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Review

VEGF-mediated signal transduction in lymphatic endothelial cells

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Abstract

The VEGF family of angiogenic ligands consists of VEGFA, VEGFB, VEGFC, VEGFD and placenta growth factor, PlGF. These growth factors bind in an overlapping pattern to three receptor tyrosine kinases, denoted VEGFR1, VEGFR2 and VEGFR3. Originally, VEGFA (the prototype VEGF) was described as a master regulator of vascular endothelial cell biology in vitro and in vivo, transducing its effect through VEGFR2. VEGFA, VEGFB and PlGF bind to VEGFR1, which is a negative regulator of endothelial cell function at least during embryogenesis. VEGFC and VEGFD were identified as lymphatic endothelial factors, acting via VEGFR3. With time, the very clear distinction between the roles of the VEGF ligands in angiogenesis/lymphangiogenesis has given way for a more complex pattern. It seems that the biology of the different VEGFR2 and VEGFR3 ligands overlaps quite extensively and that both receptor types contribute to angiogenesis as well as lymphangiogenesis. This paradigm shift in our understanding is due to the access to more sophisticated reagents and techniques revealing dynamic and plastic expression of ligands and receptors in different physiological and pathological conditions. Moreover, knowledge on the important role of VEGF coreceptors, the neuropilins, in regulating the responsiveness to VEGF has changed our perception on the mechanism of VEGF signal transduction. This review will primarily focus on the properties of VEGR3, its signal transduction and the resulting biology.

Keywords: VEGFC; VEGFD; VEGFR3; Flt4; Lymphatics; Endothelial cells; Signal transduction

Contents

1. VEGFC/D and VEGFR3 expression

VEGFC and VEGFD are produced as full length proforms, which bind only to VEGFR3. Human, but not mouse, VEGFC and VEGFD are known to be cleaved by plasmin [1], and furin and proprotein convertases denoted PC5 and PC7 [2]. Processing of VEGFC and VEGFD increases the affinity for binding to VEGFR3 but it also allows for binding to VEGFR2 (for a review, see [3]). VEGFC and VEGFD are expressed by mesenchymal cells adjacent to lymphatic vessels, where expression has been shown to co-localize with

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that, e.g. of furin [4]. In analogy with what is known for other growth factors, expression of VEGFC and VEGFD is regulated during physiological and pathological processes. In accordance, VEGFC has been shown to be upregulated by proinflammatory cytokines [5].

Expression of VEGFR3 is initiated at embryonic day 8 in developing blood vessels, but later, expression becomes more restricted to the lymphatic vasculature. VEGFR3 expression is induced in a specific subset of endothelial cells, i.e. lymphendothelial precursors, in the cardinal vein during development. Establishment of the lymphendothelial precursor cells is regulated by the transcription factors SOX18 (SRY, sex determining region Y, box 18) [6] and Prox1 (prospero homeobox 1) [7,8]. Subsequently, at E10.5, lymphendothelial precursors that express VEGFR3 bud off and migrate away from the embryonic cardinal vein towards a gradient of VEGFC, which is produced by nearby mesenchymal cells. The migrating LECs subsequently assemble into lymph sacs that extend through sprouting, to lay down the framework of the lymphatic system [9]. Later in development, VEGFR3 expression is downregulated on vascular endothelial cells, however, during angiogenesis, VEGFR3 becomes induced and expressed at higher levels [10].

VEGFR3 is also expressed in non-endothelial compartments such as in osteoblasts [11] and in neuronal progenitors [12]. VEGFC as well as VEGFR3 are furthermore expressed in macrophages [13] and VEGFC expression promotes recruitment of macrophages to sites of inflammation [14]. The potential expression of VEGFR3 on tumor cells in vitro and in vivo is disputed (see [15,16] and references therein).

VEGFR3 is markedly upregulated on vascular endothelial cells in response to activation of Notch family members, highly conserved proteins that regulate cell fate determination [17]. In vivo, Notch is coexpressed with VEGFR3 both in vascular and lymphatic vasculature.

2. VEGFC, VEGFD and VEGFR3 gene inactivation

VEGFC gene inactivation results in arrest in lymphatic vessel development and prenatal death due to tissue fluid accumulation [18]. VEGFD gene targeting on the other hand does not result in a discernable phenotype [19]. The fact that the lymphatic hypoplasia in VEGFC+/− animals can be rescued by VEGFD [19] indicates functional redundancy between the two.

VEGFR3 gene targeting results in cardiovascular failure and embryonic death at E9.5. This indicates that the initial embryonic expression of VEGFR3 on vascular endothelial cells is functional and important in embryonic development. The *vegfr3−/−* embryos are characterized by abnormal organization of vessels with defective lumen and fluid accumulation in the pericardial cavity [20].

The phenotypes of animals lacking expression of VEGFC, VEGFD or VEGFR3 are quite different from those lacking expression of VEGFA and VEGFR2, which both show early embryonic lethality, due to defective blood island formation and arrest in endothelial and hematopoietic development (see [21] and references therein).

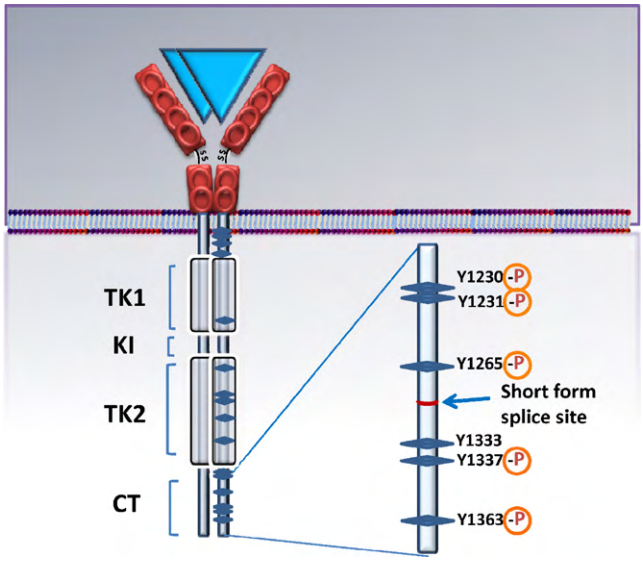

Fig. 1. Schematic outline of VEGFR3 structure and phosphorylation sites A. The extracellular domain is composed of immunoglobulin-like loops. A disulfide bridge keeps the proteolytically cleaved N-terminal part connected with the remainder of the molecule. The tyrosine kinase domain is split in two (TK1 and TK2) by the insertion of a kinase insert (KI) sequence. CT; C-terminal tail. Note that splicing of human VEGFR3 gives rise to a short and a long form. The C-terminal tail of the long form contains six tyrosine residues of which 5 serve as potential phosphorylation sites.

3. VEGFR3 structure, dimerization, activation and phosphorylation on tyrosine residues

VEGFR3 (also denoted Flt4) is a receptor tyrosine kinase composed of an extracellular ligand-binding part organized into immunoglobulin-like loops; a cleavage within the 5th loop is bridged by a disulfide bond ([22]; see Fig. 1). This is followed by a transmembrane stretch, an intracellular part equipped with a tyrosine kinase domain, which is followed by a C-terminal tail. In humans, but not in mice, a retroviral insertion between the two last exons of VEGFR3 results in two splice variants, of which the longer form extends an additional 65 amino acid residues in the C-terminus [22–24]. This stretch includes tyrosine residues Y1333, Y1337 and Y1363.

When in an unbound state, the receptor is believed to exist as a catalytically inactive monomeric protein. Binding of ligand promotes receptor dimerization, which may occur between two VEGFR3 molecules (homodimerization) or between a VEGFR3 and a VEGFR2 molecule (heterodimerization). In analogy with structural analysis of the highly related VEGFR2 extracellular domain [25], it is likely that binding of ligand to VEGFR3 leads to a change in folding of the extracellular domain and establishment of contact points between the receptors in the dimer. Such