in turn triggers intracellular signaling cascade mediated by several effectors, which are able to recognize and dock at phosphorylated tyrosine residues of the activated receptors (reviewed in ref. 11). These interactions are mediated by Src homology 2, phosphotyrosine-binding, and other domains of the signaling proteins (13).

Despite a high degree of homology within the kinase domain, the signaling properties of different VEGFRs greatly differ. Interestingly, VEGFR-1 binds VEGF with at least 10-fold higher affinity than VEGFR-2, yet it becomes poorly activated for, thus far, unclear reasons (14). A study by Gille et al. (15) of chimeric VEGF-1 and VEGFR-2 revealed that the juxtamembrane domain of VEGF-1 plays an inhibitory role in VEGFR-1.
signaling pathways, although the precise mechanism requires further investigation. The fact that VEGFR-1 is usually expressed at low levels has hampered progress in elucidating its signal transduction pathways (Fig. 1). Overexpression experiments suggested potential interacting partners of VEGFR-1, such as phospholipase C-γ, p85/phosphatidylinositol 3'-kinase, Src homology phosphatase-2, growth factor receptor-bound protein 2 (Grb2), and Nck (11). Yet, it has been difficult to link activation of VEGFR-1 to specific biological responses in cells endogenously expressing this receptor. VEGFR-1 has been shown to mediate monocyte migration (16), recruitment of endothelial cell progenitors (17), hematopoietic stem cell survival (18), and release of growth factors from liver endothelial cells (19). Importantly, VEGFR-1 signaling has been implicated in tumor metastasis. VEGFR-1-dependent induction of matrix metalloproteinase-9 expression in premetastatic lung endothelial cells and macrophages has been reported to promote lung metastasis (20). In addition, a recent study has shown that VEGFR-1-positive hematopoietic bone marrow progenitors form cellular clusters at tumor-specific premetastatic sites before the arrival of tumor cells and dictate organ-specific tumor spread (21). Moreover, it has been shown that VEGFR-1 activates extracellular signal-regulated kinase 1/2 and stress-activated protein kinase/c-Jun NH2-terminal kinase (22) and Src family kinases (23) to mediate growth and migration of human colorectal carcinoma cells. A recent study has shown that activation of VEGFR-1 in breast cancer cells supports their growth and survival (24). However, the signaling properties and biological functions of VEGFR-1 in cancer cells require further investigation.

![Fig. 1. Signal transduction and biological processes mediated by VEGFRs and various therapeutic strategies to inhibit VEGF signaling. Major tyrosine autophosphorylation sites (number) and intracellular effectors of activated VEGFR-2. Signaling cascades triggered by VEGFR-1 and VEGFR-3 are still incompletely understood. Inhibition of VEGF signaling can be achieved by monoclonal antibodies targeting VEGF (bevacizumab and ranibizumab), aptamers that bind the heparin-binding domain of VEGFR165 (pegaptanib), chimeric soluble receptors, such as VEGF-Tr, monoclonal antibodies targeting VEGFRs, various small-molecule tyrosine kinase inhibitors (sorafenib, sunitinib, and vatalanib), and anti-sense and short interfering RNA strategies (not shown). Top right inset, because pegaptanib binds to VEGF within heparin-binding domain (yellow), it inactivates only VEGFR165. In contrast, bevacizumab and ranibizumab inactivate all biologically active forms of VEGF, such as VEGFR165. eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; Grb2, growth factor receptor-bound protein 2; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; PI-3K, phosphatidylinositol 3'-kinase; PKB, protein kinase B; PKC, protein kinase C; PLC-γ, phospholipase C-γ; SHb, Src homology 2 and j domain; SHP-2, Src homology phosphatase-2; TSAd, T-cell-specific adapter; PIGF, placental growth factor.](https://www.aacrjournals.org/doi/fig/10.1158/1078-0432.CCR-05-2704)
There is now much evidence that VEGFR-2 is the major mediator of VEGF-driven responses in endothelial cells and it is considered to be a crucial signal transducer in both physiologic and pathologic angiogenesis. Its signaling pathways are relatively well understood. Y1175 in human VEGFR-2 has been identified as a major autophosphorylation site following VEGF binding (25). It serves as a docking site for phospholipase C-γ (25), which indirectly mediates activation of the mitogen-activated protein kinase pathway and thus regulates cell proliferation. Moreover, Y1175 is a binding site for other adaptor molecules, such as Src homology 2 and β cells (Shb; ref. 26). Interaction between Shb and phosphorylated Y1175 of VEGFR-2 is required for VEGF-dependent phosphatidylinositol 3’-kinase activation, and it is important for endothelial cell migration (26). A second major autophosphorylation site in human VEGFR-2 is Y1214, which is involved in activation of Cdc42 and p38 mitogen-activated protein kinase (27), a pathway regulating cell motility. Lastly, in the tumor vasculature, T-cell-specific adaptor (TSAd) binds to phosphorylated Y951. Subsequently, activated TSAd forms a complex with Src (28), which regulates cell migration. Several other signaling molecules are activated by VEGFR-2, and these include focal adhesion kinase and its substrate paxillin (29) or IQGAP1 (30). These pathways regulate endothelial cell motility. VEGFR-2-mediated activation of Src and Akt pathways also leads to increased production of nitric oxide, and thus, it may regulate vascular tone and permeability (6).

VEGFR-3 and its ligands, VEGF-C and VEGF-D, are key players in the regulation of normal and tumor lymphangiogenesis (reviewed in ref. 31). Several tyrosine residues have been predicted to become autophosphorylated on activation and dimerization of VEGFR-3 (11). However, a limited number of signaling effectors have been shown to act downstream of this receptor. Y1337 is required for Grb2 and SHC-mediated transforming capacity of VEGFR-3 (32). Moreover, VEGFR-3 mediates antiapoptotic effects as well as proliferation and migration of lymphatic endothelial cells through Akt and protein kinase C-mitogen-activated protein kinase signaling cascade (33).

VEGF Signaling and Disease—Therapeutic Approaches

Early pioneers observed over a century ago that tumor growth is accompanied by increased proliferation of blood vessels, and later on, a few investigators suggested that such vessels play a critical role in tumorogenesis because they may enable tumor cells to acquire a growth advantage relative to normal cells (reviewed in ref. 34). In 1971, Folkman (35) proposed that angiogenesis may be a novel anticancer strategy. That was followed by the discovery of several proangiogenic factors (acidic and basic fibroblast growth factors, epidermal growth factor, transforming growth factor-α, transforming growth factor-β, VEGFs, and several more; reviewed in ref. 34). This potential redundancy raised the possibility that tumor angiogenesis may require the action of numerous factors, such that blocking a single angiogenic molecule might have little or no effect on tumor growth. However, in 1993, experiments with neutralizing antibodies showed that blockade of VEGF alone can substantially suppress tumor growth and angiogenesis in mouse models (36). Subsequent studies with a dominant-negative VEGFR-2 (37) confirmed that vascularization and growth of a glioma is dependent on secretion of VEGF. These early encouraging findings were confirmed and extended to a variety of tumor models, including genetic models (reviewed in ref. 38). Importantly, in adults, VEGF-mediated angiogenesis occurs rarely (except during wound healing and in the female reproductive cycle). Therefore, targeting VEGF is expected to have relatively modest effects on physiologic processes. Several pharmacologic approaches to inhibit the VEGF axis have been described (39).

The first antiangiogenic agent approved by the Food and Drug Administration is bevacizumab (Avastin, Genentech, Inc., South San Francisco, CA), a humanized version (40) of an anti-VEGF monoclonal antibody used in early proof-of-concept studies (36). In 2004, bevacizumab was approved for the treatment of previously untreated metastatic colorectal cancer in combination with 5-fluorouracil-based chemotherapy regimens (41). Bevacizumab is presently being tested in several phase III studies in combination with chemotherapy, including relapsed metastatic colorectal cancer, non–small cell lung cancer, and previously untreated or relapsed metastatic breast cancer. Preliminary evidence of clinical benefit has been observed in all cases, except in relapsed breast cancer in combination with capcitabine (reviewed in ref. 41). Although bevacizumab was generally well tolerated, some significant toxicities were infrequently observed, including hypertension, gastrointestinal perforation, and arterial thromboembolic complications (reviewed in refs. 3, 41, 42).

Currently, other anti-VEGF agents are at various stages of clinical development (43). These include the following: VEGF-Trap (Regeneron, Tarrytown, NY), a soluble receptor targeting VEGF, VEGF-B, and placental growth factor; an antisense oligonucleotide VEGF-AS (VasGene Therapeutics, Inc., Los Angeles, CA) targeting VEGF, VEGF-C, and VEGF-D; and others.

Inhibition of VEGFR kinase activity is another approach to reduce VEGF signaling. Several small-molecule tyrosine kinase inhibitors are at various stages of clinical development (39, 42, 43). Due to the large number of molecules undergoing development, only those in advanced clinical trials will be mentioned here. Sunitinib (Sutent, Pfizer, New York, NY) is a small-molecule receptor tyrosine kinase inhibitor that, in addition to VEGFR-2, blocks activity of platelet-derived growth factor receptor-β, c-Kit receptor, and fms-related tyrosine kinase 3 and has been recently approved by the Food and Drug Administration to treat patients with gastrointestinal stromal tumors. Sorafenib ( Nexavar, Bayer, Leverkusen, Germany and Onyx Pharmaceuticals, Emeryville, CA) is a novel inhibitor of Raf kinase and VEGFR-2 tyrosine kinase that was shown to block cancer cell proliferation and angiogenesis in several different tumors. Sorafenib has been recently approved by the Food and Drug Administration for metastatic renal cell carcinoma and is undergoing clinical testing in other malignancies. Vatalanib (PTK787/ZK222584, Novartis, Basel, Switzerland) blocks kinase activity of all three VEGFRs as well as platelet-derived growth factor receptor-β and c-Kit. Clinical trials of vatalanib in combination with chemotherapy in metastatic colorectal cancer patients did not meet their end points.

Neutralizing monoclonal antibodies against VEGFRs represents an additional strategy to inhibit VEGF signaling. Recent studies with anti-VEGFR-1 and anti-VEGFR-2 antibodies have shown the ability of both reagents to decrease primary and metastatic tumor growth in some mouse models (7, 24).
Cancer is not the only pathologic condition to which anti-VEGF treatment can be applied. The neovascular form of age-related macular degeneration is a leading cause of blindness among the elderly (44). Choroidal neovascularization results in reduction or loss of central vision and legal blindness. The current Food and Drug Administration–approved age-related macular degeneration treatments involve verteporfin (Visudyne, Novartis AG) photodynamic therapy (45) and pegaptanib (Macugen, OSI Pharmaceuticals, Melville, NY, and Pfizer), an anti-VEGF aptamer that inactivates the most abundant VEGF165 isofrom through binding to its exon 7–encoded heparin-binding domain (46). Both treatments were shown to slow the progression of disease, although vision improved only in a small group of patients. Ranibizumab (Lucentis, Genentech, Inc.) is an affinity-matured Fab variant derived from bevacizumab-Fab (3). Unlike pegaptanib, which selectively neutralizes intact VEGF165, ranibizumab neutralizes the biological activities of all human VEGF isoforms and bioactive proteolytic fragments. Preliminary analysis of randomized phase III trials indicates that approximately one third of patients experienced an improvement in visual acuity following treatment for 1 year with monthly intravitreal injections of ranibizumab. On June 30, 2006, the Food and Drug Administration approved ranibizumab for the treatment of patients with the neovascular form of age-related macular degeneration. Several other agents targeting the VEGF signaling pathways in age-related macular degeneration have been developed and are in early stages of clinical tests. These include the VEGF-Trap and short interfering RNA molecules Cand5 and Sirna-027 (47, 48), which inhibit expression of VEGF and VEGFR-1, respectively.

Altered VEGF signaling occurs in several other diseases, such as inflammatory disorders, and in some pathologic conditions of female reproductive tract (38). Elevated levels of VEGF were found in rheumatoid arthritis patients, and administration of soluble VEGF-R1 attenuated the disease process in an animal model of rheumatoid arthritis (49). Increased VEGF levels have also been reported in polycystic ovary syndrome, one of the leading causes of female infertility (50).

Concluding Remarks

There is now proof that antiangiogenic therapy using VEGF inhibitors results in a clinical benefit in cancer patients. However, there are still some significant challenges to be overcome before the field may advance in a significant manner. One of the most important questions is why all patients do not respond to the anti-VEGF treatment. Therefore, a better understanding of signaling pathways in tumor angiogenesis besides VEGF and its receptors may help achieve an even more effective anticancer therapy. Furthermore, it would be desirable to have reliable markers to identify the patients who are most likely to respond to anti-VEGF treatment.

The biological functions of VEGFR-1 remain incompletely understood. The recent finding that certain tumors express VEGFR-1 (reviewed in ref. 7) implies that cancer cells may take advantage of autocrine VEGF signaling loops to survive and rapidly grow. Moreover, some tumors may also engage the VEGF-C/VEGFR-3 axis to promote migration and invasion (51). In addition, high levels of VEGF-C (as well as VEGF-D) activate lymphatic endothelial cells and enhance lymphatic metastasis (reviewed in ref. 52). Therefore, combining anti-VEGF agents with inhibitors of VEGF-C or VEGFR-3 may provide a powerful anticancer approach.

In addition to VEGF, tumor cells may secrete other members of the VEGF family and express combination of several VEGFRs, raising the possibility that they contribute to tumor growth and metastasis by autocrine and paracrine mechanisms. Although this hypothesis has not been rigorously tested yet, it argues for a better understanding of the biological responses mediated by VEGF-related growth factors.

Importantly, blocking VEGF signaling represents a promising therapy not only for cancer patients. VEGF inhibitors have been shown to slow down visual loss and, in some cases, even improve vision in age-related macular degeneration patients. Furthermore, several nonneoplastic disorders might benefit from treatment with VEGF inhibitors. Elevated levels of VEGF have been found in synovial fluids of rheumatoid arthritis patients (53). However, the function of VEGF in rheumatoid arthritis is still not completely understood. Recently, it has been suggested that VEGFR-1 mediates proliferation of bone marrow hematopoietic cells and immunity of monocytes/macrophages and promotes chronic inflammation in a murine model of rheumatoid arthritis (54). This suggests that therapeutic suppression of VEGFR-1 activity might be beneficial for rheumatoid arthritis patients.

References

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