Signal transduction by vascular endothelial growth factor receptors

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Abstract

Vascular endothelial growth factor (VEGF)/vascular permeability factor is the prototype for a growing family of dimeric growth factors, which exert their effects on vascular and lymphatic endothelial cells, as well as on a wide range of other cell types. Gene targeting shows that most, if not all, of the factors and receptors in this family serve critical functions during vascular development or in adult physiological and pathological angiogenesis. Growing tumours produce VEGF, and many different strategies for inhibiting tumour growth by inhibiting VEGF production are being tested in clinical trials at present. This review focuses on the signal transduction properties of VEGF receptor-1 and VEGF receptor-2.

Introduction: the vascular endothelial growth factor (VEGF)/vascular permeability factor family of ligands and receptors

The family of VEGFs includes VEGF-A, -B, -C, -D, -E, and placenta growth factor (reviewed in [1]). Several of these factors, notably VEGF-A, exist as different isoforms, which appear to have unique biological functions [2]. The expression of original VEGF/vascular permeability factor, which now is denoted as VEGF-A, is upregulated in hypoxic conditions, through the binding of hypoxia-inducible factor to the hypoxia-regulated element in the VEGF-A promoter. Growing tumours often have a hypoxic centre and produce VEGF-A, which stimulates growth of vessels into the tumour tissue, thereby facilitating further growth of the tumour [3]. Another unique characteristic of VEGF-A, compared with the other VEGF family members, is the ability to induce vascular leakage, by promoting formation of transcellular pores (reviewed in [4]).

The proteins of the VEGF family bind in a distinct pattern to three structurally related tyrosine kinase receptors, VEGF receptor (VEGFR)-1, -2 and -3. VEGFR-1 and -2 are expressed on vascular endothelial cells, whereas VEGFR-3 is expressed on lymphatic endothelial cells. Heparan sulphated proteoglycans, neuropilins, cadherins and integrin αvβ3 serve as co-receptors for certain, but not all VEGF proteins (reviewed in [5]). Recent results indicate that VEGFR-1 influences angiogenesis by recruiting monocytes and macrophages to sites of pathological angiogenesis, possibly by stimulating differentiation of haematopoietic precursors [6,7]. During embryogenesis, VEGFR-1 appears to have a negative regulatory role, as inactivation of the VEGFR-1 gene leads to an increased number of endothelial cells, which obstruct the vessel lumen [8]. Binding of VEGF-A to VEGFR-2 leads to survival, migration and differentiation of endothelial cells, and to the mediation of vascular permeability. VEGFR-2 gene ablation leads to an arrest in vascular development and embryonic death (reviewed in [3]).

Vasculogenesis and angiogenesis: formation of new blood vessels

The vasculature develops through a number of principal processes. During embryogenesis, blood vessels are formed by the differentiation of endothelial cells from precursors (angioblasts) (reviewed in [9]). The angioblast, which remains poorly defined molecularly, is found between the mesodermal and endodermal cell layers in the yolk sac of the mouse embryo at approx. embryonic day 7. Aggregates of mesodermal angioblasts form blood islands, which are organized as a central core of haematopoietic precursor cells and an outer lining of endothelial cell precursors. The existence of a common stem cell for these two lineages has been proposed and is referred to as a haemangioblast [10], which is known to express VEGFR-2. The cells in the outer layer of the blood islands differentiate further and connect into a primitive vascular plexus that expands through the embryo. The primitive plexus is perfused with blood upon connection of the vascular channels to the developing heart tube at embryonic day 9. The vessels are subsequently remodelled and pruned in angiogenesis, a process in which new vessels are formed from an existing capillary bed. Formation of new vessels in the adult, owing to either physiological or pathological demands, occurs by angiogenesis (reviewed in [11]), and involves the secretion of basement membrane-degrading proteases by endothelial cells, followed by migration, proliferation and differentiation of endothelial cells to form a continuous hollow tube.
Recent work has demonstrated the existence of circulating endothelial precursor cells, which contribute to pathological angiogenesis by integration and differentiation into mature endothelial cells at sites of vascular injury (reviewed in [12]). VEGF-A has been shown to promote recruitment of circulating endothelial precursors [12].

**VEGFR-1 expression, function and signal transduction**

VEGFR-1 expression is detected in angioblasts at embryonic day 8.5 [13]. Subsequently, expression declines between embryonic days 14.5. and 16.5 [13]. The expression of VEGFR-1 is increased in newborn mice and this receptor continues to be expressed at a relatively high level during the adult stages. VEGFR-1 expression is regulated by hypoxia and in agreement with this, there is increased VEGFR-1 expression in endothelial cells in tumours [14]. Many non-endothelial cells also express VEGFR-1; the most clearly established function of VEGFR-1 is in haematopoietic cells. Thus, as described above, recruitment of adult mesenchymal-derived haematopoietic stem cells [7], as well as migration of monocytes [15], appears to involve VEGFR-1. A naturally occurring soluble form of VEGFR-1 (sVEGFR-1) is expressed in the placenta [16,17]. sVEGFR-1 exerts a negative regulatory function by binding to VEGF-A with high affinity, thereby diverting the growth factor from the functional VEGFRs.

In VEGFR-1-deficient mice, endothelial cells and vessel-like structures are formed, but the vessels are non-functional and are filled with endothelial cells [8]. The primary defect resulting in this phenotype appears to be an alteration in cell fate determination, which leads to an increased number of haemangioblasts in the VEGFR-1−/− embryos. Deletion of the intracellular domain of VEGFR-1, which allows synthesis and membrane expression of its extracellular domain, allows normal vascular development [18], but pathological angiogenesis, such as tumour angiogenesis, is impaired [19]. Monocyte function is also impaired in these pathological angiogenesis, such as tumour angiogenesis, the underlying molecular mechanisms have not been resolved. This is at least in part due to the apparent inability of the receptor to respond to VEGF-A by increased kinase activity, which has hampered traditional biochemical analyses. It is possible that the low kinase activity is due to a low number of tightly regulated phosphorylation sites. In accordance with this, a repressor sequence has been identified in the VEGFR-1 juxtamembrane domain [26]; removal of this sequence allows VEGFR-1 to respond to VEGF-A stimulation. The function of the repressor sequence may be to influence the folding of the receptor and thereby the accessibility of the kinase domain active site. Alternatively, the repressor sequence may regulate interactions with phosphatases. Another complicating aspect of analysing VEGFR-1 function in vivo is the existence of sVEGFR-1. As described above, overexpression of sVEGFR-1 exerts a negative regulatory effect, either by sequestering VEGF-A, or by creating dominant-negative receptor dimers that are composed of one molecule of the sVEGFR-1 paired with one full-length receptor of either type. A number of VEGF co-receptors also modulate cellular responsiveness. Thus, the extracellular domain of neuropilin-1 binds with high affinity to immunoglobulin-like loops 3 and 4 in the extracellular domain of VEGFR-1; this prevents binding of neuropilin-1 to VEGF-A [27] and leads to decreased biological effects of the growth factor. Heparan sulphated proteoglycans bind certain VEGF-A isoforms and influence the ability of the receptors to respond to VEGF stimulation [28].

VEGFR-2 expression, function and signal transduction

VEGFR-2 is expressed in endothelial cells in most, if not all tissues in mouse and human embryos. During early embryonic development, the VEGFR-2 gene is first expressed in mesodermal blood-island progenitors at 7.0 days post coitum, and then in angioblasts that surround the blood islands [13]. The expression level of VEGFR-2 in endothelial
Figure 1 | VEGFR-1 phosphorylation sites and interaction with signal transduction molecules

The schematic outline of the intracellular domain of VEGFR-1 is shown, with the juxtamembrane domain (JM), the first tyrosine kinase domain (1st TK) the kinase insert (KI), the second tyrosine kinase domain (2nd TK) and the C-terminal tail (CT) indicated. Three SH2-domain containing molecules bind to the phosphorylation site Tyr1213, namely SHP-2, PLC\(\gamma\)1 and growth factor receptor-bound 2 (Grb2). The phosphorylation site Tyr1333 allows binding of PLC\(\gamma\)1, and the adaptor molecules Crk and Nck. The Tyr1242 and Tyr1327 are phosphorylated at very low stoichiometry and have not been shown to participate in binding of signal transduction molecules [21]. Certain tyrosine residues in the VEGFR-1 C-terminal tail are not detectably phosphorylated (indicated by ?).

The binding affinity of VEGF-A for VEGFR-2 is lower (\(K_d\) 75–125 pM) than for VEGFR-1 (\(K_d\) 10 pM) [33], but the biological activities of VEGF-A are thought to be transduced mainly by VEGFR-2. Binding of VEGF-A to VEGFR-2 causes receptor dimerization, kinase activation and autophosphorylation of specific tyrosine residues within the dimeric complex. Thus far, Tyr821, Tyr933, Tyr969, Tyr1054, Tyr1059, Tyr1175 and Tyr1214 of VEGFR-2 have been implicated as autophosphorylation sites [34,35] (Figure 2). Tyr1054 and Tyr1059 are located in the activation loop of the tyrosine kinase domain, and are required for maximal activation of the VEGFR-2 kinase. Tyr933 and Tyr969 are located in the kinase insert; Tyr969 was recently shown to be essential for VEGF-A-induced human umbilical-vein endothelial cell migration [36]. Tyr1175 and Tyr1214 are located in the C-terminal tail. It has been shown that phosphorylated Tyr1175 is a binding site for PLC\(\gamma\)1 in intact cells [34].

Several studies mainly based on the yeast two-hybrid system, reveal that SH2-domain-containing proteins directly associate with certain potentially phosphorylated tyrosine residues of VEGFR-2. In intact cells, complex formation has been difficult to demonstrate, but a number of signal transduction molecules are activated or modified in response to VEGF-A stimulation. These include phosphoinositide 3-kinase (PI 3-kinase) and its downstream substrate, the serine/threonine kinase Akt/protein kinase B (PKB), PLC\(\gamma\)1, the Src family tyrosine kinases, the Ras GTPase-activating...
Figure 2 | VEGFR-2 phosphorylation sites and interaction with signal transduction molecules

The schematic outline of the intracellular domain of VEGFR-2 is shown, with the different parts of the intracellular domain indicated as in Figure 1. Phosphorylation of Tyr\(^{951}\) allows binding of VEGFR-associated protein (VRAP), which in turn binds PI 3-kinase (PI3-K) and PLC\(\gamma\) via SH3 domain-binding to proline-rich stretches in VRAP. Phosphorylated Tyr\(^{1175}\) binds PLC\(\gamma\) via SH3 domain-binding to proline-rich stretches in VRAP. Phosphorylated Tyr\(^{1214}\) presents a binding site for Grb2. The C-terminal-tail mediates binding of focal adhesion kinase (FAK), which in turn interacts with PI3-K and paxillin. Certain tyrosine residues in the intracellular domain of VEGFR-2 have been implicated in binding of SH2-domain proteins but have not been shown to be phosphorylation sites (indicated by ?).

protein, the small adaptor molecule Nck, focal adhesion kinase, protein kinase C, extracellular signal-regulated kinase (Erk) and the p38 mitogen-activated protein kinase. Moreover, in VEGFR-2 transfected cells, Grb2 (growth factor receptor-bound 2), Shc (Src homology 2 and collagen), signal transducers and activators of transcription (STATs), and the tyrosine phosphatases SHP-1 and SHP-2 are tyrosine phosphorylated (see [5] for references). The biological significance of these interactions is often clouded; however, Akt/PKB is critical in endothelial cell survival [37] and PLC\(\gamma\)-mediated activation of the Erk pathway has been shown to be required for VEGF-induced proliferation of endothelial cells [34].

Gene targeting of different signalling molecules may lead to a vascular phenotype, but it is unclear in most cases whether this is a consequence of the elimination of a VEGFR-2-dependent signal transduction pathway. However, inactivation of the endothelial nitric oxide synthase gene has been shown to inhibit VEGF-A-induced angiogenesis and increase in vascular permeability [38]. Mice that do not express individual Src family kinases do not show vascular defects, but inactivation of the Src and Yes tyrosine kinases together results in impaired VEGF-A-induced vascular permeability and decreased infarct volumes after stroke [39]. Removal of the cytoplasmic domain of vascular endothelial cadherin in vivo results in endothelial apoptosis via the interruption of VEGF-A-induced complex formation of vascular endothelial cadherin, VEGFR-2, \(\beta\)-catenin and PI 3-kinase, leading to decreased activation of Akt and a consequent lack of upregulation of the Bcl-2 gene [40].

Conclusions

Recent results implicate VEGFR-1 as an important factor in haematopoietic function, which indirectly affects...
References

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