Cancer and Metastasis Reviews **15**: 159–163, 1996.

Biological properties of VEGF/VPF receptors

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Key words: VEGF, receptors, angiogenesis

Several experimental findings have emphasized the significant role played by Vascular Endothelial Growth Factor/Vascular Permeability Factor (VEGF/VPF) in tumor angiogenesis. For example, VEGF/VPF actions are predominantly restricted to endothelial cells [1], and the expression of VEGF/VPF is enhanced in tumor cells that lack oxygen and glucose [2]. In addition, inhibiting the interaction of VEGF/VPF with endothelial cells slows the growth of several tumors *in vivo* [3, 4]. The purpose of this review is to summarize what is known concerning one aspect of the biology of VEGF/VPF, the interaction of the growth factor with its cell surface receptors.

Structural properties of VEGF/VPF receptors

There are two known high affinity VEGF/VPF receptors; these are KDR/FLK1 [5–8] and FLT1 [9, 10]. KDR (kinase insert domain containing receptor) was cloned from a human umbilical vein endothelial cell cDNA library [5]. FLK1 (fetal liver kinase 1) is the mouse homologue of KDR and was originally cloned from a fetal liver stem cell cDNA library [7]. FLT1 (fms-like tyrosine kinase) was initially cloned from a human placenta cDNA library [9]. KDR, FLK1, and FLT1 were each discovered as orphan receptors using PCR and degenerate oligonucleotide primers designed from the kinase domains of known receptor tyrosine kinases.

There are now over 30 known receptor tyrosine kinases (RTKs); these have been categorized into 9 subtypes based upon similarity in structural features. A recent review [11] placed KDR/FLK1 and FLT1 into RTK subtype 5, which also contains the orphan receptor FLT4 [12, 13]. KDR/FLK1, FLT1, and FLT4 each contain a single membrane spanning domain, seven extracellular immunoglobulin-like domains and a kinase-insert domain. There is a 33% identical match in amino acid sequence between KDR and FLT1 in the extracellular domain, 80% match in the kinase domain, 43% match in the kinase insert domain, and a 22% match in the cytosolic tail.

Several investigators have noted structural similarities between VEGF/VPF and PDGF (platelet-derived growth factor) [14–16]. While there is a low sequence homology (18%), both growth factors are dimers and each contain conserved cysteines defining inter- and intra-disulfides. As with PDGF, there is a second gene (PIGF; placenta growth factor) encoding a growth factor with 45% sequence homology to VEGF/VPF [17]. Both the PDGF and VEGF/VPF systems contain two receptors, and there are analogies as to how the ligands bind to their respective cell surface receptors. VEGF/VPF interacts with both FLT1 and KDR (PDGF-B dimerize both PDGF-α receptor homodimers and PDGF-β receptor homodimers) and PIGF interacts with only FLT1 (PDGF-A will only dimerize PDGF-α receptor homodimers) [18–20]. A recent study has identified naturally occurring VEGF/VPF and PIGF heterodimers [21], further emphasizing the similarities between VEGF/VPF and PDGF. It may be expected that heterodimers exist between KDR and FLT1, though this has not as yet been reported.

Heparan sulfate proteoglycans are required for binding of VEGF/VPF to its receptors

Evidence supporting an essential role of heparan sulfate proteoglycans (HSPG) in the binding of VEGF to receptors is:

(1) Heparin augments binding of ^{125}I-VEGF to both Bovine Aortic Endothelial (BAE) and Human Umbilical Vein Endothelial (BAE) Cells [22].
(2) Treatment of endothelial cells with heparinase results in the abolishment of ^{125}I-VEGF binding which can be restored by adding heparin [22].
(3) Heparin is required for radioligand binding to a soluble FLK1 receptor [23].
(4) KDR-transfected CHO cells which are mutated in their ability to synthesize heparan sulfates do not bind radioligand in the absence of heparin [24].

HSPG are also required for basic fibroblast growth factor (bFGF) binding to its receptor [25, 26], although there are significant differences between the VEGF/VPF and bFGF systems. While greater that 90% of bFGF binding to cells is due to low affinity cell surface HSPG [27], there are no low affinity VEGF/VPF binding sites on endothelial cells. The size of heparin and degree of sulfation which maximally augments binding is different for VEGF/VPF than that for bFGF [28].

Preliminary evidence would suggest that heparan sulfates modulate growth factor binding to KDR and FLT1 differently. Heparin augments ^{125}I-VEGF binding to KDR-expressing melanoma cells, but inhibits radioligand binding to FLT1-expressing melanoma cells [20, 29]. Melanoma cells expressing both receptor subtypes show a biphasic sensitivity to added heparin [20]. While these results indicate that in certain cells KDR and FLT1 show a different sensitivity to heparin, a more detailed mechanistic analysis of the results is complicated by the presence of endogenous HSPG on the melanoma cell surface. Similar complications were circumvented for studies on bFGF by developing experimental systems where the effect of heparin on binding of growth factor could be monitored in the absence of cell surface HSPG [25, 30]. These systems are being adapted for studies on VEGF/VPF and it has recently been shown that heparin is required for VEGF binding to both a soluble FLK1 receptor and KDR expressed in HSPG deficient CHO cells [23, 24]. The effect of heparin on binding to FLT1 in these systems has not been studied.

It is not clear whether HSPG exert their effect by interacting with growth factor or receptor. Until recently, studies examining the effect of heparin on binding were done using the 165 amino acid alternatively spliced form [31, 32] of VEGF/VPF. VEGF/VPF_{165} is a heparin binding protein, as are two of the other three alternatively spliced variants of the growth factor. VEGF/VPF_{121}, on the other hand, does not bind heparin and a recent study has examined the requirement for heparin in VEGF/VPF_{121} binding to receptors [29]. It was shown that heperinase treatment of FLT1-expressing melanoma cells blocks binding of growth factor indicating either an essential interaction of HSPG with cell surface receptors or the presence of cell surface heparin binding proteins which regulate receptor function. Interestingly, addition of exogenous heparin to heparinase-treated cells restores VEGF/VPF_{165} binding but not VEGF/VPF_{121} binding [29] indicating that changes in the composition of cell-surface heparin-like molecules may differentially affect the interaction of various VEGF isoforms with VEGF receptors.

Signaling through VEGF/VPF receptors

The specific roles of KDR/FLK1 and FLT1 in vascular development and function is not clear, though there is some information on the signal transduction pathways induced by the specific receptor subtypes. Binding to VEGF/VPF induces autophosphorylation of both receptors [33]. To date it has proven easier to demonstrate biological consequences of VEGF/VPF binding to KDR/FLK1 than to FLT1. Waltenbergerger et al. [33] has expressed both receptor subtypes in Porcine Aortic Endothelial (PAE) cells. These investigators showed that while KDR-expressing cells showed striking changes in cell morphology, actin reorganization and membrane ruffling, chemotaxic and mitogenicity upon VEGF stimulation, FLT1 expressing cells

lacked such responses. Tyrosines 953,998, 1065, and 1061 have been identified as autophosphorylation sites on KDR [34], though the SH2-domain containing signaling protein which bind to these sites is not known.

While it has been more difficult to demonstrate cellular responses stimulated by VEGF/VPF binding to FLT1 than for KDR/FLK1, it has been easier to identify signaling proteins which interact with FLT1. From studies done in rat sinusoidal endothelial cells it was shown that VEGF/VPF binding to FLT1 leads to phosphorylation of PLCγ and GAP proteins as well as activation of MAP kinase [35]. In FLT1-expressing PAE cells VEGF/VPF induces phosphorylation of Fyn and Yes [33]. Cunningham et al. [36] has utilized a yeast two-hybrid system to demonstrate that the FLT1 tyrosine kinase domain interacts with the p85 subunit of phosphatidylinositol 3-kinase.

The cellular consequence of PIGF binding to FLT1 is at present unclear. Purified PIGF isoforms have little or no direct mitogenic or premeability-enhancing activity on adrenal cortex-derived capillary endothelial or human umbilical vein endothelial cells [18]. However, the PIGF isoforms are able to significantly augment the action of low concentration of VEGF both *in vivo* and *in vitro* [18].

Regulation of VEGF receptor expression during angiogenesis

There are now several reports documenting that the expression of both VEGF/VPF and its receptors are increased in appropriate cell types during physiological circumstances requiring neovascularization. Studies on tumor angiogenesis have shown that expression of KDR/FLK1 and/or FLT1 in endothelial cells correlates with vascularity, metastasis, and proliferation of human colon cancer [37], the progression of von Hippel-Lindau Disease-associated and sporadic hemangioblastomas [38], the growth of human gliomas [39] and correlates with human hepatic tumorigenesis [40]. The increased expression of receptor appears to be essential for the progession of these tumors since inhibition of FLK1 by either a dominant negative receptor [3], or neutralizing growth factor antibody [4] blocks tumor progression. In addition to its role in tumor angiogenesis, increased expression of VEGF/VPF receptors contributes to the progression of other physiological processes requiring neovascularization. Expression of KDR/FLK1 and/or FLT1 is increased in the developing embryonic vasculature [7, 41], in the vessels bordering healing skin wounds [41], and in the venular endothelium in delayed hypersensitivity skin reactions [42].

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