Vascular endothelial growth factors and angiogenesis

Ch. Frelin, A. Ladoux, G. D’Angelo

Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UPR 411. Sophia Antipolis, 660, route des Lucioles, 06560 Valbonne, France.

Reprint Requests: Ch. Frelin, address above.

THE GENE FAMILY AND SPLICE VARIANTS

VEGF (Vascular Endothelial Growth Factor) was initially described in 1989 both as a specific mitogen for vascular endothelial cells and as a factor that increases vascular permeability (VPF, vascular permeability factor) [8, 27]. VEGF rapidly proved to be a potent angiogenic substance. Its importance as a central regulator of vasculogenesis during early embryonic development was clearly demonstrated by targeted gene disruption experiments [6]. This review focuses on recent developments in this field and on the possible roles of VEGF in adult organisms.

Key words: VEGF, angiogenesis, pulmonary hypertension, hypoxia.
The diversity of VEGF isoforms is not matched by a corresponding diversity of receptors but receptor heterogeneity adds a further level of complexity. At least six different membrane structures recognize peptides of the VEGF family. They comprise three signalling tyrosine-kinase receptors and accessory receptors.

**SIGNALLING AND ACCESSORY RECEPTORS**

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121 and 165 forms are the predominant forms, but expression of the 189 form is observed in many tissues. Expression of the other forms is less common. Splice variants of VEGF mainly differ by the lack of or the presence of amino acids encoded by exons 6 (21 amino acids) and 7 (44 amino acids) of the VEGF gene. These sequences confer on VEGF a heparin binding ability and enable binding of the long forms of VEGF to the heparan sulphate proteoglycans of cell surfaces and in the extracellular matrix. Bound VEGF forms can be cleaved by proteases such as plasmin to yield a soluble form of VEGF (VEGF 110).

PIGF (Placental Growth Factor). This is the second form of VEGF described. Its expression is not restricted to the placenta. Three splice variants have been described. PIGF152 and PIGF131 are the two major forms present in tissues. The longer form differs from PIGF131 by inclusion of an exon-6 encoded 21 amino acid residue polycationic sequence that promotes heparin binding [30]. A third form (PIGF216) has recently been described [5].

VEGF-B. Two forms are generated by alternative splicing. VEGF-B186 corresponds to the full length transcript. Splicing of exon 6 introduces a frameshift and generates VEGF-B167 that has a widely different C-terminal sequence [34]. Recombinant VEGF-B is not mitogenic for endothelial cells [33].

VEGF-C. It was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins. VEGF-C is also highly expressed in adult mouse lung, heart and kidney [24].

VEGF-D is closely related to VEGF-C by virtue of the presence of N- and C-terminal extensions that are not found in other forms of VEGF. It is mainly expressed in lungs [17].

Viral forms of VEGF. A VEGF-like protein is encoded by the genome of Orf virus, a member of the poxvirus family. The protein product only binds to VEGF-R2 and neuropilin-1 [43]. The AIDS virus protein, HIV-1/Tat, although unrelated to VEGF's has been reported to bind and activate VEGF-R2. This mechanism may account for the development of Kaposi sarcoma in AIDS patients [1].

**SIGNALLING AND ACCESSORY RECEPTORS**

Signalling receptors are transmembrane receptor tyrosine kinases characterized by an extracellular domain with seven immunoglobulin-like domains and an intracellular split tyrosine kinase. Three receptors have been identified so far [40]:

— VEGF-R1, also known as flt-1, mainly recognizes VEGF121, VEGF165, VEGF-B and PIGF. Disruption of the gene encoding the VEGF-R1 receptor does not prevent the early differentiation of endothelial cells but impairs the development of functional blood vessels. Activation of VEGF-R1 results in an autophosphorylation of the receptor and phosphorylation of GAP, phospholipase Cγ and Shc [41]. VEGF-R1 does not activate the MAPK cascade and does not mediate mitogenic actions of VEGF. It mainly promotes cell migration [18];

— VEGF-R2, also known as KDR/flk-1, recognizes many forms of VEGF, including the viral forms but not PIGF and VEGF-B. Disruption of the VEGF-R2 gene prevents the differentiation of hemangioblasts into endothelial cells [36]. VEGF stimulation of VEGF-R2 results in the dimerization of the receptor, its autophosphorylation on tyrosine residues and phosphorylation of the Src homology (SH) domain of proteins such as phospholipase Cγ, GAP, PI-3 kinase, Nck and Shc [11, 21]. Activated VEGF-R2 also recruits the adapter molecule Grb2 [10]. Grb2 mediates the recruitment of Sos to the membrane and activates Ras and the MAP kinase cascade. Raf-1 is translocated to the membrane [9], phosphorylates MEK which in turn phosphorylates MAPK, resulting in an increased expression of early response genes and stimulation of cell proliferation;

— VEGF-R3, also known as flt-4, is a receptor specific for VEGF-C and D. Its distribution (and role) seems to be restricted to venous and lymphatic vessels [24].

Peptides of the VEGF family bind to membrane structures that are apparently devoid of signalling properties. The heparan sulphate proteoglycans bind the long forms of VEGF's (VEGF 145, 165 and 189, PIGF-2 and VEGF-B167). Glypican-1 for instance has recently been proposed to act as an extracellular chaperone for VEGF165 [19]. VEGF165 and PLGF-2 bind specifically to neuropilins that are also receptor structures for nerve repellents called semaphorins [38]. Two forms of neuropilins are now known. They have no signalling properties but improve the efficiency of VEGF binding to VEGF-R2 and promote migratory responses to VEGF165.

**HYPOXIA-DEPENDENT AND -INDEPENDENT EXPRESSIONS OF VEGF's**

VEGF expression is upregulated by a variety of extracellular factors such as Fibroblast Growth Factor-4, PDGF,
Tumor Necrosis Factor β, Keratinocyte Growth Factor, Insulin Growth Factor-1, IL1 and IL6 and nitric oxide, a major vasodilator which is produced by endothelial cells in response to VEGF [13]. VEGF expression is inhibited by IL10 and IL13. VEGF is thus part of cytokine networks that, for instance, contribute to wound healing.

Hypoxia is a major inducer of VEGF and PIGF expressions [37]. VEGF-B, C and D expressions are not regulated by hypoxia [15]. The mechanisms responsible for the hypoxic induction of VEGF have been analyzed in great detail and appear to be similar to the mechanisms by which oxygen availability regulates the expression of erythropoietin [20].

Hypoxia induces VEGF expression by two independent mechanisms. It increases transcription rates [29] and the stability of VEGF mRNAs [28]. The VEGF mRNA is intrinsically labile, but it stabilizes under hypoxic conditions. Destabilising elements are present in the 3’ and 5’ untranslated regions of the mRNAs but also in the coding sequence [12].

Hypoxia-induced transcription of VEGF mRNAs involves a still to be identified oxygen sensor which may be a heme structure [20] and specific transcription factors (Hypoxia Inducible Factors, HIF’s) of the basic loop helix/PAS domain protein family [42]. During hypoxia, HIF-1α dissociates from HSP90, binds to the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT), migrates into the nucleus and activates specific response elements (HRE) located upstream or downstream of hypoxia sensitive genes [4]. This family includes proteins that confer different protections against hypoxic episodes. A short-term protection occurs at a cellular level and includes the increased expressions of GLUT-1 glucose transporters and of glycolytic enzymes. A protection at an organ level is afforded by VEGF and the resulting angiogenesis. A protection at the organism level is afforded by the production of erythropoietin.

Actions of hypoxia can be mimicked by heavy metals such as cobalt [20, 25] but all actions of heavy metals are not necessarily mediated by the hypoxic transduction cascade [16].

Further complexity in this scheme is provided by the existence of several forms of HIF-1α and HIF-1β (ARNT). Two other forms of HIF-1α (HLF/EPAS and HIF-3) and of ARNT (ARNT-2 and ARNT-3) are now known [14, 39]. These different factors are expressed in a tissue specific manner [7]. Their expressions are also developmentally regulated. In the lung for instance, HIF-1α expression does not change during development. In contrast, HLF/EPAS, only expressed after birth, is the major form of transcription factor expressed in adult tissues. In cardiac tissues, the largest expression of HIF-1α is observed after birth, and may be responsible for the vascularization of growing vessels [26].

Preliminary evidence further suggests the possibility that the different HIF and ARNT factors combine. Whether the different heterodimers have the same efficacy to transduce a hypoxic signal and recognize the same forms of HRE is not yet known.

Expressions of VEGF receptors are also upregulated by hypoxia. Both transcriptional (VEGF-R1) and postranscriptional (VEGF-R2) regulatory mechanisms are involved. These actions may be indirect for VEGF whose expression is hypoxia-inducible, and potentiates expressions of its two receptors.

**TUMOR ANGIOGENESIS**

Growth of solid tumours is stringently dependent on an angiogenic process. As the tumor expands, cells may experience hypoxic conditions. The resulting production of VEGF may then trigger an angiogenic process and lead to tumor vascularization. It is note worthy however that in many types of tumors, VEGF production can be demonstrated in cells located at the periphery of the tumor, i.e. at locations that are not supposed to be hypoxic. It is also remarkable that the tumor vasculature is highly variable and often used as a diagnostic criteria. These observations have suggested that hypoxia is not the only mechanism that determines VEGF production in tumors and that other mechanisms may short-circuit normal hypoxia sensing. This is observed after expression of the oncogene Src [23], after inactivation of the tumor suppressor gene PS3 [32] or of the von Hippel-Lindau (vHL) gene. Cerebral hemangioblastomas and renal carcinoma are highly vascularized, and presumably non hypoxic tumors that produce high levels of VEGF. Cells isolated form these tumors have a mutated vHL gene. The normal vHL gene product inhibits basal and hypoxia induced VEGF production by forming a complex with the Sp1 transcription factor and by preventing Sp1-mediated VEGF gene transcription [31].

**IN VIVO ADAPTATIVE RESPONSES TO HYPOXIA**

Although much is known about the mechanisms by which cultured cells adapt to a hypoxic environment, the mechanisms that allow tissues or organisms to adapt to hypoxic conditions are not completely understood. Chronic exposure of rats to hypoxia (10 % O2) induces a major remodeling of the vasculature. The right ventricle of the heart undergoes hypertrophy, pulmonary arteries thicken, and a usually fatal pulmonary hypertension develops. In the right ventricle, VEGF mRNA expression increases within 12 hours of hypoxia and
then remains high during the development of cardiac hypertrophy and pulmonary hypertension. The observed VEGF production is accompanied by a cardiac angiogenesis as evidenced by an increase in the capillary density. In the left ventricle that does not undergo hypertrophy, VEGF mRNA expression increases within 12 hours and then decreases to baseline. VEGF mRNA expression in the lungs is not affected by hypoxia although this tissue senses hypoxia and responds to it as evidenced by the muscularization of pulmonary arteries [35]. Thus, in vivo, the hypoxic induction of VEGF is tissue specific. These observations suggest that cultured cells, although useful to analyze the transduction of the hypoxic signal, may not be appropriate to analyze the mechanisms of the adaptation of tissues to hypoxia.

Cardiac tissues adapt to hypoxic conditions during ischemic heart diseases. This adaptive mechanism, called preconditioning, involves a variety of mechanisms which operate on different time scales. A first «window» of protection occurs within minutes after hypoxia. It involves opening of ATP dependent K+ channels as a consequence of the exhaustion of cellular ATP reserves. A second window of protection occurs within hours after hypoxia. It involves the release of adenosine and the activation of protein kinase C [22]. A third window of protection develops later on and involves an increased expression of hypoxia sensitive genes such as the GLUT-1 glucose transporters, enzymes of the glycolysis and VEGF [2, 3]. A link between the second and third windows of cardiac protection is provided by a protein kinase C-dependent expression of HIF-1α, the transcription factor that mediates hypoxia dependent gene expression [26].

CONCLUSIONS AND FUTURE DIRECTIONS

Large amounts of information have been gathered in the past 10 years about VEGF, its receptors and their expressions during various pathological processes. The idea that hypoxia is not necessarily deleterious and that cells can adapt to hypoxic conditions by expressing new sets of genes is important and opens new avenues for the development of new strategies to limit the consequences of ischemic diseases.

It should be stressed however that our present knowledge mainly derives from in vitro experiments using cultured cells. Extrapolating this information to in vivo conditions is difficult. (i) Cells in different organs experience different oxygen tensions. (ii) Experiments performed in vitro usually compare normoxic conditions (which in vivo, would be hyperoxic) and almost anoxic conditions (1 % O2). The physiological relevance of such conditions is questionable. (iii) No specific experiment has yet been devised to define the sensitivity of the oxygen sensor(s) to oxygen.

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REFERENCES


