Biochimica et Biophysica Acta 1804 (2010) 567–580

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Structure–function analysis of VEGF receptor activation and the role of coreceptors in angiogenic signaling

Felix S. Grünewald, Andrea E. Prota, Alexandra Giese, Kurt Ballmer-Hofer *

Paul Scherrer Institut, Biomolecular Research, 5232 Villigen PSI, Switzerland

ARTICLE INFO

Article history:
Received 25 June 2009
Received in revised form 22 August 2009
Accepted 4 September 2009
Available online 15 September 2009

Keywords:
VEGF
Angiogenesis
Neuropilin
Receptor tyrosine kinase

ABSTRACT

Vascular endothelial growth factors (VEGFs) constitute a family of six polypeptides, VEGF-A, -B, -C, -D, -E and PlGF, that regulate blood and lymphatic vessel development. VEGFs specifically bind to three type V receptor tyrosine kinases (RTKs), VEGFR-1, -2 and -3, and to coreceptors such as neuropilins and heparan sulfate proteoglycans (HSPG). VEGFRs are activated upon ligand-induced dimerization mediated by the extracellular domain (ECD). A study using receptor constructs carrying artificial dimerization-promoting transmembrane domains (TMDs) showed that receptor dimerization is necessary, but not sufficient, for receptor activation and demonstrates that distinct orientation of receptor monomers is required to instigate transmembrane signaling. Angiogenic signaling by VEGF receptors also depends on cooperation with specific coreceptors such as neuropilins and HSPG. A number of VEGF isoforms differ in binding to coreceptors, and ligand-specific signal output is apparently the result of the specific coreceptor complex assembled by a particular VEGF isoform. Here we discuss the structural features of VEGF family ligands and their receptors in relation to their distinct signal output and angiogenic potential.

1. Biology of VEGF family growth factors and their receptors

1.1. Introduction to VEGF

Vascular endothelial growth factors, VEGFs, were originally discovered as vascular permeability factor, VPF, an activity released by tumor cells that promotes vascular leakage [1]. It is now clear that VPF represented a biological activity attributable to a family of polypeptide growth factors that are encoded by several genes regulating blood and lymph vessel formation during embryonic development, in wound healing, and in maintaining vessel homeostasis in adult organisms. Excess or reduced production of VEGF results in imbalanced formation of blood or lymphatic vessels and causes many human diseases. VEGFs specifically interact with hematopoietic and endothelial precursor cells such as angioblasts, and with differentiating and mature endothelial cells.

Mammalian VEGF-A, -B, -C and placenta growth factor (PlGF) are required for blood vessel formation while VEGF-C and -D regulate the formation of lymphatic vessels [2,3]. In addition, orf family parapoxviruses encode VEGF-A homologues called VEGF-E, which show a high degree of structural identity with VEGF-A [4–6]. Despite only 25–35% amino acid sequence identity with VEGF-A they bind with comparable affinity to VEGFR-2 [7–9]. Moreover, VEGF-E family members lack a heparin binding domain and vary in their abilities to bind neuropilins [7,8]. Several VEGF-like proteins, now called VEGF-F, have also been isolated from snake venoms and reported to have biological activity similar to VEGF-A_{165} [10,11]. Vammin and VR-1 from the venoms of *Vipera ammodytes ammodytes* (Western sand viper) and *Daboia russelli russelli* (Russell's viper), which share ~50% amino acid sequence identity with VEGF-A_{165}, strongly stimulate proliferation of vascular endothelial cells *in vitro* and induce arterial hypotension in rats. Another VEGF-F variant from *Trimeresurus flavoviridis* snake venom, *Tfsv*-VEGF, efficiently promotes vascular permeability similar to VEGF-A_{165}, while stimulation of endothelial cell proliferation by *Tfsv*-VEGF is negligible [12].

Proteolytic processing and alternative splicing give rise to a wide variety of VEGF isoforms with distinct biological activities [13,14]. Fig. 1 shows a schematic representation of the most common splice variants of VEGF-A. All isoforms contain exons 1–5 and either exon 8a or 8b. A 26 amino acid signal sequence (exon 1 plus 4 amino acids of exon 2) is cleaved off during secretion. The VEGFR-1 and -2 binding domain consists of amino acids 1–109 and the VEGFR interaction sites are located at opposite poles of the dimeric molecule [15,16].

Variable combinations of exons 6–8 encode additional basic sequences that mediate binding to heparan sulfate (HS) and which can be released from full length VEGFs by plasmin cleavage [17]. Exon 6a, present only in VEGF-A 206, 189, 162, 145 and partially in VEGF-A_{183}, is a highly basic stretch of 24 amino acids that confers binding to HS and, as shown for VEGF-A_{145}, to components of the extracellular matrix of corneal endothelial cells distinct from HS [18]. Exon 6b has so far only been identified in the less well characterized VEGF-A_{162} and in the longest isoform, VEGF-A_{206}. VEGF-A 165, 183, 189 and 206

* Corresponding author. Tel.: +41 56 310 4165.
E-mail address: kurt.ballmer@psi.ch (K. Ballmer-Hofer).
URL: http://mcb.web.psi.ch (K. Ballmer-Hofer).

1570-9639/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2009.09.002

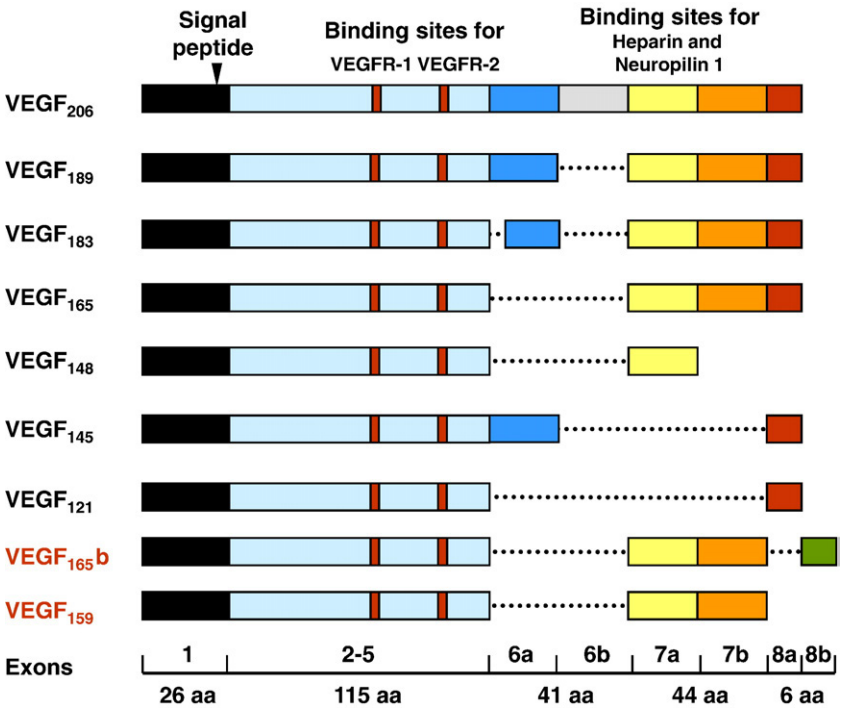

Fig. 1. Schematic representation of selected VEGF-A variants as discussed in the text.

contain an additional sequence encoded by exon 7 that also confers HS binding due to its basic properties and thus limits ligand diffusibility in tissues. The different HS binding affinities conferred by exons 6 and 7 result in specific spatial distribution of the different isoforms. VEGF-A_{189} and VEGF-A_{206} bind HS with high affinity through their exon 6a and 7 encoded basic stretches. Consequently, they remain tightly bound to the ECM, but can be released by cleavage with plasmin, urokinase or matrix metalloproteases [14,19–21] or by disruption of the HS matrix with heparinase [19]. VEGF-A_{145} [18] and VEGF-A_{165} have intermediary affinity to the ECM, while VEGF-A_{121}, lacking exons 6 and 7, is freely diffusible [19].

All VEGF-As, except VEGF-A_{148}, end with either exon 8a or 8b. Alternative splicing of exon 8 results in the formation of two families of proteins of identical length but differing in the carboxyterminal six amino acids. Exon 8b encodes the amino acids SLTRKD-COOH in place of the exon 8a sequence CDKPRR-COOH [22]. VEGF-A_{165}b, the first member of the $VEGF_{xxx}$b family to be described, was found to be present in normal tissue from kidney, but was not expressed by malignant tumor cells [22]. Additional members of the $VEGF_{xxx}$b family identified so far include VEGF-A_{121}b, $VEGF_{145}$b, VEGF-A_{189}b and unspecified larger isoforms [23] as well as VEGF-A_{183}b [24]. It is now clear that proteins of the $VEGF_{xxx}$b family make up a major fraction of VEGF-A in most normal tissues [22,24–26], whereas their expression is negligible in cancer cells [23,27,28]. At the molecular level, the anti-angiogenic effects of the $VEGF_{xxx}$b proteins can to a large extent be ascribed to reduced signaling via VEGFR-2 [23]. These isoforms are partial receptor agonists and, when coexpressed with exon 8a proteins, may exert an inhibitory function by competition for receptor binding. Interestingly, neither exon 8a nor 8b is required for binding to VEGFR-1 or -2 [23,29] and both isoforms bind to VEGF receptors with similar affinities and compete for binding to endothelial cells and to immobilized VEGFR-2 *in vitro* [23]. VEGF-A_{159}, an artificially created truncated mutant of VEGF-A_{165} lacking exon 8, retains VEGFR-1 and -2 binding. In contrast to VEGF-A_{165}b, this mutant does not inhibit angiogenesis in matrigel [29] suggesting that competitive inhibition of VEGFR-2 binding does not fully account for the anti-angiogenic effect mediated by VEGF-A_{165}b. Taken together, we propose that the exon 8b isoforms of VEGF-A are partial receptor agonists capable to elicit some of the signal output of VEGFRs such as for instance anti-apoptotic signaling or vessel permeabilization (for details see also section 2.4.) [26,30].

The complex interplay of VEGF family proteins with VEGF receptors and coreceptors is strictly required for shaping and maintaining the functionality of blood vessels. Correct spatial distribution of specific VEGF isoforms by differential ECM adherence [31] and the coordinated signal output initiated by distinct VEGFs are central for proper vessel organization.

1.2. Receptor specificity of VEGF

The biological functions of VEGF polypeptides result from binding to several cellular receptors: type V receptor tyrosine kinases (RTKs) such as VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4) [32-34], neuropilin-1 and -2 (NRP-1, -2) [35] and heparan sulfate proteoglycans (HSPG) [36]. Some VEGFs interact with multiple receptors while others show very specific receptor binding properties. PlGF and VEGF-B are specific for VEGFR-1 [37,38], VEGF-Es bind VEGFR-2 [4–6], and VEGF-C and -D bind VEGFR-2 and -3 [2,3]. Additional VEGF homologues were found in snake venoms collectively designated VEGF-F, the seventh member of the VEGF family [10]. VEGF-Fs bind to VEGFR-2 with dissociation constants similar to VEGF-A_{165}, but not to VEGFR-1, VEGFR-3 or NRP-1. More recently, a novel VEGF-like protein from the *Trimeresurus flavoviridis* snake venom (*Tfsv*-VEGF) with significantly different characteristics was described. *Tfsv*-VEGF strongly binds to VEGFR-1, but only weakly associates with VEGFR-2 [12].

In addition, VEGF-A splice variants show distinct binding patterns to coreceptors such as HSPG and neuropilins. In all VEGF-A variants exons 2–5 determine the specificity for VEGF RTKs 1–3 while exons 6 and/or 7 and 8 determine coreceptor binding. VEGFs can simultaneously bind two distinct receptors such as VEGFR-2 and neuropilin even when these receptors are expressed separately on adjacent cells [39]. This might be required for promoting endothelial cell migration and cell guidance, for instance, when vessels form along tracks defined by neural cells [40,41] or during endothelial tip cell guidance [42,43].