Minireview

Vascular Endothelial Growth Factor, a Potent and Selective Angiogenic Agent*

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The vascular system is essential for providing oxygen and nutrients, removing metabolic waste products, and furnishing efficient access of leukocytes to tissues throughout larger animals. Angiogenesis, the sprouting of new capillaries, is required for the development of the vascular system and, consequently, the growth of vertebrates. Angiogenic proteins, including several from the fibroblast growth factor family, were identified and purified in the 1980s. They were, however, found to be mitogenic not only for vascular endothelial cells but also for a wide variety of other types of cells and appeared to promote angiogenesis as part of coordinated tissue growth and repair. In the late 1980s the first selective angiogenic growth factor was purified on the basis of its ability to induce transient vascular leakage (vascular permeability factor) and endothelial cell mitogenesis (vascular endothelial growth factor (VEGF) or vascularotropin). By amino acid and cDNA sequencing, these proteins were subsequently demonstrated to be identical. The identification of VEGF2 set the stage for a rapid expansion in the understanding of what now appears to be one of the most important mediators of physiologic and pathologic angiogenesis yet discovered. Previous reviews have documented some of the initial characterization of VEGF structure and activities (1–3).3

Molecular Characterization

The originally characterized form of VEGF is an approximately 34–46-kDa homodimeric glycoprotein. The amino acid sequence is ~20% identical with platelet derived growth factor (PDGF) A and B chains (4–7) including 8 conserved Cys residues previously located within the minimal PDGF receptor-binding domain defined by truncated forms of the v-sis-derived oncogenic protein, a viral version of PDGF. In PDGF-BB homodimers, these cysteine residues participate in 3 disulfide bonds within each subunit and 2 symmetric intersubunit disulfide bonds. The amino acid sequence homology implies that the VEGF secondary and tertiary structures, arrangement of intra- and intersubunit disulfide bonds, and relative subunit orientation are similar to those of PDGF.

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2 The abbreviations used are: VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; PlGF, placenta growth factor; KDR, kinase insert domain-containing receptor; Flt, fetal liver kinase; Flt, fms-like tyrosine kinase; sFlt, soluble Flt.
3 For simplicity the VEGF nomenclature is generally used in this review to acknowledge the crucial role of this growth factor as an endothelial cell mitogen.
3 Some primary research articles cited in these reviews are not explicitly referenced within this review.

Soon after the identification of VEGF a DNA sequence encoding a dose homologue was reported. Denominated growth factor (PIGF) on the basis of its original source, it shares 53% amino acid sequence identity with VEGF, including the 8 conserved Cys residues in the putative receptor-binding domain (8), comparable with the 50% identity between the mature PDGF A and B chains. In addition to PIGF homodimers, heterodimers composed of VEGF and PlGF subunits have been recently identified and purified (9) in analogy to PDGF-AB heterodimers. Although the VEGF-PIGF heterodimer is a potent endothelial cell mitogen in vitro, PIGF homodimers exhibit only weak endothelial cell mitogenic activity under similar conditions.

The cDNA sequences of VEGF and PIGF encode N-terminal hydrophobic secretory leader sequences that promote active secretion. Like the PDGF A chain, the VEGF and PIGF genes are expressed as alternatively spliced mRNAs including forms coding polycationic regions near the C-terminal ends of the translated polypeptides as shown in Fig. 1. Three major VEGFs containing mature 121-, 165-, and 189-amino acid residue sequences and a polymerase chain reaction product inferring the existence of a minor 206-amino acid residue version have been identified.

The human VEGF gene is composed of 8 polypeptide coding exons (10). The shortest form of the protein is encoded by exons 1–5 and 8. Inclusion of the cationic polypeptide sequence encoded by exon 7 generates the apparently predominant 165-amino acid form that, in contrast to VEGF121, binds to isolated heparin and to heparan proteoglycans distributed on cellular surfaces and within extracellular matrices. Addition of the very cationic 24-amino acid residue sequence (Fig. 1) encoded by exon 6 promotes even tighter binding of VEGF189 to these endogenous polyanions (11). The PIGF gene contains 7 coding exons (12) that can generate two alternatively spliced forms. PIGF152 differs from the shorter PIGF131 by inclusion of an exon 6-encoded 21-amino acid residue polycationic sequence (Fig. 1) in an equivalent C-terminal location to the VEGF inserts (12). This cationic sequence also promotes heparin binding in vitro (13) and presumably facilitates heparan proteoglycan binding in vivo. Therefore, alternative mRNA splicing appears to modulate VEGF and PIGF binding to endogenous heparan proteoglycans, thus controlling diffusion from cellular sites of synthesis and determining the extent of local storage.

The heparan proteoglycan binding forms of VEGF can be released from cellular surfaces and extracellular matrices by heparinases and by plasmin, a protease that is proteolytically activated during tissue remodeling by plasminogen activators (14). Plasmin-mobilized VEGF, a truncated active form similar in size to VEGF121, does not bind heparin, indicating that this proteolytic treatment probably removes much of the heparin binding C-terminal sequence encoded by the alternatively spliced exons.

VEGF Receptors and Signal Transduction

Two homologous VEGF receptors, KDR (or Flk-1 from mouse) and Flt-1, are expressed by vascular endothelial cells in vitro and in vivo beginning during early vascular embryonic development. As shown in Fig. 2, KDR (15) and Flt-1 (16), each ~1300 amino acid residues long, are composed of 7 extracellular Ig-like domains containing the ligand-binding region, a single short membrane-spanning sequence, and an intracellu
results. Although eliminating either receptor is lethal by day 8–10 of gestation, the phenotypes immediately prior to death are different. Few, if any, vascular endothelial cells are observed in KDR knockout mice compatible with the role of this receptor as a critical mediator of endothelial cell mitosis (19). In contrast, Flt-1 knockout mice contain endothelial cells, but they exist in poorly organized vessels. Therefore, activation of this second receptor by VEGF and PIGF might modulate the interaction of these cells with each other or the basement membrane on which they reside (20).

The Flt-1 receptor mRNA can be spliced to generate forms encoding either the full-length membrane-spanning receptor or a soluble form, denoted sFlt-1, that is truncated on the C-terminal side of the sixth extracellular Ig-like domain (21) as shown in Fig. 1. Pure sFlt-1 retains its specific high affinity binding for VEGF and PIGF (17). However, the soluble receptor fully inhibits VEGF-stimulated endothelial cell mitogenesis at concentrations that are substoichiometric to VEGF so it does not appear to act simply by sequestering the growth factor.

Like other growth factor transmembrane tyrosine kinase receptors, VEGF receptors presumably undergo ligand-induced dimerization. Formation of dimers between sFlt-1 and full-length VEGF receptors could account for the ability of the truncated receptor to override the activity of membrane-spanning receptors because sFlt-1-containing heterodimers would not trigger signal transduction dependent on intracellular tyrosine kinase dimerization. For sFlt-1 to efficiently inhibit mitogenesis by such a "dominant negative" mechanism it would be expected to dimerize not only with Flt-1 but also with the mitogenically competent KDR receptor. Similar inhibitory heterodimerization has been shown to occur between artificially truncated tyrosine kinase-deficient KDR and full-length VEGF receptors (22). Modulation of the relative expression of Flt-1 and sFlt-1 might provide a means by which endothelial cells could regulate their response to VEGF and PIGF.

Ligand-induced growth factor receptor dimerization triggers signal transduction by promoting either autophosphorylation or transphosphorylation of the adjacent receptor subunit and by binding and phosphorylating specific downstream signal transduction protein mediators. In several other growth factor receptors insert sequences within the tyrosine kinase domains contain tyrosine residues that upon phosphorylation generate docking sites for complexation with downstream signal transduction proteins. KDR and Flt-1 kinase domains contain 70 (15) and 66 (16) amino acid residue insert sequences, respectively. At least 4 tyrosine residues in the KDR cytoplasmic domains are subject to either auto- or transphosphorylation, or transphosphorylation of the adjacent receptor subunit and participate several cytoplasmic proteins including some that contain receptor phosphotyrosine-binding SH2 domains and can participate in downstream signal transduction. These tyrosine-phosphorylated proteins include phosphatidylinositol 3-kinase, which phosphorylates phosphatidylinositol 4,5-bisphosphate at the 3-position of the inositol ring to produce potential second messengers, and phospholipase C, an enzyme that hydrolyzes phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate and 1,2-diacylglycerol, which stimulate Ca2+ release and activation of protein kinase C, respectively. In addition, the Ras GTPase-activating protein GAP and Nck, a protein containing one SH2 and three SH3 docking domains that might couple cell surface receptors to other downstream effectors, are phosphorylated (24). The differences in KDR and Flt-1 signal transduction that account for the disparity in mitogenic signal generation are not yet known.

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**Fig. 1. Structures of VEGF and PIGF subunits.** Polypeptide subunits translated from alternatively spliced mRNAs are shown schematically as horizontal bars followed by amino acid residue sequence lengths of the mature processed human subunits after removal of secretory leader sequences. Horizontal lines denote the positions of N-terminal secretory leader signal peptide (SP) sequences and the minimum receptor-binding region of the homologous PDGF B sequence mapped from truncated versions of its v-sis oncogene homologue (MINIMUM V-SIS). Asn-linked oligosaccharides are shown as branched Y symbols. Amino acid residue sequence inserts encoded by alternatively spliced exons are filled by stipples and diagonal lines. The human amino acid residue sequences and net charges of the polycationic matrix targeting signal regions are listed below the corresponding insert bar segments.

**Fig. 2. Structures of VEGF/PIGF receptors.** The full-length VEGF-specific KDR receptor and the homologous Flt-1 receptor, which binds both VEGF and PIGF, are each composed of 7 extracellular Ig-like domains containing the ligand-binding region, a single plasma membrane-spanning sequence, and intracellular tyrosine kinase domains containing a kinase insert sequence. An alternatively expressed soluble truncated form of Flt-1, denoted sFlt-1, contains the N-terminal 6 Ig-like domains followed by a unique 31-amino acid residue C-terminal sequence functions as an inhibitor of VEGF mitogenic activity. Human amino acid (aa) residue sequence lengths are given in parentheses.

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**VEGF**

| SP | MINIMUM V-SIS | Mature subunit (amino acid length) |
|----|---------------|-----------------------------------|
|    |               |                                   |
| Y  |               | 121                               |
| Y  |               | 165                               |
| Y  |               | 189                               |

**PIGF**

| SP | MINIMUM V-SIS |               |
|----|---------------|---------------|
| Y  |               | 131           |
| Y  |               | 152           |

**Matrix targeting signal (-12)**

**Matrix targeting signal (+8.5)**

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**VEGF-activated endothelial cell receptors also phosphorylate several cytoplasmic proteins including some that contain receptor phosphotyrosine-binding SH2 domains and can participate in downstream signal transduction. These tyrosine-phosphorylated proteins include phosphatidylinositol 3-kinase, which phosphorylates phosphatidylinositol 4,5-bisphosphate at the 3-position of the inositol ring to produce potential second messengers, and phospholipase C, an enzyme that hydrolyzes phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate and 1,2-diacylglycerol, which stimulate Ca2+ release and activation of protein kinase C, respectively. In addition, the Ras GTPase-activating protein GAP and Nck, a protein containing one SH2 and three SH3 docking domains that might couple cell surface receptors to other downstream effectors, are phosphorylated (24). The differences in KDR and Flt-1 signal transduction that account for the disparity in mitogenic signal generation are not yet known.
**Distribution and Control of Expression**

Angiogenesis is minimal in healthy adult males but is a prominent activity associated with the female estrus cycle. VEGF expression and neovascularization of ovarian follicles increase immediately prior to ovulation whereas the corpus luteum expresses VEGF shortly after ovulation. Repair of the endometrium at the end of each estrus cycle is dependent on vascular regeneration, and VEGF mRNA expression is elevated in estrogen-responsive epithelial cells lining the oviducts and uterus. VEGF mRNA is found in extraembryonic giant trophoblast cells at the sites of implanted fertilized eggs (25) and persists during early postimplantation. It falls to low levels through the initial stages of embryonic development and then increases during organ growth (26).

VEGF mRNA remains detectable in several adult organs and cell types in vivo. In adult rats VEGF mRNA is present in lung alveolar cells and kidney glomerular and proximal tubules (27). Lower levels are found in liver hepatocytes and brain. In addition, VEGF mRNA is expressed in all cells of the adrenal cortex and testosterone-producing Leydig cells of the testes (25). PlGF mRNA is abundantly expressed within placenta and by human vascular endothelial cells (28). It is also present in several transformed cell lines (9, 12) and in lower levels in adult heart, brain, lung, and skeletal muscle (12), perhaps reflecting endothelial cell expression in vivo.

Transcription of VEGF mRNA is induced by a variety of factors. Serum-derived and paracrine growth factors and cytokines, including PDGF-BB (29), keratinocyte growth factor (fibroblast growth factor-7), epidermal growth factor, tumor necrosis factor (30), transforming growth factor-β1 (29–31), and interleukin-1 (32), can each induce expression of VEGF from 3- to 20-fold in a variety of cultured cells. With the exception of PDGF-BB, none of these factors are directly mitogenic for microvascular endothelial cells in culture; thus their observed angiogenic activities could reflect induction of VEGF expression.

In addition to protein growth factors, some small mediators have been shown to modulate VEGF expression. Phorbol esters increase VEGF protein levels more than 5-fold in human keratinocytes. Prostaglandin E2 increases VEGF mRNA and protein levels in a preproangiogenic cell line in a manner promoted by the differentiation inducer retinoic acid and inhibited by dexamethasone, which suppresses bone formation in vivo (33). Thus one of the mechanisms of prostaglandin promotion of bone growth, a process dependent on angiogenesis, could be its induction of VEGF in osteoblasts.

Hypoxia is known to induce angiogenesis, thereby providing a compensatory mechanism by which tissues can increase oxygenation. Therefore, diminished O2 is one of the most intriguing transcriptional inducers of VEGF (34) and its receptors (35) in normal and transformed cells. Hypoxic induction of VEGF appears to be a general response since many types of cultured cells have been observed to increase VEGF mRNA levels by approximately 10–50-fold as a consequence of lowering the percent O2 from ambient 21% to the range of 0.3%.

Similar induction of VEGF at reduced pO2 levels is seen in vivo including within hypoxic regions of tumors (34). Occlusion of coronary arteries induces myocardial ischemia and VEGF mRNA expression in porcine hearts (36). In response to exposure of rats to chronic hypobaric hypoxia for 1 month VEGF mRNA and protein are elevated in lung alveolar cells, and both KDR and Flt-1 mRNA levels increase in lung vascular endothelial cells along with DNA synthesis indicative of mitosis (35).

The mechanism by which hypoxia increases expression of VEGF is only partially understood. Induction of VEGF is stimulated by CoCl2 and inhibited by CO (37). An analogous CoCl2 stimulation of hypoxia-inducible erythropoietin expression is proposed to act by replacement of iron with cobalt in the porphyrin ring of a putative heme-containing protein oxygen sensor, decreasing its affinity for O2 and favoring the deoxy conformation. In contrast, CO could bind tightly at the O2 site and lock the sensor in the oxy conformation, thus inhibiting hypoxic responses. Either the same or similar regulatory proteins could modulate transcription of VEGF and erythropoietin. In fact, two potential regulatory DNA enhancer sequences that are 90% homologous with the human erythropoietin hypoxia-response element are located 5’ to the transcriptional start site of the VEGF gene (37). In addition, a functional 5’ enhancer of apparently unique sequence has been mapped to a 100-base pair segment — 800 base pairs upstream of the VEGF transcriptional start site (38).

**Biological Activities**

Endothelial cells throughout the vascular system can respond mitogenically to VEGF. No other normally differentiated major cell types have been confirmed to divide in response to VEGF, consistent with the restricted endothelial cell expression of the mitogenically functional KDR receptor. Even if other less commonly studied cells are eventually shown to respond to VEGF, it remains the most selective vascular endothelial cell mitogen known.

VEGF also elicits non-mitogenic responses by vascular endothelial cells including chemotaxis (39) and the expression of plasminogen activators (40) and collagenases (41) that facilitate penetration of growing capillaries into tissues. A single intradermal injection of VEGF, but not PlGF, can induce vascular leakage in 5 min that is largely eliminated within 20–30 min. Endogenous paracrine expression of VEGF adjacent to fenestrated endothelium could contribute to a persistent increase in vascular permeability such as is observed in kidney and brain choroid plexus (27). However, vascular leakage has not been detected in response either to intravascular injections of VEGF, expression from transfected cells (42), or expression in vascularized neural tissue such as the cerebellar granule cell layer containing an intact vascular blood brain barrier that is not associated with persistent permeability (27). Therefore, additional unknown factors might modulate VEGF-induced permeability.

Angiogenesis is an integral feature of normal tissue repair. In rodents, VEGF mRNA is maximally expressed by surface epidermal keratinocytes soon after dermal injury. However, in healing-impaired diabetic rodents it is abnormally low when highly vascular new tissue would develop in normal animals (30). Exogenous VEGF can induce new blood vessel formation and increase perfusion in ischemic rabbit limbs (43) and in response to decreased blood flow in porcine coronary arteries (44). VEGF also promotes the repair of damaged rat carotid artery endothelial monolayers concomitantly inhibiting pathological thickening of the underlying smooth muscle layers, thereby maintaining lumen diameter and blood flow (45).

Elevated expression of VEGF can also contribute to progression of several diseases. The sustained growth of solid tumors appears to be dependent on angiogenesis. Human tumor biopsies exhibit enhanced expression of VEGF mRNAs by malignant cells and VEGF receptor mRNAs in adjacent endothelial cells. VEGF expression appears to be greatest in regions of tumors adjacent to avascular areas of necrosis (34) consistent with the possibility that tumor angiogenesis might be driven, at least in part, by hypoxic induction of VEGF regardless of the particular genetic mutations leading to transformation. Monoclonal anti-VEGF antibodies substantially inhibit the vascularization and growth of human tumors in nude mice but do not...
influence growth of the same tumor cells in culture (46). Therefore, the tumor growth advantage conferred by VEGF expression appears to be a consequence of paracrine stimulation of angiogenesis. Viral expression of a VEGF-budding construct of the mouse KDR receptor, truncated to eliminate the cytoplasmic tyrosine kinase domains, virtually abolishes the growth of a transplantable tumor in mice (22), presumably by the previously described dominant negative mechanism of heterodimer formation with membrane-spanning VEGF receptors.

Pathological neoangiogenesis is a defining feature of a family of human ocular diseases in which vascular growth in the retina leads to visual degeneration culminating in blindness. VEGF accounts for most of the angiogenic activity produced in or near the retina in diabetic retinopathy (47). Elevated VEGF expression also has been observed in several inflammatory conditions typically characterized by increased angiogenesis. Rheumatoid arthritic synovial tissue contains high levels of VEGF mRNA and protein associated with macrophages along the synovial lining (39, 48). Increased VEGF, KDR, and Flt-1 mRNAs are also detected in psoriatic skin (49) and in contact dermatitis, a delayed dermal hypersensitivity reaction (50). The deduced VEGF amino acid sequence is 44% identity with their putative mammalian ancestor and are nearly 50% identical to each other, probably reflecting divergence of rapidly evolving viral genes. An analogous situation was previously recognized in the homologous PDGF system in which the primate retroviral v-sis oncogene presumably arose by the integration and divergence of a host PDGF B gene.

Perspective

The unique vascular endothelial cell selectivity and hypoxic induction of VEGF have contributed to the growing recognition of its physiologic importance as an angiogenic agent. Molecular characterization of the VEGF system has already revealed selective molecular agents and targets that could provide specific therapeutic tools for either enhancing or inhibiting angiogenesis. Further elucidation of the factors and conditions that modulate the expression of VEGF, PIGF, and their receptors will increase our understanding of not only the biological chemistry of this specific molecular system but also of vascular development, growth, and repair.

REFERENCES

1. Neufeld, G., Tesler, S., Gitay-Goren, H., Cohen, T., and Levi, B.-Z. (1994) J. Immunol. 152, 1480–1487
2. Harada, K., Friedman, M., Lopez, J. J., Wang, S. Y., Prasad, P. P., Pearlman, J. D., Edelman, E. R., Sellek, F. W., and Simons, M. (1996) Am. J. Physiol. (Heart Circ. Physiol.) in press
3. Asahara, T., Bauters, C., Pastore, C., Kearney, M., Rossow, S., Bunting, S., Ferrara, N., Symes, J., and Isner, J. M. (1995) J. Vasc. Surg. 314–325
4. Takahashi, S., Arai, A., Takahashi, K., and Goto, S. (1995) J. Vasc. Surg. 21, 314–325
5. Harada, K., Friedman, M., Lopez, J. J., Wang, S. Y., Prasad, P. P., Pearlman, J. D., Edelman, E. R., Sellek, F. W., and Simons, M. (1996) Am. J. Physiol. (Heart Circ. Physiol.) in press
6. Asahara, T., Bauters, C., Pastore, C., Kearney, M., Rossow, S., Bunting, S., Ferrara, N., Symes, J., and Isner, J. M. (1995) J. Vasc. Surg. 314–325
7. Takahashi, S., Arai, A., Takahashi, K., and Goto, S. (1995) J. Vasc. Surg. 21, 314–325
8. Harada, K., Friedman, M., Lopez, J. J., Wang, S. Y., Prasad, P. P., Pearlman, J. D., Edelman, E. R., Sellek, F. W., and Simons, M. (1996) Am. J. Physiol. (Heart Circ. Physiol.) in press
9. Asahara, T., Bauters, C., Pastore, C., Kearney, M., Rossow, S., Bunting, S., Ferrara, N., Symes, J., and Isner, J. M. (1995) J. Vasc. Surg. 314–325
10. Takahashi, S., Arai, A., Takahashi, K., and Goto, S. (1995) J. Vasc. Surg. 21, 314–325
11. Park, J. E., Chen, H. H., Winer, J., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25646–25654
12. Maglione, D., Guerriero, V., Viglietto, G., Ferrara, M. G., Aprilekova, O., Altalito, K., Del Vecchio, S., Lei, K.-J., Chou, J.-Y., and Persico, M. G. (1993) Oncogene 8, 925–931
13. Park, J. E., Chen, H. H., Winer, J., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25646–25654
14. Tisdale, J. M., DiSalvo, J. P., and Symes, J. F. (1995) J. Vasc. Surg. 314–325
15. Takahashi, S., Arai, A., Takahashi, K., and Goto, S. (1995) J. Vasc. Surg. 21, 314–325
16. Park, J. E., Chen, H. H., Winer, J., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25646–25654
17. Maglione, D., Guerriero, V., Viglietto, G., Ferrara, M. G., Aprilekova, O., Altatito, K., Del Vecchio, S., Lei, K.-J., Chou, J.-Y., and Persico, M. G. (1993) Oncogene 8, 925–931
18. Park, J. E., Chen, H. H., Winer, J., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25646–25654