Chapter 18
VEGF Signal Transduction in Angiogenesis

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Abstract: The development of a number of novel tumor therapies targeting the function of vascular endothelial growth factors (VEGFs) and their receptors has promoted an interest in understanding signal transduction regulating angiogenesis, i.e. formation of new blood vessels. The VEGFRs regulate many if not all aspects of endothelial cell function during active angiogenesis, and mediate survival signals during endothelial cell quiescence. Most tumors produce VEGF as a consequence of the hypoxic tumor microenvironment, leading to persistent stimulation of angiogenesis necessary for an expansion of the tumor as well as tumor spread through the circulation. Increased understanding of VEGFR signal transduction properties may allow development of fine-tuned therapy, targeting pathways critical in formation of new tumor vessels while preserving pathways required for survival of endothelial cells in normal vessels.

Introduction to Vascular Endothelial Growth Factor (VEGF) Signal Transduction

The term “VEGF” denotes both the prototype family member now named VEGF-A, and the family of five structurally related, homodimeric polypeptides of 40kDa; VEGF-A, -B, -C, -D and placenta growth factor, PlGF. VEGF-A is alternately spliced to generate VEGF-A121, VEGF-A145, VEGF-A165 and VEGF-A189 (indicating the number of amino acid residues in the human splice variants; mouse variants are each one amino acid shorter) endowed with different biological properties [1]. A newly discovered splice variant, denoted VEGF-A165b, contains exon 8b, encoding a unique stretch of 5-amino acid residues. VEGF-A165b binds to VEGFR2 with high affinity but fails to transduce biological responses and may be an antagonist of VEGF-A165 [2]. VEGF-like proteins from the ORF virus family, denoted VEGF-E, cause contagious pustular dermatitis in sheep and goats and is transmissible to humans by direct contact [3, 4]. Snake venom-derived VEGF-like proteins (denoted VEGF-F) have unique structural features [5]. The mammalian VEGFs bind to different extents to three receptor tyrosine kinases, VEGF receptor-1, -2 and -3 [6].

The VEGF receptors are transmembrane glycoproteins with an extracellular ligand-binding domain, which in VEGFR1 and VEGFR2 is organized in 7 immunoglobulin-like loops. In VEGFR3, one of the loops is replaced by a disulfide bridge. The intracellular domain of each receptor is endowed with a ligand-activated kinase domain, which is split in two parts by the insertion of a “kinase insert” sequence of 70 amino acid residues.

In addition to the full-length receptor tyrosine kinase, VEGFR1 occurs as a soluble splice variant composed of the extracellular domain only [7]. The full-length form is expressed on a number of different cell types, including monocytes/macrophages and vascular endothelial cells (ECs) [8]. The soluble splice variant is highly expressed during gestation and has been associated with pre-eclampsia [9]. Deletion of the vegfr1 gene leads to embryonic lethality at embryonic day (E) 11.5 due to excessive proliferation of ECs [10]. Thus, VEGFR1 is thought to serve as a negative regulator of VEGFR2, in part through the soluble variant which acts as a trap for VEGF-A. It cannot be excluded that the activated VEGFR1 kinase domain induces negative regulatory signaling in the target endothelial cell [11, 12]. However, priming cells by activation of VEGFR1 has been shown to enhance subsequent signal transduction via VEGFR2 [13]. The full length VEGFR1 mediates migration of hematopoietic precursors and monocytes (for a review, see

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VEGFR2 is implicated in most if not all aspects of vascular endothelial cell biology. A number of signal transduction pathways induced downstream of VEGFR2 have been identified (see below). During development, VEGFR2 is the first specific endothelial marker to be expressed on hematopoietic/endothelial progenitors [15]. Subsequently, expression of VEGFR2 is turned off in hematopoietic cells. Expression of VEGFR2 on ECs declines during the third trimester, but is induced again in conjunction with active angiogenesis [16, 17]. Inactivation of the vegfr2 gene leads to embryonic death at mouse E 8.5–9.5, due to lack of proper differentiation and/or migration of ECs [18].

VEGFR3 is found primarily on lymphatic endothelial cells, and is critical for lymphatic EC development and function [19]. VEGFR3 may also be expressed on fenestrated capillaries, tumor ECs and on monocytes/macrophages [20, 21]. Mice deficient for VEGFR3 die at E9.5 due to defective remodeling and maturation of the primitive blood vascular plexus into larger vessels [22]. VEGFR3 is the only VEGFR for which naturally occurring mutations have been described [23].

Regulation of VEGF/VEGFR Expression

Hypoxia, i.e. low oxygen tension, is an important regulator of physiological and pathological angiogenesis. In hypoxia, the transcription factor hypoxia-inducible factor (HIF)-1 accumulates, allowing increased transcription of a multitude of genes through binding of HIF-1 to the hypoxia-responsive element (HRE). HIF-1 may also be induced by a number of other stimuli, such as growth factors, under normoxic conditions [24]. Such activation involves several different signal transduction pathways, including the phosphoinositide 3’ kinase (PI3K), extracellular regulated kinase (Erk) 1/2 and PKC pathways, which act through increasing HIF-1 translation or through regulatory phosphorylation [24, 25] (see chapters 15 & 16 for detailed discussion). The VEGFR2 promoter appears to lack a classical HRE, but has been shown to be regulated by the related HIF-2 [26].

Members of the Ets (E26 transforming sequence in avian erythroblastosis virus) family of transcription factors are expressed in endothelial cells and modify expression of several genes implicated in angiogenesis and inflammation; for example, Ets-1 regulates the expression of VEGFR1 and VEGFR2. Transcriptional activity by Ets is regulated e.g. by Erk1/2-mediated serine phosphorylation as well as through a number of other mechanisms (for a review, see [27]).

Activation of VEGFRs

Binding of VEGF leads to dimerization of receptor molecules followed by activation of the intrinsic tyrosine kinase. The VEGFRs have been shown to form both homo- and heterodimers in vitro [28–30]. The activated receptor molecules in the dimers transphosphorylate each other, on tyrosine residues. An initial phosphorylation on positive regulatory tyrosine residue(s) in the kinase activation loop precedes full activation of the kinase. This is followed by phosphorylation on other tyrosine residues in the intracellular domain of the receptor to create binding sites for signaling intermediates, thereby initiating signaling cascades.

Numerous tyrosine phosphorylation sites have been identified on VEGFR1 [31], VEGFR2 [32–34], and VEGFR3 [28] (Fig. 18.1). It is noteworthy that VEGFR1 lacks phosphorylation on positive regulatory tyrosine residues [31], due to replacement of a conserved residue in the activation loop, from Asp to Asn at position 1050 [35]. This may explain why VEGFR1 kinase activity is difficult to induce. Positive regulatory tyrosine phosphorylation is found both in VEGFR2 and VEGFR3. Interestingly, for all three VEGF receptors, certain tyrosine phosphorylation sites are used selectively. Thus, for VEGFR1, phosphorylation site usage is dictated by the particular activating VEGF ligand, such as PIGF [13] and VEGF-A [31], which have been shown to induce different phosphorylation site patterns. For VEGFR2, the Y951 phosphorylation site, located in the insert region between the two parts of the kinase domain, is used primarily when the receptor is expressed in endothelial cells engaged in active angiogenesis [34]. Thirdly, C-terminal sites in VEGFR3 are phosphorylated in VEGFR3 homodimers, but not when VEGFR3 is heterodimerized with VEGFR2 [28]. The implication of these findings is that VEGFR tyrosine phosphorylation is both highly dynamic and tightly regulated, in agreement with the versatility of these receptors in endothelial biology.

Trimeric Gq/G11 proteins have been implicated as important regulators of VEGFR2 signaling. Thus, antisense-mediated suppression of Gq/11 expression completely attenuated VEGFR2 tyrosine phosphorylation and signal transduction through a mechanism involving direct association between the receptor and the trimeric G-proteins [36].

Down-regulation of VEGFR Activity

How are VEGFRs turned off in order to halt signal transduction? One important mechanism involves dephosphorylation by phosphotyrosine phosphatases (PTPs; [37]). One interesting example is the receptor type PTP denoted vascular endothelial (VE)-PTP (also denoted PTP receptor type B; PTPRβ), which is required for maintenance and remodeling of blood vessels during development [38]. VEGFRs do not appear to be direct substrates for VE-PTP; however. The broadly expressed transmembrane PTP denoted density-enhanced phosphatase-1 (DEP1)/CD148, has been implicated in regulation of endothelial cell junctional integrity and silencing of VEGFR2 in dense cells [39]. Moreover, the Src Homology-2 (SH2) domain-containing PTP SHP-2, which is a ubiquitously expressed...