Role of vascular endothelial growth factor in regulation of physiological angiogenesis

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Ferrara, Napoleone. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 280: C1358–C1366, 2001.—Evidence accumulating over the last decade has established the fundamental role of vascular endothelial growth factor (VEGF) as a key regulator of normal and abnormal angiogenesis. The biological effects of VEGF are mediated by two tyrosine kinase receptors, Flt-1 (VEGFR-1) and KDR (VEGFR-2). The signaling and biological properties of these two receptors are strikingly different. VEGF is essential for early development of the vasculature to the extent that inactivation of even a single allele of the VEGF gene results in embryonic lethality. VEGF is also required for female reproductive functions and endochondral bone formation. Substantial evidence also implicates VEGF as an angiogenic mediator in tumors and intraocular neovascular syndromes, and numerous clinical trials are presently testing the hypothesis that inhibition of VEGF may have therapeutic value.

BIOLOGICAL EFFECTS OF VEGF

VEGF is a mitogen for vascular endothelial cells derived from arteries, veins, and lymphatics (17, 33, 34, 87). VEGF also elicits a pronounced angiogenic response in a wide variety of in vivo models (68, 87). There is also strong evidence that VEGF is a survival factor for endothelial cells, both in vitro and in vivo (4, 11, 42, 44, 115). VEGF withdrawal has been shown to result in regression of vasculature in several physiological and pathological circumstances. It has been proposed that pericyte coverage is the critical event that determines whether endothelial cells require VEGF for survival in vivo (10). Consistent with a prosurvival activity, VEGF induces expression of the antiapoptotic proteins Bel-2 and A1 in human endothelial cells (42). Also, the prosurvival activity of VEGF requires the phosphatidylinositol 3-kinase (PI 3-kinase)/Akt signal transduction pathway (44).
Recent studies have reported mitogenic effects of VEGF also on a few non-endothelial cell types in culture, such as retinal pigment epithelial cells (52), pancreatic duct cells (79), and Schwann cells (104). It remains to be established whether this is also true in vivo.

VEGF is known also as vascular permeability factor (VPF) on the basis of its ability to induce vascular leakage in the guinea pig skin (25, 96). These early studies focused on protein extravasation. More recently, Bates and Curry (7) showed that VEGF also induces an increase in hydraulic conductivity of isolated microvessels and that such an effect is mediated by increased calcium influx (8).

Other studies have suggested that another important role of VEGF in the regulation of microvascular permeability is the induction of a fenestrated phenotype in at least certain endothelial cells (28, 92, 93).

Dvorak (24) proposed that an increase in microvascular permeability to proteins is a crucial step in angiogenesis. According to this hypothesis, leakage of plasma proteins and formation of an extravascular fibrin gel would be sufficient for endothelial cell growth; the role of mitogenic growth factors would be primarily to augment this process (26). This hypothesis has been tested recently by Eliceiri et al. (27). According to these authors, Src activity is required for VEGF-dependent activities. Members of the Src family appear to be differentially involved in mediating VEGF-dependent permeability vs. angiogenesis (27). Mice deficient in src or yes showed no VEGF-induced vascular permeability, while fyn+/− mice displayed a normal permeability response. However, mice deficient in any of these kinases did not show any defect in VEGF-mediated angiogenesis, suggesting that this function requires multiple members of the Src kinase family. In contrast, the vascular permeability-enhancing activity of VEGF specifically depends on src or yes (27). Because mice lacking src and yes not only have a normal angiogenic response to exogenous VEGF but also develop normally, the conclusion is that enhanced vascular permeability is not a requirement for VEGF-dependent angiogenesis.

**VEGF ISOFORMS**

By alternative exon splicing of a single gene consisting of eight exons (108), several VEGF isoforms can be generated. Initially, four human VEGF isoforms were described: VEGF121, VEGF165, VEGF189, and VEGF206 (55, 68, 99, 108). Mouse and rat VEGF isoforms are shorter by one amino acid (17, 33). More recently, several additional isoforms have been reported, including VEGF145 (88) and VEGF183 (57), representing less frequent splice variants. VEGF121 is weakly acidic and fails to bind to heparin, while VEGF165 is a basic, heparin-binding protein (56). VEGF189 and VEGF206 are more basic and bind to heparin with even greater affinity than VEGF165 (56). VEGF121 is a freely diffusible protein; VEGF165 is also secreted, although a significant fraction remains bound to the cell surface and the extracellular matrix (ECM). In contrast, VEGF189 and VEGF206 are almost completely sequestered in the ECM (85). However, these isoforms may be released in a soluble form by heparin or heparinase, suggesting that their binding site is represented by proteoglycans containing heparin-like moieties. The long forms may be released also by plasmin following cleavage at the COOH terminus (60). These findings suggest that the VEGF proteins may become available to endothelial cells by at least two different mechanisms: as freely diffusible proteins (VEGF121, VEGF165) or following protease activation and cleavage of the longer isoforms. However, loss of heparin binding, whether due to alternative splicing of RNA or plasmin cleavage, results in a substantial loss of mitogenic activity for vascular endothelial cells (60). In agreement with such conclusions, a very recent study has shown that only VEGF164 is able to fully rescue a tumorigenic phenotype in VEGF−/− cells (51). VEGF120 partially rescued and VEGF188 failed completely to rescue tumor expansion, indicating that VEGF164/165 has optimal characteristics of bioavailability combined with high biological potency. Recent studies have demonstrated that VEGF121 fails to bind neuropilin-1, an isoform-specific VEGF receptor that presents VEGF165 to its signaling receptors in a manner that enhances the effectiveness of the signal transduction cascade, providing a further explanation for the lower bioactivity of VEGF121 relative to that of other isoforms (103). Table 1 summarizes the properties of the VEGF isoforms.

Other studies have suggested that VEGF isoforms also play a differential role in the development of hemorrhage associated with brain tumors (16). Overexpression of VEGF121 or VEGF165 but not of VEGF189 resulted in significant intracerebral bleeding, suggesting again an important biological difference among the VEGF isoforms (16).

**VEGF RECEPTORS**

**Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2)**

**Tyrosine Kinases**

**Binding characteristics and structural properties.** The Flt-1 (fms-like tyrosine kinase) (23) and KDR (kinase domain region) (107) receptors bind VEGF.

**Table 1. Properties of the principal VEGF isoforms**

<table>
<thead>
<tr>
<th>VEGF121</th>
<th>VEGF165</th>
<th>VEGF189</th>
</tr>
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<tbody>
<tr>
<td>Heparin affinity</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Biological potency</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Diffusibility</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Binding to NRP1</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Summary of some of the properties of the vascular endothelial growth factor (VEGF) isoforms. As discussed in text, there is an inverse relationship between heparin affinity and diffusibility. VEGF121 has the lowest affinity for heparin and the highest bioavailability. VEGF165 is tightly bound to heparin-like moieties in the cell surface and probably plays a role in angiogenesis mainly as a proteolytic fragment, which becomes diffusible. The heparin-binding domain is important for biological activity. Recent studies have indicated that binding to neuropilin-1 (NRP1), which is mediated by the heparin-binding domain, may, at least in part, explain the greater potency of VEGF165 relative to VEGF121. NRP1 presents VEGF to the signaling receptor in a manner that enhances the effectiveness of the signal transduction cascade.
with high affinity. Both Flt-1 and Flk-1/KDR have seven immunoglobulin (Ig)-like domains in the extracellular domain, a single transmembrane region, and a tyrosine kinase (TK) domain, which is interrupted by a kinase-insert domain (74, 98, 106). Flt-1 has the highest affinity for recombinant human VEGF165, with a dissociation constant (Kd) of ~10–20 pM (23). KDR has a lower affinity for VEGF, with a Kd of ~75–125 pM (107). As indicated in **Signal transduction**, there is compelling evidence that KDR is the key signaling receptor, while Flt-1 functions, at least in some circumstances, as a decoy receptor. A cDNA coding an alternatively spliced soluble form of Flt-1 (sFlt-1), lacking the seventh Ig-like domain, transmembrane sequence, and cytoplasmic domain, has been identified in human umbilical vein endothelial cells (HUVEC) (58, 59).

Previous studies have mapped the binding site for VEGF to the second Ig-like domain of Flt-1 and KDR (5, 20, 39). Deletion of the second domain of Flt-1 abolished the binding of VEGF. Introduction of the second domain of KDR into an Flt-1 mutant lacking the homologous domain restored VEGF binding (20, 21). However, the ligand specificity was characteristic of the KDR receptor, since the mutant failed to bind PlGF. Wiesmann et al. (113) solved the crystal structure of a VEGF-Flt-1 domain 2 complex. These studies have shown domain 2 in a predominantly hydrophobic interaction with the poles of the VEGF dimer.

**Signal transduction.** Several studies have demonstrated that Flt-1 and KDR have different signal transduction properties (95, 112). Porcine aortic endothelial cells devoid of VEGF receptors display chemotaxis and mitogenesis in response to VEGF when transfected with a plasmid coding for KDR but not Flt-1 (112). Flk-1/KDR undergoes strong ligand-dependent tyrosine phosphorylation in intact cells, while Flt-1 revealed a weak or undetectable response (95, 112). In further support of this conclusion, VEGF mutants that bind selectively to Flk-1/KDR are able to induce mitogenesis and chemotaxis in normal endothelial and angiogenesis, as well as permeability, in vivo (46, 61). In contrast, Flt-1-selective VEGF mutants are devoid of such activities (46, 61). Also, anti-idiotypic antibodies that activate Flk-1/KDR promote tumor angiogenesis (83). Furthermore, Flk-1/KDR activation has been shown to be required for the antiapoptotic effects of VEGF for HUVEC in serum-free conditions (44). These findings agree with those of other studies showing that PlGF, which binds with high affinity to Flt-1 but not to Flk-1/KDR (Table 2), lacks direct mitogenic or permeability-enhancing properties or the ability to effectively stimulate tyrosine phosphorylation in endothelial cells (84). Interestingly, high concentrations of PlGF expected to saturate the Flt-1 sites are able to potentiate the activity of VEGF, both in vivo and in vitro (84). These findings led to the hypothesis that Flt-1 may not be primarily a signaling receptor but, rather, a “decoy” receptor, able to regulate the activity of VEGF on the vascular endothelium in a negative fashion by sequestering and rendering this ligand less available to Flk-1/KDR (84). Recent studies have provided more evidence in support of this hypothesis. Hiratsuka et al. (54) showed that a targeted mutation resulting in a Flt-1 receptor lacking the TK domain but able to bind VEGF does not result in lethality or any defect in embryonic development and angiogenesis in mice, while complete inactivation of the receptor results in early embryonic lethality (see *Embryonic and early postnatal development*). Furthermore, endothelial cells isolated from Flt-1(TK−/−) animals display a normal mitogenic response to VEGF. These results suggest that Flt-1 plays its main role in angiogenesis as a ligand-binding molecule, rather than as a signal transducing receptor, at least as defined by conventional criteria. Interestingly, recent studies have shown that the juxtaplamembrane region of Flt-1 contains a repressor sequence that constitutively inhibits VEGF-dependent signal transduction (47). Deletion of such a repressor sequence confers on Flt-1 the ability to mediate VEGF-dependent endothelial cell migration and PI 3-kinase activation (47). Recently, Ogawa et al. (80) isolated a polypeptide with ~25% amino acid identity to mammalian VEGF, encoded by a gene previously identified as a parapoxvirus orf virus, which affects sheep, goats, and, occasionally, humans. This polypeptide, VEGF-E, is a potent mitogen and permeability-enhancing factor. Interestingly, VEGF-E binds Flk-1/KDR (Table 2) and induces its autophosphorylation to almost the same extent as VEGF165 but fails to bind Flt-1 (75, 80). Together, these studies strongly support the hypothesis that, while the role of Flt-1 in the adult vasculature is still unclear, interaction with Flk-1/KDR is essential to induce the full spectrum of VEGF biological responses. Nevertheless, several studies indicated that Flt-1 is able to interact with various signal transducing proteins, including the p85 subunit of the PI 3-kinase and the mitogen-activated protein kinase, and in some circumstances generates a mitogenic signal, at least in transfected cell lines (19, 67, 112).

Soker et al. (103) demonstrated the existence of an additional VEGF receptor that binds VEGF165 but not VEGF121. This isoform-specific VEGF receptor is iden-

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**Table 2. Binding of VEGF family members to the known VEGF tyrosine kinase receptors**

<table>
<thead>
<tr>
<th>VEGF</th>
<th>Flt-1 (Flt-1)</th>
<th>Flk-1/KDR (Flk-1/KDR)</th>
<th>Flt-4 (Flt-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>VEGF-B</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>VEGF-C</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>VEGF-D</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>VEGF-E</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>PlGF</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Summary of the interaction of several members of the VEGF gene family with the three known VEGF tyrosine kinase receptors. VEGFR-3 (Flt-4), which binds VEGF-C and VEGF-D, appears to be primarily involved in the regulation of lymphatic angiogenesis and is not a receptor for VEGF-A. Placenta growth factor (PlGF) and VEGF-B only bind Flt-1/VEGFR-1 and appear to have little direct mitogenic/angiogenic activity. VEGF-E, a protein encoded by a strain of the parapoxvirus orf virus, appears to be a selective ligand of KDR/VEGFR-2 and strongly induces angiogenesis and vascular permeability.
tical to human neuropilin-1, a receptor for the collapsin/semaphorin family that regulates neuronal cell guidance (40). When coexpressed in cells with KDR, neuropilin-1 enhanced the binding of VEGF<sub>165</sub> to KDR and VEGF<sub>165</sub>-mediated chemotaxis. Conversely, inhibition of VEGF<sub>165</sub> binding to neuropilin-1 inhibits its binding to KDR and its mitogenic activity for endothelial cells. These findings suggest that neuropilin-1 may present VEGF<sub>165</sub> to the KDR receptor in a manner that enhances the effectiveness of KDR-mediated signal transduction. So far there is no evidence that neuropilin-1 signals after VEGF binding. As previously mentioned, these findings may contribute to the explanation of the greater mitogenic potency of VEGF<sub>165</sub> compared with VEGF<sub>121</sub>. Migdal et al. (77) showed that PI GF-2, a heparin-binding isoform of PI GF (71), also binds to neuropilin-1.

**Role of VEGF and Its Receptors in Physiological Angiogenesis**

**Embryonic and early postnatal development.** Gene targeting studies have demonstrated that both Flt-1 and Flk-1/KDR are essential for development of the embryonic vasculature in mice (36, 97). However, their respective roles in endothelial cell proliferation and differentiation appear to be distinct. Mouse embryos homozygous for a targeted mutation in the Flt-1 locus died in utero between days 8.5 and 9.5 (36). Endothelial cells developed in both embryonic and extraembryonic sites but failed to organize in normal vascular channels. More recent studies have shown that an excessive proliferation of endothelial progenitors is the main factor leading to disorganization (37). As previously noted, more recent studies revealed that normal blood vessel development and survival may occur in the absence of the TK domain of Flt-1 (54). Flk-1<sup>−/−</sup> mice lacked vasculogenesis and also failed to develop blood islands. Hematopoietic precursors were severely disrupted and organized blood vessels failed to develop throughout the embryo or the yolk sac, resulting in death in utero between days 8.5 and 9.5 (97).

Two independent studies (14, 31) have generated direct evidence for the role played by VEGF in embryonic vasculogenesis and angiogenesis. Inactivation of a single VEGF allele in mice resulted in embryonic lethality between days 11 and 12. The VEGF<sup>+/−</sup> embryos were growth retarded and also exhibited a number of developmental anomalies. The forebrain region appeared significantly underdeveloped. In the heart region, the outflow region was grossly malformed; the dorsal aortas were rudimentary, and the thickness of the ventricular wall was markedly decreased. The yolk sac revealed a markedly reduced number of nucleated red blood cells within the blood islands. Significant defects in the vasculature of other tissues and organs, including the placenta and nervous system, were observed. In situ hybridization demonstrated the expression of VEGF mRNA in heterozygous embryos. Thus the VEGF<sup>+/−</sup> phenotype is due to gene dosage and not maternal imprinting.

More recently, Carmeliet et al. (15) reported an isoform-specific knockout of the VEGF gene. They generated mice that express exclusively VEGF<sub>120</sub>. Fifty percent of the VEGF<sub>120/129</sub> mice died shortly after delivery, while the remainder died within 2 wk. The survivors demonstrated impaired myocardial contractility, heart enlargement, and defective angiogenesis leading to ischemic cardiomyopathy. These findings suggest that the action of the heparin-binding isoforms of VEGF cannot be replaced by VEGF<sub>120</sub>.

To selectively investigate the role of VEGF in the heart, a heart-specific VEGF knockout was performed (48). Using Cre-loxP technology, mice with cardiomyocyte-specific deletion of exon 3 in the gene encoding VEGF were generated. Cardiomyocytes represent less than one-third of the total cell number in the heart, yet knockout mice had reduced body weights, and their hearts were thin walled, dilated, and hypovascular and displayed definitive basal contractile dysfunction. Furthermore, the hearts of these mice had fewer coronary microvessels, thinned ventricular walls, depressed basal contractile function, induction of hypoxia-responsive genes, and an abnormal response to β-adrenergic stimulation. These findings establish a critical role for the cardiomyocyte as a secretory cell.

To determine the role of VEGF in postnatal development, two independent strategies were employed. Inducible, Cre-loxP-mediated gene targeting or administration of a soluble VEGF receptor chimeric protein [mFlt(1–3)-IgG] was used to inactivate VEGF in early postnatal life (43). Mice containing the “floxed” VEGF allele were bred to a strain transgenic for Cre recombinase controlled by an interferon-inducible promoter (MX-1) (65). Partial inhibition of VEGF achieved by this inducible gene targeting system resulted in increased mortality, stunted body growth, and impaired organ development, most notably of the liver. Administration of mFlt(1–3)-IgG, which achieves a higher degree of VEGF inhibition, resulted in nearly complete growth arrest when the treatment was initiated at day 1 or day 8 postnatally. Such treatment was also accompanied by rapid lethality. Decreased levels of proliferation of various cell types were detected in all organs studied. Ultrastructural analysis documented alterations in endothelial and other cell types. The liver of mFlt(1–3)-IgG-treated neonates had fewer endothelial cells and focal loss of integrity of the space of Disse. Histological and biochemical changes consistent with liver and renal failure were observed. Endothelial cells isolated from the liver of mFlt(1–3)-IgG-treated neonates demonstrated an increased apoptotic index, indicating that VEGF is required not only for proliferation but also for survival of endothelial cells. Furthermore, these findings are consistent with a critical dependence of glomerular development on VEGF (63, 102, 109). However, VEGF inhibition resulted in less significant alterations as the animal matured, and the dependence on VEGF was eventually lost sometime after the fourth postnatal week. Administration of mFlt(1–3)-IgG to juvenile mice failed to induce apoptosis in liver endothelial cells (43).
Endochondral bone formation. Endochondral bone formation is a fundamental mechanism for longitudinal bone growth during vertebrate development (89). Cartilage, an avascular tissue, is replaced by bone in a process termed endochondral ossification. During this process, the epiphysial growth plate undergoes morphogenesis. A region of resting chondrocytes differentiates into a zone of proliferating chondrocytes that then hypertrophies and, finally, undergoes apoptotic cell death, while being replaced by bone. Such a sequence of events relies on the precise coupling of chondrogenesis (cartilage production) with osteogenesis (bone formation). During this process, blood vessel invasion from the metaphysis coincides with mineralization of the ECM, apoptosis of hypertrophic chondrocytes, ECM degradation, and bone formation.

Recently, the role of VEGF in endochondral bone formation was examined. The VEGF mRNA is expressed by hypertrophic chondrocytes in the epiphysial growth plate (13, 45). To determine the functional significance of VEGF, a soluble VEGF receptor was administered systemically to 24-day-old mice (45). After such treatment, blood vessel invasion was almost completely suppressed, concomitant with impaired trabecular bone formation. Recruitment and/or differentiation of chondroclasts, which express gelatinase B/matrix metalloproteinase-9 (111), and resorption of terminal chondrocytes was substantially decreased. Although proliferation, differentiation, and maturation of chondrocytes were apparently normal, resorption of hypertrophic chondrocytes was inhibited, resulting in a three- to sixfold expansion of the hypertrophic chondrocyte zone (45).

Interestingly, a similar, albeit less dramatic, phenotype was obtained when VEGF was deleted in the cartilage of developing mice by means of Cre-loxP-mediated, tissue-specific gene inactivation (53). In this model system, the cartilage specific collagen 2a promoter drives the expression of Cre recombinase, which in turn induces the VEGF knockout. loxP sites were introduced into the genome of mice flanking exon 3 of the mouse VEGF gene (43). In this model, a two- to threefold increase in the number of hypertrophic chondrocytes at embryonic day 17.5 was observed (53).

These findings indicate that VEGF-dependent blood vessels are essential for coupling cartilage resorption with bone formation. Therefore, the vasculature carries the essential cellular and humoral signals required for correct growth plate morphogenesis. However, cessation of the anti-VEGF treatment was followed by capillary invasion, restoration of bone growth, resorption of the hypertrophic cartilage, and normalization of the growth plate architecture. Interestingly, VEGF receptor expression was not localized to only vascular endothelial cells. Osteoblasts strongly expressed Flt-1 but not Flk-1/KDR. In this context, it is interesting to point out that an earlier study (76) found VEGF to have chemotactic effects on bovine osteoblasts. It is tempting to speculate that these effects are mediated by Flt-1. These findings indicate that VEGF-mediated capillary invasion is a critical signal that regulates growth plate morphogenesis and triggers cartilage remodeling.

Female reproductive tract angiogenesis. Follicular growth and the development and endocrine function of the ovarian corpus luteum (CL) are dependent on the proliferation of new capillary vessels (6). Subsequently, the blood vessels regress, suggesting the coordinated action of inducers and inhibitors of angiogenesis in the course of the ovarian cycle (49, 72). Earlier studies suggested the release of angiogenic factors from developing CL (50). Therefore, the identification of the regulators of CL angiogenesis has been the object of intense investigation. Previous studies have shown that the VEGF mRNA is temporally and spatially related to the proliferation of blood vessels in the rat, mouse, and primate ovary and in the rat uterus, suggesting that VEGF may be a mediator of the cyclical growth of blood vessels that occurs in the female reproductive tract (18, 86, 91, 100). Very recently, the availability of an effective inhibitor of rodent VEGF, such as a truncated soluble Flt-1 receptor [mFlt(1–3)-IgG], made it possible to directly test this hypothesis (20, 32). Treatment with mFlt(1–3)-IgG resulted in virtually complete suppression of CL angiogenesis in a rat model of hormonally induced ovulation (32). This effect was associated with inhibition of CL development and progesterone release. Also, failure of maturation of the endometrium was observed, probably reflecting suppression of ovarian steroid production plus a direct inhibition of locally produced VEGF. Areas of ischemic necrosis were demonstrated in the CL of treated animals. However, no effect on the preexisting ovarian vasculature was observed. These findings indicate that VEGF-mediated angiogenesis is essential for CL development and endocrine function. A similar inhibition of CL and uterine angiogenesis has been observed in a toxicological study in cynomolgus monkeys treated with a humanized anti-VEGF monoclonal antibody (rhuMAb VEGF) (94). Very recently, Fraser et al. (38) recently showed that administration of an anti-VEGF neutralizing antibody also inhibits CL development in the marmoset. Treatment, initiated at the time of ovulation and continued for 3 or 10 days, resulted in blockade of development of the normally extensive capillary bed. Luteal function was also markedly compromised by the treatment. These findings support the notion that VEGF-mediated angiogenesis is essential for luteal function.

Role of VEGF in Pathological Angiogenesis

There is considerable evidence that VEGF is a major tumor angiogenesis factor. The VEGF mRNA is up-regulated in a large number of tumor types (for reviews, see Refs. 25 and 33). There is an extensive body of data documenting that inhibition of VEGF activity results in suppression of growth of a wide variety of tumor cell lines in murine models (30, 33, 62). Furthermore, clinical trials in cancer patients are ongoing with several VEGF inhibitors, including a humanized monoclonal antibody (90), and various small molecules inhibiting signal transduction (105, 114).
Diabetes mellitus, occlusion of central retinal vein, or prematurity with subsequent exposure to oxygen can all be associated with intraocular neovascularization (41). By virtue of its regulation by hypoxia (101), VEGF was a potential mediator of such neovascularization. In agreement with this hypothesis, elevations of VEGF levels in the aqueous and vitreous of eyes with proliferative retinopathy have been described (1, 2, 73). In a large series where eye fluids from 164 patients were examined, a strong correlation was found between levels of immunoreactive VEGF in the aqueous and vitreous humors and active proliferative retinopathy associated with diabetes, occlusion of central retinal vein, or prematurity (2).

Neovascularization is a major cause of visual loss also in age-related macular degeneration (AMD), the overall leading cause of blindness (41). Several studies have demonstrated the immunohistochemical localization of VEGF in surgically resected choroidal neovascular membranes from AMD patients (66, 69). These findings suggest a role for VEGF in the progression of AMD-related choroidal neovascularization, raising the possibility that a pharmacological treatment with VEGF inhibitors may constitute a therapy for this condition. Clinical trials using a humanized anti-VEGF Fab are ongoing.

**PERSPECTIVES**

The role of VEGF in developmental and pathological angiogenesis is well documented. However, numerous questions are incompletely answered, including the mechanisms of signal transduction of the VEGF receptors and, in particular, the significance of Flt-1. The recent finding that key signaling functions in Flt-1 are constitutively inhibited by repressor motifs emphasizes the unusual characteristics of this receptor and, furthermore, raises the question as to how such a poorly signaling receptor can play a critical role in the development of the vascular system. This is likely to be the subject of further studies. Also, an important issue is the significance of other members of the VEGF family that selectively bind Flt-1, such as PIGF (84) and VEGF-B (81). In this context, recent studies indicate that VEGF-B inactivation in mice results in reduced heart size and impaired recovery from experimentally induced myocardial ischemia, although the animals develop normally and fail to display any overt deficit (9).

The high expression of VEGF mRNA in human tumors, the presence of the VEGF protein in ocular fluids of individuals with proliferative retinopathies and in the synovial fluid of rheumatoid arthritis patients, and the localization of VEGF in AMD lesions support the hypothesis that VEGF is a key mediator of angiogenesis associated with various disorders. This resulted in extensive clinical testing of such a hypothesis, using a variety of inhibitors (30). The results of phase II studies performed with the use of a humanized anti-VEGF monoclonal antibody in cancer patients have been recently reported (12, 22), and the treatment shows early evidence of clinical efficacy to warrant definitive phase III studies.

**REFERENCES**


