The splice variants of vascular endothelial growth factor (VEGF) and their receptors

Christopher J. Robinson and Sally E. Stringer*

The Cancer Research Campaign Department of Drug Development and the CRC and University of Manchester Department of Medical Oncology, Paterson Institute for Cancer Research, Christie Hospital, Manchester, M20 4BX, UK

*Author for correspondence (e-mail: sstringer@picr.man.ac.uk)

Journal of Cell Science 114, 853-865 © The Company of Biologists Ltd

Summary

Vascular endothelial growth factor (VEGF) is a secreted mitogen highly specific for cultured endothelial cells. In vivo VEGF induces microvascular permeability and plays a central role in both angiogenesis and vasculogenesis. VEGF is a promising target for therapeutic intervention in certain pathological conditions that are angiogenesis dependent, most notably the neovascularisation of growing tumours. Through alternative mRNA splicing, a single gene gives rise to several distinct isoforms of VEGF, which differ in their expression patterns as well as their biochemical and biological properties. Two VEGF receptor tyrosine kinases (VEGFRs) have been identified, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 seems to mediate almost all observed endothelial cell responses to VEGF, whereas roles for VEGFR-1 are more elusive. VEGFR-1 might act predominantly as a ligand-binding molecule, sequestering VEGF from VEGFR-2 signalling. Several isoform-specific VEGF receptors exist that modulate VEGF activity. Neuropilin-1 acts as a co-receptor for $VEGF_{165}$, enhancing its binding to VEGFR-2 and its bioactivity. Heparan sulphate proteoglycans (HSPGs), as well as binding certain VEGF isoforms, interact with both VEGFR-1 and VEGFR-2. HSPGs have a wide variety of functions, such as the ability to partially restore lost function to damaged $VEGF_{165}$ and thereby prolonging its biological activity.

Key words: VEGF, Angiogenesis, Hypoxia, VEGFR, Heparan sulphate proteoglycan, Neuropilin

Introduction

Angiogenesis is the complex process by which new blood vessels arise from the pre-existing vasculature (reviewed by Folkman, 1997). Localised breakdown of the extracellular matrix (ECM) precedes the proliferation, migration and tissue infiltration of capillary endothelial cells. In time these cells remodel back into capillary structures, and a new ECM is deposited. Angiogenesis is essential in embryonic development and wound healing as well as in the female reproductive cycle and the formation and growth of bone. However, its dysregulation contributes to several pathological conditions, such as diabetic retinopathy, rheumatoid arthritis and the development of solid tumours (Folkman, 1990; Folkman and Hanahan, 1991).

The switch from the normal quiescent vasculature to angiogenesis is induced by factors released predominantly by surrounding pericytes and lymphocytes. Such angiogenic factors include acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF) and thymidine phosphorylase (TP), which are directly angiogenic, as well as transforming growth β (TGF-β) and tumour necrosis factor α (TNF-α), which act indirectly. However, the only growth factor that is observed almost ubiquitously at sites of angiogenesis and whose levels correlate most closely with the spatial and temporal events of blood vessel growth is vascular endothelial growth factor (VEGF).

Roles of VEGF in angiogenesis

VEGF receptor expression is almost exclusive to vascular endothelial cells. Therefore, in contrast to other angiogenic factors, the proliferative action of VEGF is predominantly restricted to endothelial cells. Although VEGF is mitogenic for lymphocytes (Praloran et al., 1991), retinal pigment epithelial cells (Guerrin et al., 1995) and Schwann cells (Sondell et al., 1999), binding of VEGF to other non-endothelial cells seems to have alternative functions, such as the induction of monocyte migration (Clauss et al., 1990).

VEGF is essential to vasculogenesis, the de novo formation of blood vessels from vascular precursor cells: loss of a single *VEGF* allele in mouse models leads to gross developmental deformities in the forming vasculature and embryonic death between days 11 and 12 (Carmeliet et al., 1996; Ferrara et al., 1996). Mice lacking either VEGFR-1 or VEGFR-2 die even earlier, between embryonic days 8.5 and 9.5 (Fong et al., 1995; Shalaby et al., 1995).

As well as inducing endothelial cell proliferation and migration VEGF has several other pro-angiogenic activities. It induces endothelial expression of proteases such as interstitial collagenase and the urokinase-type and tissue-type plasminogen activators (uPA and tPA) (Pepper et al., 1991; Unemori et al., 1992). These proteases release cells from anchorage, allowing migration, and can generate by-products that themselves affect angiogenesis. VEGF also stimulates microvascular leakage (which allows tissue infiltration of plasma proteins – hence the alternative name vascular permeability factor (VPF); Senger et al., 1983; Connolly et al., 1989; Keck et al., 1989) and hexose transport (to meet increasing energy demands; Pekala et al., 1990). In addition VEGF participates in the continued survival of nascent

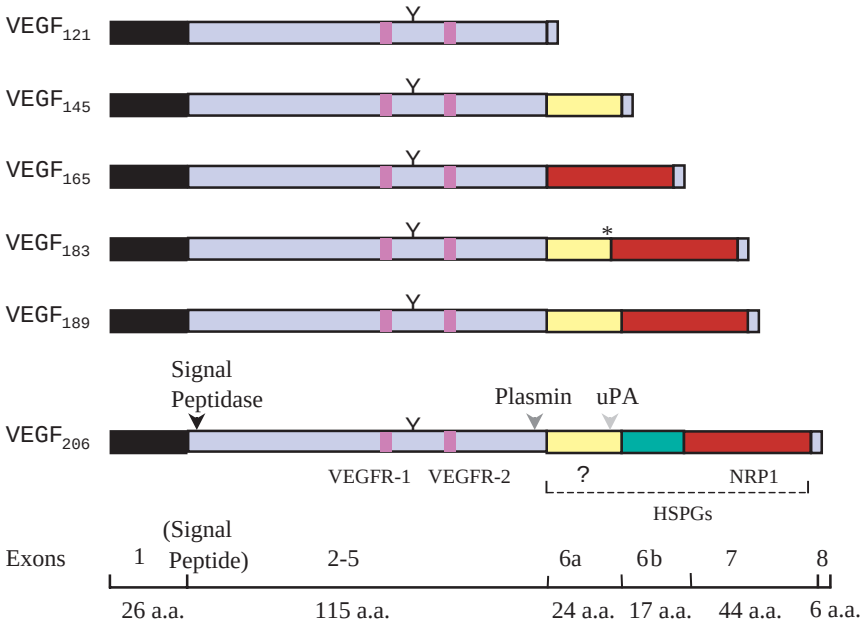

Fig. 1. The splice variants of human VEGF. The human *VEGF* gene, through alternative mRNA splicing, produces six isoforms, which differ by the presence or absence of sequences encoded by exons 6 and 7. Domain sizes (a.a., amino acid residues) and the exons from which they derive are shown at the foot of the figure. Sites of interaction with VEGFRs, NRP1 and HSPGs are indicated on the $VEGF_{206}$ isoform, as are certain enzyme cleavage sites. Note that it is not known where uPA cleaves the exon-6-encoded region in relation to the truncation point of this domain within $VEGF_{183}$ (*). The potential *N*-glycosylation site is indicated by 'Y', whereas '?' represents binding to unknown components at the cell surface and in the ECM.

endothelial cells (Alon et al., 1995; Benjamin and Keshet, 1997).

Most tumour types overexpress VEGF mRNA. This expression directly correlates with regions of angiogenesis and high vascular density (see Ferrara and Davis-Smyth, 1997). High levels of VEGF are generally associated with hypoxia, an excess of soluble inducing factors or unregulated VEGF expression. Vascular endothelial cells in the tumour vicinity also appear to upregulate expression of VEGF receptors. The newly formed blood vessels are inherently leaky, which enhances the likelihood of metastasis. Studies relating VEGF expression to tumour aggressiveness, metastatic potential and the probability of relapse indicate that high levels of VEGF expression correlate with poor prognosis (Toi et al., 1994; Maeda et al., 1996).

The VEGF gene and protein structure

The human gene for VEGF resides on chromosome 6p21.3 (Vincenti et al., 1996). The coding region spans ~14 kb and contains eight exons (Houck et al., 1991; Tischer et al., 1991). Alternative splicing of a single pre-mRNA generates several distinct VEGF species (Fig. 1).

X-ray crystallography of a VEGF fragment (residues 8-109) to 1.9 Å resolution showed that VEGF belongs to the dimeric cysteine-knot growth factor superfamily (Muller et al., 1997). Each monomer is characterised by an intrachain disulphide bonded knot motif at one end of a four-stranded β sheet (McDonald and Hendrickson, 1993; Murray-Rust et al., 1993; Sun and Davies, 1995). The superfamily is subdivided; VEGF belongs to the platelet-derived growth factor (PDGF) family, in which the monomers are held in a 'side-by-side' orientation, the two β sheets lying perpendicular to the twofold-symmetry axis. The structure of the $VEGF_{165}$ heparin-binding region (residues 111-165) has been solved separately by NMR and represents a novel type of heparin-binding domain (Fairbrother et al., 1998).

All VEGF isoforms are secreted as covalently linked homodimers. Monomers associate initially through hydrophobic interactions and are then stabilised by disulphide bonding between Cys51 of one chain and Cys61 of the other (Potgens et al., 1994). The signal peptide (exon 1 and four residues of exon 2), which includes an amphipathic α-helix (residues 12-19) essential for this dimerisation, is cleaved off during secretion (Leung et al., 1989; Keck et al., 1989, Siemeister et al., 1998b). A potential *N*-glycosylation site exists at Asn74 that appears to have no effect on VEGF function but is required for efficient secretion (Peretz et al., 1992; Claffey et al., 1995).

Site-directed mutagenesis identified three acidic residues (Asp63, Glu64 and Glu67) in exon 3 and three basic residues (Arg82, Lys84 and His86) in exon 4 essential for binding to VEGFR-1 and VEGFR-2, respectively. Three highly flexible loops are clustered at each pole of VEGF at the dimer interface. Loop II contains the VEGFR-1 binding determinants and lies close to loop III of the opposing monomer, which binds to VEGFR-2 (Keyt et al., 1996a). The positioning of these receptor-binding interfaces at each pole of VEGF seems to facilitate receptor dimerisation, which is essential for transphosphorylation and signalling, because mutant dimers that have only one receptor-binding site antagonise native VEGF activity (Siemeister et al., 1998a).

VEGF expression

Many cytokines and growth factors upregulate VEGF mRNA or induce VEGF release. These include PDGF, TNF-α, TGF-α, TGF-β, FGF-4, keratinocyte growth factor (KGF/FGF-7), epidermal growth factor (EGF), IL-1α, IL-1β, IL-6 and insulin-like growth factor 1 (IGF-1). Several lack direct angiogenic effects but exert angiogenic activity through VEGF and bFGF (Brogi et al., 1994; Pertovaara et al., 1994). The cytoplasmic tail of tissue factor, a receptor for coagulation factor VII/VIIa, also regulates VEGF production and may be important at sites of wound healing (Abe et al., 1999).

Hypoxia induces a rapid and strong increase in VEGF

mRNA levels, which is particularly noticeable around necrotic areas of tumours (Shweiki et al., 1992; Plate et al., 1992; Minchenko et al., 1994). Interestingly, the other VEGF family members and bFGF are not induced by hypoxia; therefore VEGF might be the main mediator of hypoxia-induced neovascularisation (Brogi et al., 1994; Enholm et al., 1997). Adenosine released by hypoxic cells binds adenosine A_2 receptors and upregulates VEGF through the cAMP-dependent protein kinase (PKA) pathway (Takagi et al., 1996).

A hypoxia response element (HRE) acts upstream of the *VEGF* gene as an enhancer (Levy et al., 1995; Liu et al., 1995). This HRE contains a consensus binding site for hypoxia-inducible factor 1 (HIF-1), a heterodimer of the bHLH-type transcription factors HIF-1α and ARNT (Madan and Curtin, 1993; Forsythe et al., 1996). Low oxygen tension increases HIF-1 levels at a post-transcriptional level and increases its DNA-binding ability (Jiang et al., 1996; Salceda et al., 1996; Semenza et al., 1997).

The von Hippel-Lindau tumour suppressor (VHL) negatively regulates hypoxia-induced genes, including *VEGF* (Siemester et al., 1996; Iliopoulos et al., 1996). VHL cytoplasmically sequesters PKCζ and PKCδ, preventing their translocation to the cell membrane, subsequent MAPK activation and induction of VEGF (Pal et al., 1997). Changes in cell signalling through differentiation might also influence VEGF expression through control of PKC and cAMP/PKA pathways (Claffey et al., 1992; Garrido et al., 1993). The *VEGF* promoter contains potential binding sites for the transcription factors Sp1, AP-1 and AP-2, through which PKC and PKA can influence gene expression (Tischer et al., 1991).

Hypoxia increases the half-life of VEGF mRNA, which is intrinsically labile owing to the presence of three synergistic sequence elements within the 5′ and 3′ untranslated regions (Dibbens et al., 1999). Binding of a hypoxia-induced stability factor (HuR) increases the half-life of this mRNA three- to eight-fold (Levy et al., 1998). An alternative transcription-initiation site allows VEGF mRNA translation from a downstream ribosomal entry site. This might be advantageous under hypoxic stress, when cap-dependent translation can be inhibited (Stein et al., 1998; Akiri et al., 1998).

Several specific transformation events can also induce *VEGF* expression, such as inactivation of the *VHL* gene (Stratmann et al., 1997; Mukhopadhyay et al., 1997), activatory mutations of p53 (Kieser et al., 1994, Mukhopadhyay et al., 1995) and oncogenic mutation or amplification of *RAS* (Rak et al., 1995; Grugel et al., 1995: Mazure et al., 1996).

The VEGF splice variants

To date, six isoforms of human VEGF have been identified (see Fig. 1), which range in length from 121 to 206 amino acid residues ($VEGF_{121\text{-}206}$) (Leung et al., 1989; Tischer et al., 1991; Houck et al., 1991; Poltorak et al., 1997; Lei et al., 1998). Murine immortal fibroblasts encode a shorter form, $VEGF_{115}$, which has a novel 37-residue C terminus, but no human homologue has been isolated (Sugihara et al., 1998). Most VEGF-producing cells appear preferentially to express $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$. $VEGF_{183}$ also has a wide tissue distribution and may have avoided earlier detection through confusion with $VEGF_{189}$ (Lei et al., 1999; Jingjing et al., 1999). In contrast, $VEGF_{145}$ and $VEGF_{206}$ are comparatively rare, seemingly restricted to cells of placental origin (Anthony et al., 1994; Cheung et al., 1995). Bacic et al. demonstrated tissue-specific VEGF mRNA splicing in rats but the mechanisms that determine the relative expression levels are as yet undetermined (Bacic et al., 1995). Interestingly human skin mast cells normally express $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ but can be induced to express $VEGF_{206}$ as well by incubation with phorbol myristate acetate (PMA), which stimulates PKC activity (Grutzkau et al., 1998).

Through alternative mRNA splicing, the VEGF isoforms differ by the presence or absence of sequences encoded by exons 6 and 7 (Tischer et al., 1991). $VEGF_{165}$ is secreted as ~46-kDa homodimers, which have a basic character and moderate affinity for heparin, owing to the presence of 15 basic amino acids within the 44 residues encoded by exon 7 (Ferrara and Henzel, 1989; Gospodarowicz et al., 1989; Plouet et al., 1989). In contrast, $VEGF_{121}$, which lacks this region, is a weakly acidic protein and does not bind heparin. $VEGF_{121}$ is freely released from producing cells, whereas 50-70% of $VEGF_{165}$ remains cell and ECM associated, probably owing to heparan sulphate proteoglycan (HSPG) interactions (Houck et al., 1992). $VEGF_{189}$ and $VEGF_{206}$ contain additional sequence encoded by exon 6 and bind heparin strongly. These isoforms are completely sequestered in the ECM and to a lesser extent at the cell surface (Houck et al., 1992; Park et al., 1993).

The exon-6a-encoded sequence of $VEGF_{145}$ confers an affinity for heparin similar to that of the exon-7-encoded sequence of $VEGF_{165}$ (Poltorak et al., 1997). However, this sequence also mediates binding to components of the ECM that is independent of heparin or heparan sulphate. ECM-bound $VEGF_{145}$ remains active as an endothelial cell mitogen (Poltorak et al., 1997). The 24-residue exon 6 peptide contains 12 basic amino acids, including the sequence ^{126}K-R-K-R-K-K^{131} identified as a cell surface retention consensus sequence (CRS). 60-72-kDa high-affinity CRS-binding proteins have been identified on the cell surface that appear to be derived from the same gene product, CRS-BP1; however, their significance is unknown (Boensch et al., 1995). Since $VEGF_{145}$ is freely released from producing cells, it is thought that a combination of CRS-BP1 binding and heparan sulphate interactions with the extended region derived from exons 6 and 7 is responsible for the cell-surface retention of $VEGF_{189}$ and $VEGF_{206}$ (Poltorak et al., 1997; Jonca et al., 1997). Recently the sequence encoded by exon 6 has also been shown to release bioactive bFGF from the ECM and cell surface and thus confers on $VEGF_{189}$ the ability to exert some of its biological effects through bFGF signalling pathways (Jonca et al., 1997).

VEGF isoforms in the ECM constitute a reservoir of growth factor that can be slowly released by exposure to heparin, heparan sulphate and heparinases or more rapidly mobilised by specific proteolytic enzymes such as plasmin and urokinase-type plasminogen activator (uPA) (Houck et al., 1992; Plouet et al., 1997). These enzymes already contribute to angiogenesis through ECM depolymerisation and, as well as releasing sequestered VEGF from the cell surface and ECM, might also regulate VEGF bioactivity. Recombinant $VEGF_{189}$ and $VEGF_{206}$ are unable to stimulate endothelial cell mitogenesis (Houck et al., 1991), because protein folding in these larger isoforms obscures regions responsible for receptor binding. $VEGF_{189}$ binds VEGFR-1 but requires enzyme maturation by uPA or plasmin to bind VEGFR-2 and exert its mitogenic

effects on endothelial cells (Plouet et al., 1997; Fig. 1). uPA cleavage towards the C-terminal end of the exon-6-encoded region generates a truncated factor (uPA-$VEGF_{189}$) that has an endothelial cell mitogenicity equivalent to that of $VEGF_{165}$. Although not all VEGF isoforms contain a site for uPA cleavage, they can all be cleaved by plasmin. This Pl-VEGF ($VEGF_{110}$), by comparison with $VEGF_{165}$ and uPA-$VEGF_{189}$, elicits a 50-fold reduced mitogenic effect on endothelial cells, which is similar to that observed for $VEGF_{121}$ (Keyt et al., 1996b; Plouet et al., 1997). This demonstrates that the VEGF sequences encoded by exons 6 and 7 do more than just regulate the bioavailability of VEGF through HSPG binding: they actually enhance mitogenic signalling.

VEGF-related proteins

VEGF shares ~20% amino acid sequence identity with PDGF. Members of the PDGF/VEGF family contain eight conserved cysteine residues and therefore have a common mode of dimerisation and a similar structure within their receptor-binding domains (see above). Several growth factors that have significant sequence homology to VEGF share the same receptors as VEGF and often impinge upon VEGF activity.

Placenta growth factor (PlGF) (Persico et al., 1999) shares 53% amino acid sequence identity with the PDGF-like region of human VEGF (Maglione et al., 1991). Its expression is restricted to the placenta, where alternative mRNA splicing generates three isoforms: PlGF-1 ($PlGF_{131}$), PlGF-2 ($PlGF_{152}$) and PlGF-3 ($PlGF_{183}$) (Maglione et al., 1993; Cao et al., 1997). Exon 6 encodes a highly basic 21 amino acid sequence unique to PlGF-2, which confers binding to both heparin and the neuropilin-1 receptor (Hauser and Weich, 1993; Park et al., 1994; Migdal et al., 1998). The only signalling receptor identified for PlGF is VEGFR-1 (Park et al., 1994; Kendall et al., 1994). VEGF-PlGF heterodimers exist in vivo but have weaker mitogenic activity than VEGF homodimers and might represent a mechanism for modulating VEGF bioactivity (DiSalvo et al., 1995; Cao et al., 1996).

VEGF-B/VEGF-related-factor (Joukov et al., 1997b) resembles PlGF in many aspects. VEGF-B has a wide tissue distribution but is particularly abundant in the heart and skeletal muscle (Olofsson et al., 1996a). Two isoforms exist, owing to the use of alternative splice acceptor sites within exon 6 that utilise different reading frames (Grimmond et al., 1996; Olofsson et al., 1996b). The VEGF-B_{167} C terminus retains homology to VEGF and mediates binding to ECM and cell surface HSPGs. However, VEGF-B_{186} contains a novel hydrophobic sequence, rich in proline, serine and threonine residues and *O*-glycosylated, possibly to aid its solubility (Olofsson et al., 1996b). The VEGF-B isoforms bind and activate VEGFR-1 (Olofsson et al., 1998) and can also bind the neuropilin-1 receptor (Makinen et al., 1999). VEGF can heterodimerise with both VEGF-B isoforms when coexpressed (Olofsson et al., 1996a; Olofsson et al., 1996b). VEGF-B_{167}–VEGF heterodimers remain cell-surface associated, perhaps indirectly controlling VEGF release and bioavailability.

VEGF-C/VEGF-related protein contains a region sharing ~30% amino acid identity with $VEGF_{165}$ (Lee et al., 1996; Joukov et al., 1996). However, it is more closely related to VEGF-D/c-*fos*-induced growth factor, with which it forms a subfamily of the VEGF-related proteins (Orlandini et al., 1996; Yamada et al., 1997; Achen et al., 1998). Both proteins bind and activate VEGFR-3 (Flt-4; a member of the VEGFR family that does not bind VEGF) as well as VEGFR-2, and are mitogenic for cultured endothelial cells (Lee et al., 1996; Joukov et al., 1996; Achen et al., 1998). They are synthesised as precursor proteins requiring proteolytic processing at the C- and N-termini to release the VEGF-homology domain (Joukov et al., 1996). This might control receptor specificity, because incompletely processed VEGF-C binds VEGFR-3 with lower affinity but does not bind VEGFR-2 at all (Joukov et al., 1997a). VEGF-C is thought to participate in both vascular and lymphatic development, but in adult tissues probably acts as a paracrine factor involved in lymphangiogenesis and maintenance of the lymphatic vessels (Kukk et al., 1996; Jeltsch et al., 1997; Dumont et al., 1998). Although much less mitogenic than VEGF, VEGF-C might also regulate angiogenesis (Witzenbichler et al., 1998; Cao et al., 1998; Pepper et al., 1998). Less is known of VEGF-D functions, but expression patterns in mice indicate an important role in lung development (Farnebo et al., 1999).

VEGF-E is the collective term for a group of proteins having significant homology to VEGF that are encoded by certain strains of the *orf* virus, a pathogen of sheep, goats and occasionally humans (Lyttle et al., 1994; Meyer et al., 1999). Presumably the *VEGF* gene was acquired from a host genome and has subsequently undergone genetic drift. Some biological functions appear to be retained, because viral infection results in skin lesions with acute microvascular proliferation and dilation. All VEGF-E variants studied bind VEGFR-2, but not VEGFR-1 or VEGFR-3 (Ogawa et al., 1998; Meyer et al., 1999; Wise et al., 1999). One variant was further shown to bind neuropilin-1. These viral proteins seem to be as potent as $VEGF_{165}$ at stimulating endothelial cell proliferation despite lacking heparin-binding ability.

The VEGF receptors

VEGFR-1 and VEGFR-2 are both high-affinity receptors for VEGF that, along with VEGFR-3, form the *flt* subfamily of receptor tyrosine kinases. These are characterised by seven extracellular immunoglobulin (Ig)-like domains followed by a membrane-spanning region and a conserved intracellular tyrosine kinase domain interrupted by a kinase insert sequence (Fig. 2) (Shibuya et al., 1990; Matthews et al., 1991; Terman et al., 1991; Pajusola et al., 1992).

The ~180-kDa glycoprotein VEGFR-1, the first VEGFR to be identified (*fms*-like tyrosine kinase receptor/Flt-1; Shibuya et al., 1990; de Vries et al., 1992), has the highest affinity for VEGF (K_d 10-30 pM; de Vries et al., 1992; Quinn et al., 1993; Waltenberger et al., 1994). VEGFR-1 is also shared by the related growth factors PlGF and VEGF-B. Its expression in mice is localised to the endothelium in adult and embryonic tissues, as well as to the neovasculature of healing skin wounds (Peters et al., 1993). The presence of VEGFR-1 mRNA in quiescent as well as proliferating endothelial cells suggests a continued role in endothelial maintenance. VEGFR-1 is essential during vasculogenesis: *VEGFR-1*$^{-/-}$ mice die at embryonic day 8.5 (Fong et al., 1995). In these mice, endothelial cells form normally at both embryonic and extra-embryonic sites but fail to assemble correctly into organised

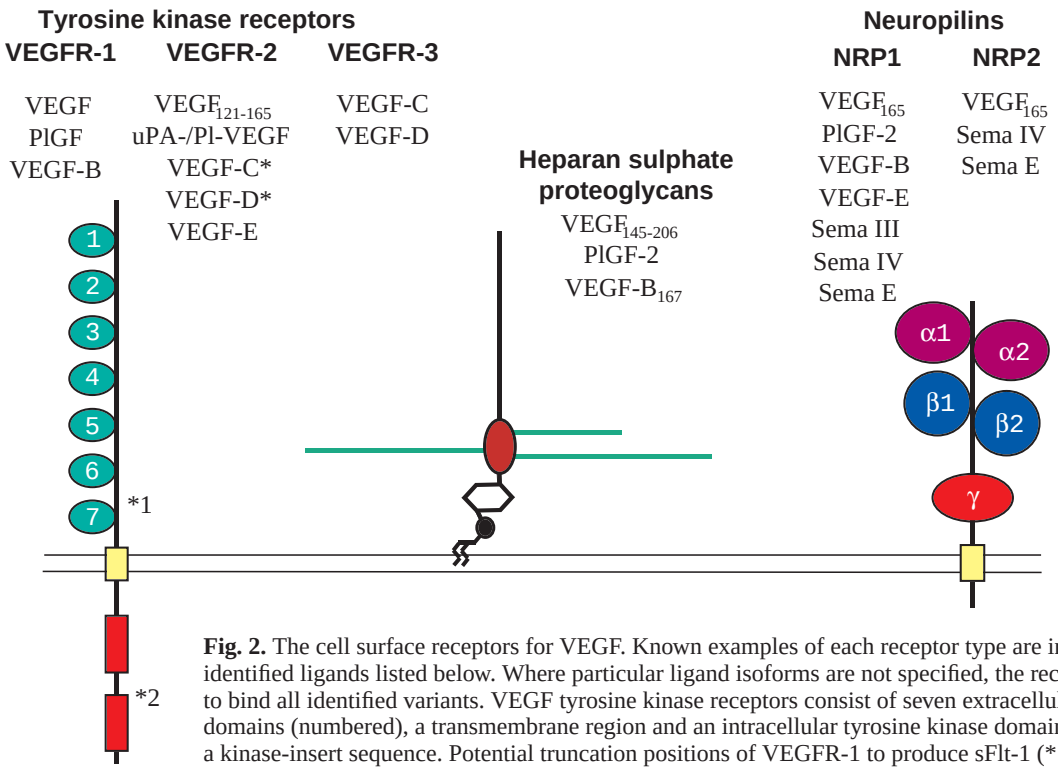

Fig. 2. The cell surface receptors for VEGF. Known examples of each receptor type are indicated and identified ligands listed below. Where particular ligand isoforms are not specified, the receptor is believed to bind all identified variants. VEGF tyrosine kinase receptors consist of seven extracellular Ig-like domains (numbered), a transmembrane region and an intracellular tyrosine kinase domain interrupted by a kinase-insert sequence. Potential truncation positions of VEGFR-1 to produce sFlt-1 (*1) and of VEGFR-2 observed in the rat retina (*2) are shown. Heparan sulphate proteoglycans are represented here by glypican-1, an endothelial cell HSPG known to interact with $VEGF_{165}$. Ligands are bound through electrostatic interactions with specific sequences of sulphation within the HS chains (shown in green). In the case of glypican-1 these HS chains are all attached to their core protein at a site close to the cell membrane (shown in red). The neuropilins are isoform-specific receptors for certain VEGF family members. NRP1 acts as a co-receptor for VEGFR-2, enhancing the binding and biological activity of $VEGF_{165}$. The α1-α2 region has homology to components of the complement system; β1-β2 shares homology with coagulation factors V and VIII, whereas the γ domain contains a MAM (Meprin, A5/neuropilin, Mu) domain, a protein sequence also found in the meprin metalloproteinases and receptor tyrosine phosphatase μ.

blood vessels. This defect appears to be a consequence of increased commitment of mesenchymal cells to becoming hemangioblasts, the common precursors of both blood cells and endothelial cells (Fong et al., 1999).

Tyrosine phosphorylation of VEGFR-1 in response to VEGF stimulation is hard to detect, and, in endothelial cells, no direct proliferative, migratory or cytoskeletal effects mediated by this receptor are apparent (Park et al., 1994; Waltenberger et al., 1994; Seetharam et al., 1995). However, VEGFR-1 has been implicated in upregulated endothelial expression of tissue factor, urokinase-type plasminogen activator and plasminogen activator inhibitor 1 (Clauss et al., 1996; Landgren et al., 1998; Olofsson et al., 1998). In other cell types VEGFR-1 has different roles, such as tissue factor induction and chemotaxis in monocytes, and enhancing matrix metalloproteinase expression by vascular smooth muscle cells (Barleon et al., 1996; Clauss et al., 1996; Wang and Keiser, 1998).

VEGFR-2 (also known as kinase-insert-domain-containing receptor, KDR) is a 200-230-kDa high-affinity receptor for VEGF (K_d 75-760 pM; Terman et al., 1992; Quinn et al., 1993; Waltenberger et al., 1994), as well as for VEGF-C and VEGF-D. Identified in humans through screening endothelial cDNA for tyrosine kinase receptors (Terman et al., 1991), this receptor shares 85% sequence identity with the previously discovered mouse fetal liver kinase 1 (Flk-1) (Matthews et al., 1991; Millauer et al., 1993; Quinn et al., 1993). *VEGFR-2*$^{-/-}$ mice die by embryonic day 9.5, exhibiting defects in the development of endothelial and haematopoietic precursors (Shalaby et al., 1995). VEGFR-2 is normally expressed in such precursors, as well as in endothelial cells, nascent haematopoietic stem cells and the umbilical cord stroma. However, in quiescent adult vasculature, VEGFR-2 mRNA appears to be downregulated (Millauer et al., 1993; Quinn et al., 1993; Eichmann et al., 1997). Although VEGFR-1 has the greater affinity for VEGF, VEGFR-2 is tyrosine phosphorylated much more efficiently upon ligand binding and in endothelial cells leads to mitogenesis, chemotaxis and changes in cell morphology (Quinn et al., 1993; Waltenberger et al., 1994).

Recent work has begun to elucidate the specific roles of individual Ig-like domains in VEGFR function. The second and third Ig-like domains of both receptors appear to be necessary for high-affinity ligand binding (Davis-Smyth et al., 1996; Barleon et al., 1997a; Wiesmann et al., 1997; Fuh et al., 1998; Shinkai et al., 1998; Piossek et al., 1999). Deletion of the second Ig-like domain in VEGFR-1 abolishes ligand binding, whereas in VEGFR-2 the third Ig-like domain is most critical. The fourth Ig-like domain is believed to mediate receptor dimerisation in VEGFR-1 (Barleon et al., 1997a); this may also apply to VEGFR-2. The fifth and sixth Ig-like domains of VEGFR-2 seem to be required for VEGF retention after binding, whereas the first Ig-like domain might regulate ligand binding because its removal improves VEGF association (Shinkai et al., 1998). Both VEGF receptors are

glycosylated; this is not essential for VEGFR-1 ligand binding (Barleon et al., 1997a), but only the mature glycosylated form of VEGFR-2 can efficiently autophosphorylate (Takahashi and Shibuya, 1997).

A soluble truncated form of VEGFR-1 (sFlt-1) that contains only the first six Ig-like domains has been cloned from a HUVEC cDNA library (Kendall and Thomas, 1993; Kendall et al., 1996). sFlt-1 binds to VEGF as strongly as does full-length VEGFR-1 and inhibits VEGF activity by sequestering it from signalling receptors and by forming non-signalling heterodimers with VEGFR-2. Particularly high levels of sFlt-1 occur in the placenta, where it might control VEGF activity at particular stages of pregnancy (Clark et al., 1998; He et al., 1999). A truncated form of VEGFR-2, lacking the C-terminal half of the kinase domain, is expressed in normal rat retina (Wen et al., 1998). This truncated form is expressed at lower levels than full-length VEGFR-2 in these cells, but seems to be activated by VEGF at least as efficiently.

VEGFR-3 (Flt-4) is also a member of the *flt* subfamily of receptor tyrosine kinases, but its expression becomes restricted mainly to the lymphatic endothelium of adult tissues (Pajusola et al., 1992; Finnerty et al., 1993; Kaipainen et al., 1995). VEGFR-3 binds VEGF-C and VEGF-D, but not VEGF, and is thought to control lymphangiogenesis (see above).

Receptor expression

Upstream control elements confer endothelial-cell-specific transcription on both *VEGFR-1* and *VEGFR-2* (Morishita et al., 1995; Patterson et al., 1995). Not surprisingly, hypoxia, as well as inducing VEGF release, is also a potent stimulator of VEGFR-1 and VEGFR-2 expression in vivo (Tuder et al., 1995; Li et al., 1996). In common with *VEGF*, *VEGFR-1* has a HIF-1 consensus in its promoter region (Gerber et al., 1997). *VEGFR-2* has no similar sequence and transcription might be slightly downregulated by hypoxia (Thieme et al., 1995; Gerber et al., 1997). However, VEGFR-2 is thought to be upregulated at the post-transcriptional level by an unidentified paracrine factor released from ischaemic tissues (Brogi et al., 1996; Waltenberger et al., 1996).

Several growth factors, including VEGF (Wilting et al., 1996; Barleon et al., 1997b), affect VEGF receptor expression. VEGF stimulation of VEGFR-2 both upregulates expression of the *VEGFR-2* gene and increases cellular VEGFR-2 levels (Shen et al., 1998). bFGF is known to synergise with VEGF in inducing angiogenesis and one mechanism for this is through upregulation of VEGFR-2 in endothelial cells (Pepper and Mandriota, 1998). Interestingly cell-cell and cell-matrix contact might also affect VEGF receptor expression: VEGFR-2 levels vary according to cell density and the geometry of the culture conditions (Pepper and Mandriota, 1998b), and expression of both VEGFRs is influenced by the levels of platelet endothelial cell adhesion molecule 1 (PECAM-1; Sheibani and Frazier, 1998).

Signal transduction

Separate but overlapping VEGFR-1-binding and VEGFR-2-binding sites exist at each pole of VEGF at the dimer interface. The interaction of receptor monomers at these sites on VEGF induces receptor dimerisation and subsequent activation through transphosphorylation (Wiesmann et al., 1997; Fuh et al., 1998). VEGFR-1 and VEGFR-2, as well as forming homodimers, might also heterodimerise: sFlt-1 and VEGFR-2 can form non-signalling complexes (Kendall et al., 1996), and PlGF-VEGF heterodimers that are both mitogenic and chemotactic for endothelial cells exist in vivo (Cao et al., 1996).

VEGFR-2 seems to mediate almost all observed endothelial cell responses to VEGF. In contrast, although VEGFR-1 can induce monocyte migration (Barleon et al., 1996), endothelial cell migration in response to VEGFR-1 has not been demonstrated unequivocally. The lack of significant endothelial cell responses to VEGFR-1 stimulation has led some to speculate that its main role is as a decoy receptor that sequesters VEGF from signalling through VEGFR-2 (Park et al., 1994). Indeed mice embryos expressing VEGFR-1 that lacks the tyrosine kinase domain show normal development and angiogenesis; only VEGF-mediated macrophage migration is affected (Hiratsuka et al., 1998). If VEGFR-1 acts predominantly as a ligand-binding molecule, PlGF and VEGF-B might exist mainly to free VEGF for VEGFR-2 binding. Such competition would explain the potentiating activity of PlGF on the bioactivity of low concentrations of VEGF (Park et al., 1994). However, VEGFR-1 signalling should not be dismissed, because site-directed mutagenesis has identified several specific phosphorylated residues capable of interacting with SH2-domain proteins (Igarashi et al., 1998).

Signal transduction events associated with VEGF have been difficult to elucidate, owing to cell-specific and species-specific differences in expression of VEGFRs and signal transduction components. The presence of potential modulatory species, such as HSPGs and the neuropilins, further complicates the picture. Current understanding of VEGF signalling is beyond the scope of this Commentary, but details can be found elsewhere (Zachary, 1998; Petrova et al., 1999; Larrivee and Karsan, 2000).

Neuropilin-1 and other VEGF-binding molecules

Crosslinking experiments using ^{125}I-labelled VEGF indicate receptors for VEGF that do not correspond to the VEGFRs exist (Gitay-Goren et al., 1996; Soker et al., 1996; Omura et al., 1997). These receptors recognise the exon-7-encoded domain of VEGF and therefore bind $VEGF_{165}$ but not $VEGF_{121}$ (Soker et al., 1996). One such receptor is neuropilin-1 (NRP1) (Fig. 2; Soker et al., 1998), a 120-130-kDa glycoprotein previously characterised as a neuronal receptor for certain secreted members of the collapsin/semaphorin family (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997). NRP1 has a wide tissue distribution that includes some tumour-derived cells and endothelial cells (Soker et al., 1998). NRP-1 on embryonic neurons is implicated in the control of axon growth and guidance as a consequence of binding certain members of the semaphorin family (Kawakami et al., 1996; Takagi et al., 1995). Studies on mouse embryos suggest it also has a role in angiogenesis and vasculogenesis. NRP1 overexpression causes death at day 17.5 with an excessively high density of blood vessels, which are dilated and prone to hemorrhaging (Kitsukawa et al., 1995). *NRP1*$^{-/-}$ mice die at embryonic day 10.5-12.5 from cardiovascular anomolies (Kitsukawa et al., 1997).

NRP1 acts as a co-receptor for $VEGF_{165}$, enhancing its binding to VEGFR-2 and its bioactivity (Soker et al., 1998). Interestingly both VEGFR-2 and NRP1 on endothelial cells are upregulated by the indirectly acting angiogen TNF-α (Giraudo et al., 1998). NRP1 has only a very short intracellular domain, and no reponses to $VEGF_{165}$ were observed on cells without VEGFR-2 (Soker et al., 1998). However, Cai and Reed (1999) recently identified a widely expressed NRP1-interacting protein (NIP) that was previously implicated in membrane trafficking. NRP1 signaling might also contribute to hematopoiesis, given that $VEGF_{165}$ binding leads to increased erythropoietin and Flt-3 ligand expression in bone marrow stromal cells (Tordjman et al., 1999).

Other VEGF-related growth factors also share the NRP1 receptor (Fig. 2). Both PlGF-2 and $VEGF\text{-}B_{167}$ bind NRP1 and HSPGs through regions homologous to the exon-7-encoded domain of VEGF. However, the non-heparin-binding $VEGF\text{-}B_{186}$ requires proteolytic cleavage of its unique C-terminal region to expose a short proline-rich sequence for NRP1 interaction (Makinen et al., 1999). The biological role of binding of NRP1 to VEGF-B and PlGF-2 is unclear, because these growth factors bind only to VEGFR-1, not VEGFR-2. PlGF-2 has no effect upon the proliferation of endothelial cells expressing NRP1 and is no more efficient than PlGF-1 at stimulating VEGFR-1-mediated migration (Migdal et al., 1998).

The NRP1 receptor plays roles in the diverse processes of angiogenesis, vasculogenesis and the development of the nervous system. Interestingly $VEGF_{165}$ might act as a neurotrophic factor stimulating axonal growth along with the proliferation and survival of cells in the peripheral nervous system (Sondell et al., 1999). Likewise the chemorepulsive semaphorin Sema III, which binds NRP1 and inhibits axonal motility, also inhibits endothelial cell motility and competes with $VEGF_{165}$ for NRP1-binding (Miao et al., 1999).

NRP1 can homomultimerise or form heteromultimers with neuropilin-2 (NRP2), although the functional significance of these complexes is not clear (Giger et al., 1998). NRP2 (Chen et al., 1997) was identified during expression cloning of NRP1 from breast carcinoma cells, and therefore represents a separate cell-surface $VEGF_{165}$-receptor (Soker et al., 1998). Further receptors might also exist, given that Omura et al. identified a 190-kDa $VEGF_{165}$-specific receptor expressed on a glioma cell line (Omura et al., 1997). Like NRP1, the binding of $VEGF_{165}$ to this receptor does not seem to lead to tyrosine phosphorylation.

Heparin and heparan sulphate proteoglycans

Heparin is a highly sulphated linear polysaccharide released by mast cells that has well documented procoagulant activity. Heparan sulphate (HS) is a closely related molecule that has a much lower sulphate content and is found on the cell surface, and in the ECM of almost all mammalian cells as a component of heparan sulphate proteoglycans (HSPGs). The structures of heparin and HS are key to their biological function because distinct patterns of sulphation upon the polysaccharide chains are recognised and bound by specific protein ligands. This can serve simply to immobilise or protect bound ligands. However, in several cases, such binding has been shown to modulate the biological activity of proteins directly (Stringer and Gallagher, 1997; Lindahl et al., 1998).

Gitay-Goren et al. demonstrated that binding of $VEGF_{165}$ to its receptors on vascular endothelial cells is dependent on cell-surface-associated heparin-like molecules (Gitay-Goren et al., 1992). Low concentrations (0.1-10 μg/ml) of heparin strongly potentiate $VEGF_{165}$ binding, whereas higher concentrations are inhibitory. Depletion of cell surface heparin/HS by heparinase treatment severely reduces such binding but can be restored by the addition of exogenous heparin. These results were subsequently found also to apply when $VEGF_{165}$ binds to VEGFR-2 or NRP1 (Tessler et al., 1994; Terman et al., 1994; Soker et al., 1996; Soker et al., 1998). In contrast, the $VEGF_{121}$–VEGFR-2 interaction is unaffected by exogenous heparin or heparinase (Gitay-Goren et al., 1996). The possible participation of HS in $VEGF_{165}$–VEGFR-2 complexes might explain why some groups report this isoform as a more potent endothelial cell mitogen than $VEGF_{121}$ (Keyt et al., 1996b). Interestingly, rather than affecting the dissociation constant for $VEGF_{165}$ binding, heparin/HS seems to have a receptor-unmasking effect, increasing levels of VEGFR-2 available for $VEGF_{165}$ (Gitay-Goren et al., 1992; Terman et al., 1994).

The VEGFR-1 receptor behaves quite differently: both $VEGF_{165}$ and $VEGF_{121}$ binding are potently inhibited by exogenous heparin, even at concentrations lower than those required for potentiation of $VEGF_{165}$-VEGFR-2 binding (Terman et al., 1994; Cohen et al., 1995). However, heparin-like molecules are essential for VEGFR-1 function, because heparinase treatment of cells abolishes the ability of VEGFR-1 to bind both $VEGF_{121}$ and $VEGF_{165}$. Interestingly, whereas exogenous heparin partially restores $VEGF_{165}$ binding to such cells, $VEGF_{121}$ binding remains lost (Cohen et al., 1995). Since $VEGF_{165}$, unlike $VEGF_{121}$, is a heparin-binding protein, heparin bound to $VEGF_{165}$ must be able to complement the loss of cell surface HS in a way that free heparin cannot to allow some $VEGF_{165}$–VEGFR-1 binding. It seems that HSPGs are essential for VEGF binding to VEGFR-1, and presumably exogenous heparin interferes with the normal functioning of HSPGs at this receptor. Changes in local heparin concentration or the levels and composition of cell surface HS might modulate the interactions of the VEGF isoforms with VEGFR-1.

Since $VEGF_{121}$ has no affinity for heparin/HS, some of these effects of exogenous heparin and heparinase on VEGFR-1 must be mediated at the receptor level (Cohen et al., 1995). Cell-surface heparin receptors are thought to exist and may influence VEGFR functions (Gitay-Goren et al., 1992). However, VEGFR-1 is a heparin-binding protein itself, containing a potential heparin-binding domain in its fourth Ig-like extracellular loop (Kendall et al., 1993; Park and Lee, 1999). Interestingly, this domain is implicated in VEGFR-1 dimerisation, and heparin/HS interactions might account for the observed effects of these species on VEGF binding. VEGFR-2 also appears to interact directly with heparin/HS (Chiang and Flanagan, 1995; Dougher et al., 1997), perhaps through the hexapeptide sequence RKTKKR between the sixth and seventh Ig-like domains. Clearly the regulatory effects of heparin/HS on VEGF binding to its receptors depend both on the particular receptor subtype and the VEGF isoform involved.

Heparin/HS affinity can also affect VEGF function through several other mechanisms. HSPGs affect VEGF bioavailability through sequestration at the cell surface/ECM. This VEGF

reservoir may be protected from enzymatic degradation (Houck et al., 1992), although ECM-bound $VEGF_{189}$ does not appear to be protected from uPA or plasmin maturation (Plouet et al., 1997). VEGF can displace other HSPG-bound growth factors from the ECM, most notably bFGF, which can then synergise with VEGF in stimulating angiogenesis (Asahara et al., 1995; Jonca et al., 1997). Heparin/HS also protects the heparin-binding isoforms from natural inhibitory and degradatory processes by preventing their interaction with proteins such as α_2-macroglobulin and platelet factor 4 (Soker et al., 1993; Gengrinovitch et al., 1995).

A novel function of heparin may be in the restoration of function to damaged VEGF. Oxidising agents and free radicals, both common around areas of inflammation and wound healing, can inactivate VEGF. Under such conditions, heparin binding by $VEGF_{165}$ could prolong its biological activity compared with $VEGF_{121}$ by partially restoring lost function (Gitay-Goren et al., 1996). In fact, glypican-1, an HSPG known to be present on endothelial cells, has been shown to play a chaperone-like function by partially restoring $VEGF_{165}$ activity after oxidative damage and promoting VEGFR-2 binding (Gengrinovitch et al., 1999). Since most experiments use recombinant $VEGF_{165}$, restoration of activity lost through damage may explain some of the observed effects of exogenous heparin.

Concluding remarks

Here, we have atempted to highlight the complexity of VEGF signaling – from the various properties of the VEGF splice variants to the wide range of molecules that can interact with and modulate the activity of these different isoforms. Some fundamental gaps in our knowledge are only now being addressed through VEGF research. Why several isoforms of VEGF exist and how they differ functionally have never been fully explained. Spatial and temporal expression studies of VEGF in mouse models may provide further insights. The signaling events downstream of VEGFR activation are being elucidated, but the cellular responses they elicit, at least as far as VEGFR-1 is concerned, are still contentious. Is the primary function of VEGFR-1 on endothelial cells to act as a decoy receptor for VEGF and thereby reduce signaling through VEGFR-2? Such a role would be unique among the receptor tyrosine kinases.

The discovery of NRP1 as an isoform-specific co-receptor for VEGF has opened up a whole new field of VEGF research and has even revealed potentially new roles for VEGF in hematopoiesis and axonal growth in the CNS. However, there are certain aspects of this fascinating molecule that we are still ignorant about – for example, what role does NRP1 play in binding growth factors that do not interact with VEGFR2?

HSPGs at the cell surface and in the ECM carry out a variety of distinct functions as a consequence of their interactions with VEGF (all isoforms except $VEGF_{121}$), VEGFR-1 and VEGFR-2. Their recently identified ability to partially restore $VEGF_{165}$ function lost due to oxidative damage represents a novel function for these ubiquitous proteoglycans and underlines the need for further study.

A great deal has been achieved since the discovery of VEGF over a decade ago, but we are still a long way off understanding how events both inside and outside cells work together to control and coordinate VEGF activity. Such an understanding will be vital in the future design of therapeutic agents to control angiogenesis.

REFERENCES

Abe, K., Shoji, M., Chen, J., Bierhaus, A., Danave, I., Micko, C., Casper, K., Dillehay, L., Nawroth, P. P. and Rickles, F. R. (1999). Regulation of vascular endothelial growth factor production and angiogenesis by the cytoplasmic tail of tissue factor. *Proc. Nat. Acad. Sci. USA* **96**, 8663-8668.

Achen, M. G., Jeltsch, M., Kukk, E., Makinen, T., Vitali, A., Wilks, A. F., Alitalo, K. and Stacker, S. A. (1998). Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk-1) and VEGF receptor 3 (Flt-4). *Proc. Nat. Acad. Sci. USA* **95**, 548-553.

Akiri, G., Nahari, D., Finkelstein, Y., Le, S. Y., Elroy-Stein, O. and Levi, B. Z. (1998). Regulation of vascular endothelial growth factor (VEGF) expression is mediated by internal initiation of translation and alternative initiation of transcription. *Oncogene* **17**, 227-236.

Alon, T., Hemo, I., Itin. A., Pe'er. J., Stone, J. and Keshet, E. (1995). Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat. Med.* **10**, 1024-1028.

Anthony, F. W., Wheeler, T., Elcock, C. L., Pickett, M. and Thomas, E. J. (1994). Short report: identification of a specific pattern of vascular endothelial growth factor mRNA expression in human placenta and cultured placental fibroblasts. *Placenta* **15**, 557-561.

Asahara, T., Bauters, C., Zheng, L. P., Takeshita, S., Bunting, S., Ferrara, N., Symes, J. F. and Isner, J. M. (1995). Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. *Circulation* **92**, 365-371

Bacic, M., Edwards, N. A. and Merrill, M. J. (1995). Differential expression of vascular endothelial growth factor (vascular permeability factor) forms in rat tissues. *Growth Factors* **12**, 11-15.

Barleon, B., Sozzani, S., Zhou, D., Weich, H. A., Mantovani, A. and Marme, D. (1996). Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor Flt-1. *Blood* **87**, 3336-3343.

Barleon, B., Totzke, F., Herzog, C., Blanke, S., Kremmer, E., Siemeister, G., Marme, D. and Martiny-Baron, G. (1997a). Mapping of the sites for ligand binding and receptor dimerization at the extracellular domain of the vascular endothelial growth factor receptor FLT-1. *J. Biol. Chem.* **272**, 10382-10388.

Barleon, B., Siemeister, G., Martiny-Baron, G., Weindel, K., Herzog, C. and Marme, D. (1997b). Vascular endothelial growth factor up-regulates its receptor *fms*-like tyrosine kinase 1 (FLT-1) and a soluble variant of FLT-1 in human vascular endothelial cells. *Cancer Res.* **57**, 5421-5425.

Benjamin, L. E. and Keshet, E. (1997). Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. *Proc. Nat. Acad. Sci. USA* **94**, 8761-8766.

Boensch, C., Kuo, M. D., Connolly, D. T., Huang, S. S. and Huang, J. S. (1995). Identification, purification, and characterization of cell-surface retention sequence-binding proteins from human SK-Hep cells and bovine liver plasma membranes. *J. Biol. Chem.* **270**, 1807-1816.

Brogi, E., Wu, T., Namiki, A. and Isner, J. M. (1994). Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only. *Circulation* **2**, 649-652.

Brogi, E., Schatteman, G., Wu, T., Kim, E. A., Varticovski, L., Keyt, B. and Isner, J. M. (1996). Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. *J. Clin. Invest.* **97**, 469-476.

Cai, H. and Reed, R. R. (1999). Cloning and characterization of neuropilin-1-interacting protein: a PSD-95/Dlg/ZO-1 domain-containing protein that interacts with the cytoplasmic domain of neuropilin-1. *J. Neurosci.* **19**, 6519-6527.

Cao, Y., Chen, H., Zhou, L., Chiang, M. K., Anand-Apte, B., Weatherbee, J. A., Wang, Y., Fang, F., Flanagan, J. G. and Tsang, M. L. (1996). Heterodimers of placenta growth factor/vascular endothelial growth factor. Endothelial activity, tumor cell expression, and high affinity binding to Flk-1/KDR *J. Biol. Chem.* **271**, 3154-3162.

Cao, Y., Ji, W. R., Qi, P., Rosin, A. and Cao, Y. (1997). Placenta growth factor: identification and characterization of a novel isoform generated by RNA alternative splicing. *Biochem. Biophys. Res. Commun.* **235**, 493-498.

Cao, Y., Linden, P., Farnebo, J., Cao, R., Eriksson, A., Kumar, V., Qi, J. H. Claesson-Welsh, L. and Alitalo, K. (1998). Vascular endothelial growth factor C induces angiogenesis in vivo. *Proc. Nat. Acad. Sci. USA* **95**, 14389-14394.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., Declercq, C., Pawling, J., Moons, L., Collen, D., Risau, W. and Nagy, A. (1996). Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* **380**, 435-439.

Chen, H., Chedotal, A., He, Z., Goodman, C. S. and Tessier-Lavigne, M. (1997). Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema III. *Neuron* **19**, 547-559.

Cheung, C. Y., Singh, M., Ebaugh, M. J. and Brace, R. A. (1995). Vascular endothelial growth factor gene expression in ovine placenta and fetal membranes. *Am. J. Obstet. Gynecol.* **173**, 753-759.

Chiang, M. K. and Flanagan, J. G. (1995). Interactions between the Flk-1 receptor, vascular endothelial growth factor, and cell surface proteoglycan identified with a soluble receptor reagent. *Growth Factors* **12**, 1-10.

Claffey, K. P., Wilkison, W. O. and Spiegelman, B. M. (1992). Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. *J. Biol. Chem.* **267**, 16317-16322.

Claffey, K. P., Senger, D. R. and Spiegelman, B. M. (1995). Structural requirements for dimerization, glycosylation, secretion, and biological function of VPF/VEGF. *Biochim. Biophys. Acta* **1246**, 1-9.

Clark, D. E., Smith, S. K., He, Y., Day, K. A., Licence, D. R., Corps, A. N. Lammoglia, R. and Charnock-Jones, D. S. (1998). A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol. Reprod.* **59**, 1540-1548

Clauss, M., Gerlach, M., Gerlach, H., Brett, J., Wang, F., Familletti, P. C., Pan, Y. C., Olander, J. V., Connolly, D. T. and Stern, D. (1990). Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J. Exp. Med.* **172**, 1535-1545.

Clauss, M., Weich, H., Breier, G., Knies, U., Rockl, W., Waltenberger, J. and Risau, W. (1996). The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. *J. Biol. Chem.* **271**, 17629-17634.

Cohen, T., Gitay-Goren, H., Sharon, R., Shibuya, M., Halaban, R., Levi, B. Z., Neufeld, G. (1995). $VEGF_{121}$, a vascular endothelial growth factor (VEGF) isoform lacking heparin binding ability, requires cell-surface heparan sulfates for efficient binding to the VEGF receptors of human melanoma cells. *J. Biol. Chem.* **270**, 11322-11326.

Connolly, D. T., Olander, J. V., Heuvelman, D., Nelson, R., Monsell, R., Siegel, N., Haymore, B. L., Leimgruber, R. and Feder, J. (1989). Human vascular permeability factor. Isolation from U937 cells. *J. Biol. Chem.* **264**, 20017-20024.

Davis-Smyth, T., Chen, H., Park, J., Presta, L. G. and Ferrara, N. (1996). The second immunoglobulin-like domain of the VEGF tyrosine kinase receptor Flt-1 determines ligand binding and may initiate a signal transduction cascade. *EMBO J.* **15**, 4919-4927.

de Vries, C., Escobedo, J. A., Ueno, H., Houck, K., Ferrara, N. and Williams, L. T. (1992). The *fms*-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science* **55**, 989-991.

Dibbens, J. A., Miller, D. L., Damert, A., Risau, W., Vadas, M. A. and Goodall, G. J. (1999). Hypoxic regulation of vascular endothelial growth factor mRNA stability requires the cooperation of multiple RNA elements. *Mol. Biol. Cell* **10**, 907-919.

DiSalvo, J., Bayne, M. L., Conn, G., Kwok, P. W., Trivedi, P. G., Soderman, D. D., Palisi, T. M., Sullivan, K. A. and Thomas, K. A. (1995). Purification and characterization of a naturally occurring vascular endothelial growth factor/placenta growth factor heterodimer. *J. Biol. Chem.* **270**, 7717-7723

Dougher, A. M., Wasserstrom, H., Torley, L., Shridaran, L., Westdock, P., Hileman, R. E., Fromm, J. R., Anderberg, R., Lyman, S., Linhardt, R. J., Kaplan, J. and Terman, B. I. (1997). Identification of a heparin binding peptide on the extracellular domain of the KDR VEGF receptor. *Growth Factors* **14**, 257-268.

Dumont, D. J., Jussila, L., Taipale, J., Lymboussaki, A., Mustonen, T., Pajusola, K., Breitman, M. and Alitalo, K. (1998). Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. *Science* **282**, 946-949

Eichmann, A., Corbel, C., Nataf, V., Vaigot, P., Breant, C. and Le-Douarin, N. M. (1997). Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc. Nat. Acad. Sci. USA* **94**, 5141-5146.

Enholm, B., Paavonen, K., Ristimaki, A., Kumar, V., Gunji, Y., Klefstrom, J., Kivinen, L., Laiho, M., Olofsson, B., Joukov, V., Eriksson, U. and Alitalo, K. (1997). Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factors, oncoproteins and hypoxia. *Oncogene* **14**, 2475-2483.

Fairbrother, W. J., Champe, M. A., Christinger, H. W., Keyt, B. A. and Starovasnik, M. A. (1998). Solution structure of the heparin-binding domain of vascular endothelial growth factor. *Structure* **6**, 637-648.

Farnebo, F., Piehl, F. and Lagercrantz, J. (1999). Restricted expression pattern of VEGF-D in the adult and fetal mouse: high expression in the embryonic lung. *Biochem. Biophys. Res. Commun.* **257**, 891-894.

Ferrara, N. and Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.* **161**, 851-858.

Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K. S., Powell-Braxton, L., Hillan, K. J., Moore, M. W. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* **380**, 439-442.

Ferrara, N. and Davis-Smyth, T. (1997). The biology of vascular endothelial growth factor. *Endocr. Rev.* **18**, 4-25.

Finnerty, H., Kelleher, K., Morris, G. E., Bean, K., Merberg, D. M., Kriz, R., Morris, J. C., Sookdeo, H., Turner, K. J. and Wood, C. R. (1993). Molecular cloning of murine FLT and FLT4. *Oncogene* **8**, 2293-2298.

Folkman, J. (1990). What is the evidence that tumors are angiogenesis dependent? *J. Nat. Cancer Inst.* **82**, 4-6.

Folkman, J. and Hanahan, D. (1991). Switch to the angiogenic phenotype during tumorigenesis. *Princess Takamatsu Symp.* **22**, 339-347.

Folkman, J. (1997). Angiogenesis and angiogenesis inhibition: an overview. *EXS* **79**, 1-8.

Fong, G. H., Rossant, J., Gertsenstein, M. and Breitman, M. L. (1995). Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* **376**, 66-70.

Fong, G. H., Zhang, L., Bryce, D. M. and Peng, J. (1999). Increased hemangioblast commitment, not vascular disorganization, is the primary defect in flt-1 knock-out mice. *Development* **126**, 3015-3025.

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D. and Semenza, G. L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* **16**, 4604-4613.

Fuh, G., Li, B., Crowley, C., Cunningham, B. and Wells, J. A. (1998). Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. *J. Biol. Chem.* **273**, 11197-11204.

Garrido, C., Saule, S. and Gospodarowicz, D. (1993). Transcriptional regulation of vascular endothelial growth factor gene expression in ovarian bovine granulosa cells. *Growth Factors* **8**, 109-117.

Gengrinovitch, S., Greenberg, S. M., Cohen, T., Gitay-Goren, H., Rockwell, P., Maione-T. E., Levi, B. Z. and Neufeld, G. (1995). Platelet factor-4 inhibits the mitogenic activity of $VEGF_{121}$ and $VEGF_{165}$ using several concurrent mechanisms. *J. Biol. Chem.* **270**, 15059-15065.

Gengrinovitch, S., Berman, B., David, G., Witte, L., Neufeld, G. and Ron, D. (1999). Glypican-1 is a $VEGF_{165}$ binding proteoglycan that acts as an extracellular chaperone for $VEGF_{165}$. *J. Biol. Chem.* **274**, 10816-10822.

Gerber, H. P., Condorelli, F., Park, J. and Ferrara, N. (1997). Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J. Biol. Chem.* **272**, 23659-23667.

Giger, R. J., Urquhart, E. R., Gillespie, S. K., Levengood, D. V., Ginty, D. D. and Kolodkin, A. L. (1998). Neuropilin-2 is a receptor for semaphorin IV: insight into the structural basis of receptor function and specificity. *Neuron* **21**, 1079-1092.

Giraudo, E., Primo, L., Audero, E., Gerber, H. P., Koolwijk, P., Soker, S., Klagsbrun, M., Ferrara, N. and Bussolino, F. (1998). Tumor necrosis factor-alpha regulates expression of vascular endothelial growth factor receptor-2 and of its co-receptor neuropilin-1 in human vascular endothelial cells. *J. Biol. Chem.* **273**, 22128-22135.

Gitay-Goren, H., Soker, S., Vlodavsky, I. and Neufeld, G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell surface-associated heparin-like molecules. *J. Biol. Chem.* **267**, 6093-6098.

Gitay-Goren, H., Cohen, T., Tessler, S., Soker, S., Gengrinovitch, S., Rockwell, P., Klagsbrun, M., Levi, B. Z. and Neufeld, G. (1996).

Selective binding of $VEGF_{121}$ to one of the three vascular endothelial growth factor receptors of vascular endothelial cells. *J. Biol. Chem.* **271**, 5519-5523.

Gospodarowicz, D., Abraham, J. A. and Schilling, J. (1989). Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. *Proc. Nat. Acad. Sci. USA* **86**, 7311-7315.

Grimmond, S., Lagercrantz, J., Drinkwater, C., Silins, G., Townson, S., Pollock, P., Gotley, D., Carson, E., Rakar, S., Nordenskjold, M., Ward, L., Hayward, N. and Weber, G. (1996). Cloning and characterization of a novel human gene related to vascular endothelial growth factor. *Genome Res.* **6**, 124-131.

Grugel, S., Finkenzeller, G., Weindel, K., Barleon, B. and Marme, D. (1995). Both v-Ha-Ras and v-Raf stimulate expression of the vascular endothelial growth factor in NIH 3T3 cells. *J. Biol. Chem.* **270**, 25915-25919.

Grutzkau, A., Kruger-Krasagakes, S., Baumeister, H., Schwarz, C., Kogel, H., Welker, P., Lippert, U., Henz, B. M. and Moller, A. (1998). Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of $VEGF_{206}$. *Mol. Biol. Cell* **9**, 875-884.

Guerrin, M., Moukadiri, H., Chollet, P., Moro, F., Dutt, K., Malecaze, F. and Plouet, J. (1995). Vasculotropin/vascular endothelial growth factor is an autocrine growth factor for human retinal pigment epithelial cells cultured in vitro. *J. Cell. Physiol.* **164**, 385-394.

Hauser, S. and Weich, H. A. (1993). A heparin-binding form of placenta growth factor (PlGF-2) is expressed in human umbilical vein endothelial cells and in placenta. *Growth Factors* **9**, 259-268.

He, Y., Smith, S. K., Day, K. A., Clark, D. E., Licence, D. R. and Charnock-Jones, D. S. (1999). Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT-1) pre-mRNA is important for the regulation of VEGF activity. *Mol. Endocrinol.* **13**, 537-545.

He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell* **90**, 739-751.

Hiratsuka, S., Minowa, O., Kuno, J., Noda, T. and Shibuya, M. (1998). Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. *Proc. Nat. Acad. Sci. USA* **95**, 9349-9354.

Houck, K. A., Ferrara, N., Winer, J., Cachianes, G., Li, B. and Leung, D. W. (1991). The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol. Endocrinol.* **5**, 1806-1814.

Houck, K. A., Leung, D. W., Rowland, A. M., Winer, J. and Ferrara, N. (1992). Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. *J. Biol. Chem.* **267**, 26031-26037.

Igarashi, K., Isohara, T., Kato, T., Shigeta, K., Yamano, T. and Uno, I. (1998). Tyrosine-1213 of Flt-1 is a major binding site of Nck and SHP-2. *Biochem. Biophys. Res. Commun.* **246**, 95-99.

Iliopoulos, O., Levy, A. P., Jiang, C., Kaelin, W. G. Jr and Goldberg, M. A. (1996). Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc. Nat. Acad. Sci. USA* **93**, 10595-10599.

Jeltsch, M., Kaipainen, A., Joukov, V., Meng, X., Lakso, M., Rauvala, H., Swartz, M., Fukumura, D., Jain, R. K. and Alitalo, K. (1997). Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* **276**, 1423-1425.

Jiang, B. H., Semenza, G. L., Bauer, C. and Marti, H. H. (1996). Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. *Am. J. Physiol.* **271**, 1172-1180.

Jingjing, L., Xue, Y., Agarwal, N. and Roque, R. S. (1999). Human Muller cells express $VEGF_{183}$, a novel spliced variant of vascular endothelial growth factor. *Invest. Ophthalmol. Vis. Sci.* **40**, 752-759.

Jonca, F., Ortega, N., Gleizes, P. E., Bertrand, N. and Plouet, J. (1997). Cell release of bioactive fibroblast growth factor-2 by exon 6-encoded sequence of vascular endothelial growth factor. *J. Biol. Chem.* **272**, 24203-24209.

Joukov, V., Pajusola, K., Kaipainen, A., Chilov, D., Lahtinen, I., Kukk, E., Saksela, O., Kalkkinen, N. and Alitalo, K. (1996). A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt-4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J.* **15**, 290-298.

Joukov, V., Sorsa, T., Kumar, V., Jeltsch, M., Claesson-Welsh, L., Cao, Y., Saksela, O., Kalkkinen, N. and Alitalo, K. (1997a). Proteolytic processing regulates receptor specificity and activity of VEGF-C. *EMBO. J.* **16**, 3898-3911.

Joukov, V., Kaipainen, A., Jeltsch, M., Pajusola, K., Olofsson, B., Kumar, V., Eriksson, U. and Alitalo, K. (1997b). Vascular endothelial growth factors VEGF-B and VEGF-C. *J. Cell. Physiol.* **173**, 211-215.

Kaipainen, A., Korhonen, J., Mustonen, T., van-Hinsbergh, V. W., Fang, G. H., Dumont, D., Breitman, M. and Alitalo, K. (1995). Expression of the *fms*-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc. Nat. Acad. Sci. USA* **92**, 3566-3570.

Kawakami, A., Kitsukawa, T., Takagi, S. and Fujisawa, H. (1996). Developmentally regulated expression of a cell surface protein, neuropilin, in the mouse nervous system. *J. Neurobiol.* **29**, 1-17.

Keck, P. J., Hauser, S. D., Krivi, G., Sanzo, K., Warren, T., Feder, J. and Connolly, D. T. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* **246**, 1309-1312.

Kendall, R. L. and Thomas, K. A. (1993). Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc. Nat. Acad. Sci. USA* **90**, 10705-10709.

Kendall, R. L., Wang, G., DiSalvo, J. and Thomas, K. A. (1994). Specificity of vascular endothelial cell growth factor receptor ligand binding domains. *Biochem. Biophys. Res. Commun.* **201**, 326-330.

Kendall, R. L., Wang, G. and Thomas, K. A. (1996). Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR *Biochem. Biophys. Res. Commun.* **226**, 324-238.

Keyt, B. A., Nguyen, H. V., Berleau, L. T., Duarte, C. M., Park, J., Chen, H. and Ferrara, N. (1996a). Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective VEGF variants by site-directed mutagenesis. *J. Biol. Chem.* **271**, 5638-5646.

Keyt, B. A., Berleau, L. T., Nguyen, H. V., Chen, H., Heinsohn, H., Vandlen, R. and Ferrara, N. (1996b). The carboxyl-terminal domain (111-165) of vascular endothelial growth factor is critical for its mitogenic potency. *J. Biol. Chem.* **271**, 7788-7795.

Kieser, A., Weich, H. A., Brandner, G., Marme, D. and Kolch, W. (1994). Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. *Oncogene.* **9**, 963-969.

Kitsukawa, T., Shimono, A., Kawakami, A., Kondoh, H. and Fujisawa, H. (1995). Overexpression of a membrane protein, neuropilin, in chimeric mice causes anomalies in the cardiovascular system, nervous system and limbs. *Development.* **121**, 4309-4318.

Kitsukawa, T., Shimizu, M., Sanbo, M., Hirata, T., Taniguchi, M., Bekku, Y., Yagi, T. and Fujisawa, H. (1997). Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. *Neuron* **19**, 995-1005.

Kolodkin, A. L., Levengood, D. V., Rowe, E. G., Tai, Y. T., Giger, R. J. and Ginty, D. D. (1997). Neuropilin is a semaphorin III receptor. *Cell* **90**, 753-762

Kukk, E., Lymboussaki, A., Taira, S., Kaipainen, A., Jeltsch, M., Joukov, V. and Alitalo, K. (1996). VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* **122**, 3829-3837.

Landgren, E., Schiller, P., Cao, Y. and Claesson-Welsh, L. (1998). Placenta growth factor stimulates MAP kinase and mitogenicity but not phospholipase C-gamma and migration of endothelial cells expressing Flt 1. *Oncogene* **16**, 359-367.

Larrivee, B. and Karsan, A. (2000). Signaling pathways induced by vascular endothelial growth factor (review). *Int. J. Mol. Med.* **5**, 447-456.

Lee, J., Gray, A., Yuan, J., Luoh, S. M., Avraham, H. and Wood, W. I. (1996). Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt-4. *Proc. Nat. Acad. Sci. USA* **93**, 1988-1992.

Lei, J., Jiang, A. and Pei, D. (1998). Identification and characterization of a new splicing variant of vascular endothelial growth factor: $VEGF_{183}$. *Biochim. Biophys. Acta* **1443**, 400-406.

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V. and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306-1309.

Levy, A. P., Levy, N. S., Wegner, S. and Goldberg, M. A. (1995). Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J. Biol. Chem.* **270**, 13333-13340.

Levy, N. S., Chung, S., Furneaux, H. and Levy, A. P. (1998). Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *J. Biol. Chem.* **273**, 6417-6423.

Li, J., Brown, L. F., Hibberd, M. G., Grossman, J. D., Morgan, J. P. and Simons, M. (1996). VEGF, Flk-1, and Flt-1 expression in a rat myocardial infarction model of angiogenesis. *Am. J. Physiol.* **270**, 1803-1811.

Lindahl, U., Kusche-Gullberg, M. and Kjellen, L. (1998). Regulated diversity of heparan sulfate. *J. Biol. Chem.* **273**, 24979-24982.

Liu, Y., Cox, S. R., Morita, T. and Kourembanas, S. (1995). Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5′ enhancer. *Circ. Res.* **77**, 638-643.

Lyttle, D. J., Fraser, K. M., Fleming, S. B., Mercer, A. A. and Robinson, A. J. (1994). Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. *J. Virol.* **68**, 84-92

Madan, A. and Curtin, P. T. (1993). A 24-base-pair sequence 3′ to the human erythropoietin gene contains a hypoxia-responsive transcriptional enhancer. *Proc. Nat. Acad. Sci. USA* **90**, 3928-3932.

Maeda, K., Chung, Y. S., Ogawa, Y., Takatsuka, S., Kang, S. M., Ogawa, M., Sawada, T. and Sowa, M. (1996). Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. *Cancer* **77**, 858-863.

Maglione, D., Guerriero, V., Viglietto, G., Delli-Bovi, P. and Persico, M. G. (1991). Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc. Nat. Acad. Sci. USA* **88**, 9267-9271.

Maglione, D., Guerriero, V., Viglietto, G., Ferraro, M. G., Aprelikova, O., Alitalo, K., Del-Vecchio, S., Lei, K. J., Chou, J. Y. and Persico, M. G. (1993). Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14. *Oncogene* **8**, 925-931.

Makinen, T., Olofsson, B., Karpanen, T., Hellman, U., Soker, S., Klagsbrun, M., Eriksson, U. and Alitalo, K. (1999). Differential binding of vascular endothelial growth factor B splice and proteolytic isoforms to neuropilin-1. *J. Biol. Chem.* **274**, 21217-21222.

Matthews, W., Jordan, C. T., Gavin, M., Jenkins, N. A., Copeland, N. G. and Lemischka, I. R. (1991). A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. *Proc. Nat. Acad. Sci. USA* **88**, 9026-9030.

Mazure, N. M., Chen, E. Y., Yeh, P., Laderoute, K. R. and Giaccia, A. J. (1996). Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. *Cancer Res.* **56**, 3436-3440.

McDonald, N. Q. and Hendrickson, W. A. (1993). A structural superfamily of growth factors containing a cystine knot motif. *Cell* **73**, 421-424.

Meyer, M., Clauss, M., Lepple-Wienhues, A., Waltenberger, J., Augustin, H. G., Ziche-M., Lanz, C., Buttner, M., Rziha, H. J. and Dehio, C. (1999). A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. *EMBO J.* **18**, 363-374.

Miao, H. Q., Soker, S., Feiner, L., Alonso, J. L., Raper, J. A. and Klagsbrun, M. (1999). Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility: functional competition of collapsin-1 and vascular endothelial growth factor-165. *J. Cell Biol.* **146**, 233-242.

Migdal, M., Huppertz, B., Tessler, S., Comforti, A., Shibuya, M., Reich, R., Baumann, H. and Neufeld, G. (1998). Neuropilin-1 is a placenta growth factor-2 receptor. *J. Biol. Chem.* **273**, 22272-22278.

Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N. P., Risau, W. and Ullrich, A. (1993). High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* **72**, 835-846.

Minchenko, A., Bauer, T., Salceda, S. and Caro, J. (1994). Hypoxic stimulation of vascular endothelial growth factor expression in vitro and in vivo. *Lab. Invest.* **71**, 374-379.

Morishita, K., Johnson, D. E. and Williams, L. T. (1995). A novel promoter for vascular endothelial growth factor receptor (*flt-1*) that confers endothelial-specific gene expression. *J. Biol. Chem.* **270**, 27948-27953.

Mukhopadhyay, D., Tsiokas, L. and Sukhatme, V. P. (1995). Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. *Cancer Res.* **55**, 6161-6165.

Mukhopadhyay, D., Knebelmann, B., Cohen, H. T., Ananth, S. and Sukhatme, V. P. (1997). The von Hippel-Lindau tumor suppressor gene product interacts with Sp1 to repress vascular endothelial growth factor promoter activity. *Mol. Cell. Biol.* **17**, 5629-5639.

Muller, Y. A., Christinger, H. W., Keyt, B. A. and de Vos, A. M. (1997). The crystal structure of vascular endothelial growth factor (VEGF) refined to 1. 93 Å resolution: multiple copy flexibility and receptor binding. *Structure* **5**, 1325-1338.

Murray-Rust, J., McDonald, N. Q., Blundell, T. L., Hosang, M., Oefner, C., Winkler, F. and Bradshaw, R. A. (1993). Topological similarities in TGF-β2, PDGF-BB and NGF define a superfamily of polypeptide growth factors. *Structure* **1**, 153-159.

Ogawa, S., Oku, A., Sawano, A., Yamaguchi, S., Yazaki, Y. and Shibuya, M. (1998). A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain. *J. Biol. Chem.* **273**, 31273-31282.

Olofsson, B., Pajusola, K., Kaipainen, A., von-Euler, G., Joukov, V., Saksela, O., Orpana, A., Pettersson, R. F., Alitalo, K. and Eriksson, U. (1996a). Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc. Nat. Acad. Sci. USA* **93**, 2576-2581.

Olofsson, B., Pajusola, K., von-Euler, G., Chilov, D., Alitalo, K. and Eriksson, U. (1996b). Genomic organization of the mouse and human genes for vascular endothelial growth factor B (VEGF-B) and characterization of a second splice isoform. *J. Biol. Chem.* **271**, 19310-19317.

Olofsson, B., Korpelainen, E., Pepper, M. S., Mandriota, S. J., Aase, K., Kumar, V., Gunji, Y., Jeltsch, M. M., Shibuya, M., Alitalo, K. and Eriksson, U. (1998). Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. *Proc. Nat. Acad. Sci. USA* **95**, 11709-11714.

Omura, T., Miyazawa, K., Ostman, A. and Heldin, C. H. (1997). Identification of a 190-kDa vascular endothelial growth factor 165 cell surface binding protein on a human glioma cell line. *J. Biol. Chem.* **272**, 23317-23322.

Orlandini, M., Marconcini, L., Ferruzzi, R. and Oliviero, S. (1996). Identification of a c-*fos*-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family. *Proc. Nat. Acad. Sci. USA* **93**, 11675-11680.

Pajusola, K., Aprelikova, O., Korhonen, J., Kaipainen, A., Pertovaara, L., Alitalo, R. and Alitalo, K. (1992). FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. *Cancer Res.* **52**, 5738-5743.

Pal, S., Claffey, K. P., Dvorak, H. F. and Mukhopadhyay, D. (1997). The von Hippel-Lindau gene product inhibits vascular permeability factor/vascular endothelial growth factor expression in renal cell carcinoma by blocking protein kinase C pathways. *J. Biol. Chem.* **272**, 27509-27512.

Park, J. E., Keller, G. A. and Ferrara, N. (1993). The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. *Mol. Biol. Cell* **4**, 1317-1326.

Park, J. E., Chen, H. H., Winer, J., Houck, K. A. and Ferrara, N. (1994). Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR *J. Biol. Chem.* **269**, 25646-25654.

Park, M. and Lee, S. T. (1999). The fourth immunoglobulin-like loop in the extracellular domain of FLT-1, a VEGF receptor, includes a major heparin-binding site. *Biochem. Biophys. Res. Commun.* **264**, 730-734.

Patterson, C., Perrella, M. A., Hsieh, C. M., Yoshizumi, M., Lee, M. E. and Haber, E. (1995). Cloning and functional analysis of the promoter for KDR/*flk*-1, a receptor for vascular endothelial growth factor. *J. Biol. Chem.* **270**, 23111-23118.

Pekala, P., Marlow, M., Heuvelman, D. and Connolly, D. (1990). Regulation of hexose transport in aortic endothelial cells by vascular permeability factor and tumor necrosis factor-alpha, but not by insulin. *J. Biol. Chem.* **265**, 18051-18054.

Pepper, M. S., Ferrara, N., Orci, L. and Montesano, R. (1991). Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. *Biochem. Biophys. Res. Commun.* **181**, 902-906.

Pepper, M. S. and Mandriota, S. J. (1998). Regulation of vascular endothelial growth factor receptor-2 (Flk-1) expression in vascular endothelial cells. *Exp. Cell. Res.* **241**, 414-425.

Pepper, M. S., Mandriota, S. J., Jeltsch, M., Kumar, V. and Alitalo, K. (1998). Vascular endothelial growth factor (VEGF)-C synergizes with basic fibroblast growth factor and VEGF in the induction of angiogenesis in vitro and alters endothelial cell extracellular proteolytic activity. *J. Cell. Physiol.* **177**, 439-452.

Peretz, D., Gitay-Goren, H., Safran, M., Kimmel, N., Gospodarowicz, D. and Neufeld, G. (1992). Glycosylation of vascular endothelial growth factor is not required for its mitogenic activity. *Biochem. Biophys. Res. Commun.* **182**, 1340-1347.

Persico, M. G., Vincenti, V. and DiPalma, T. (1999). Structure, expression and receptor-binding properties of placenta growth factor (PlGF). *Curr. Top. Microbiol. Immunol.* **237**, 31-40.

Pertovaara, L., Kaipainen, A., Mustonen, T., Orpana, A., Ferrara, N., Saksela, O. and Alitalo, K. (1994). Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *J. Biol. Chem.* **269**, 6271-6274.

Peters, K. G., De Vries, C. and Williams, L. T. (1993). Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. *Proc. Nat. Acad. Sci. USA* **90**, 8915-8919.

Petrova, T. V., Makinen, T. and Alitalo, K. (1999). Signaling via vascular endothelial growth factor receptors. *Exp. Cell. Res.* **253**, 117-130.

Piossek, C., Schneider-Mergener, J., Schirner, M., Vakalopoulou, E., Germeroth, L. and Thierauch, K. H. (1999). Vascular endothelial growth factor (VEGF) receptor II-derived peptides inhibit VEGF. *J. Biol. Chem.* **274**, 5612-5619.

Plate, K. H., Breier, G., Weich, H. A. and Risau, W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature* **359**, 845-848.

Plouet, J., Schilling, J. and Gospodarowicz, D. (1989). Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. *EMBO J.* **8**, 3801-3806.

Plouet, J., Moro, F., Bertagnolli, S., Coldeboeuf, N., Mazarguil, H., Clamens, S. and Bayard, F. (1997). Extracellular cleavage of the vascular endothelial growth factor 189-amino acid form by urokinase is required for its mitogenic effect. *J. Biol. Chem.* **272**, 13390-13396.

Poltorak, Z., Cohen, T., Sivan, R., Kandelis, Y., Spira, G., Vlodavsky, I., Keshet, E. and Neufeld, G. (1997). $VEGF_{145}$, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. *J. Biol. Chem.* **272**, 7151-7158.

Potgens, A. J., Lubsen, N. H., van-Altena, M. C., Vermeulen, R., Bakker, A., Schoenmakers, J. G., Ruiter, D. J. and de-Waal, R. M. (1994). Covalent dimerization of vascular permeability factor/vascular endothelial growth factor is essential for its biological activity. Evidence from Cys to Ser mutations. *J. Biol. Chem.* **269**, 32879-32885.

Praloran, V., Mirshahi, S., Favard, C., Moukadiri, H. and Plouet, J. (1991). Mitogenic activity of vasculotropin for peripheral human lymphocytes. *C. R. Acad. Sci. III* **313**, 21-26

Quinn, T. P., Peters, K. G., de Vries, C., Ferrara, N. and Williams, L. T. (1993). Fetal liver kinase-1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. *Proc. Nat. Acad. Sci. USA* **90**, 7533-7537.

Rak, J., Filmus, J., Finkenzeller, G., Grugel, S., Marme, D. and Kerbel, R. S. (1995). Oncogenes as inducers of tumor angiogenesis. *Cancer Metast. Rev.* **14**, 263-277.

Salceda, S., Beck, I. and Caro, J. (1996). Absolute requirement of aryl hydrocarbon receptor nuclear translocator protein for gene activation by hypoxia. *Arch. Biochem. Biophys.* **334**, 389-394.

Seetharam, L., Gotoh, N., Maru, Y., Neufeld, G., Yamaguchi, S. and Shibuya, M. (1995). A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. *Oncogene* **10**, 135-147.

Semenza, G. L., Agani, F., Booth, G., Forsythe, J., Iyer, N., Jiang, B. H., Leung, S., Roe, R., Wiener, C. and Yu, A. (1997). Structural and functional analysis of hypoxia-inducible factor 1. *Kidney Int.* **51**, 553-555.

Senger, D. R., Galli, S. J., Dvorak, A. M., Perruzzi, C. A., Harvey, V. S. and Dvorak, H. F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* **219**, 983-985.

Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L., Schuh, A. C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1 deficient mice. *Nature* **376**, 62-66.

Sheibani, N. and Frazier, W. A. (1998). Down-regulation of platelet endothelial cell adhesion molecule-1 results in thrombospondin-1 expression and concerted regulation of endothelial cell phenotype. *Mol. Biol. Cell* **9**, 701-713.

Shen, B. Q., Lee, D. Y., Gerber, H. P., Keyt, B. A. Ferrara, N. and Zioncheck, T. F. (1998). Homologous up-regulation of KDR/Flk-1 receptor expression by vascular endothelial growth factor in vitro. *J. Biol. Chem.* **273**, 29979-29985.

Shibuya, M., Yamaguchi, S., Yamane, A., Ikeda, T., Tojo, A., Matsushime, H. and Sato, M. (1990). Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (*flt*) closely related to the *fms* family. *Oncogene* **5**, 519-524.

Shinkai, A., Ito, M., Anazawa, H., Yamaguchi, S., Shitara, K. and Shibuya, M. (1998). Mapping of the sites involved in ligand association and dissociation at the extracellular domain of the kinase insert domain-containing receptor for vascular endothelial growth factor. *J. Biol. Chem.* **273**, 31283-31288.

Shweiki, D., Itin, A., Soffer, D. and Keshet, E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* **359**, 843-845.

Siemeister, G., Weindel, K., Mohrs, K., Barleon, B., Martiny-Baron, G. and Marme, D. (1996). Reversion of deregulated expression of vascular endothelial growth factor in human renal carcinoma cells by von Hippel-Lindau tumor suppressor protein. *Cancer Res.* **56**, 2299-2301.

Siemeister, G., Schirner, M., Reusch, P., Barleon, B., Marme, D. and Martiny-Baron, G. (1998a). An antagonistic vascular endothelial growth factor (VEGF) variant inhibits VEGF-stimulated receptor autophosphorylation and proliferation of human endothelial cells. *Proc. Nat. Acad. Sci. USA* **95**, 4625-4629.

Siemeister, G., Marme, D. and Martiny-Baron, G. (1998b). The alpha-helical domain near the amino terminus is essential for dimerization of vascular endothelial growth factor. *J. Biol. Chem.* **273**, 11115-11120.

Soker, S., Svahn, C. M. and Neufeld, G. (1993). Vascular endothelial growth factor is inactivated by binding to alpha 2-macroglobulin and the binding is inhibited by heparin. *J. Biol. Chem.* **268**, 7685-7691.

Soker, S., Fidder, H., Neufeld, G. and Klagsbrun, M. (1996). Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind $VEGF_{165}$ via its exon 7-encoded domain. *J. Biol. Chem.* **271**, 5761-5767.

Soker, S., Takashima, S., Miao, H. Q., Neufeld, G. and Klagsbrun, M. (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* **92**, 735-745.

Sondell, M., Lundborg, G. and Kanje, M. (1999). Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. *J. Neurosci.* **19**, 5731-5740.

Stein, I., Itin, A., Einat, P., Skaliter, R., Grossman, Z. and Keshet, E. (1998). Translation of vascular endothelial growth factor mRNA by internal ribosome entry: implications for translation under hypoxia. *Mol. Cell. Biol.* **18**, 3112-3119.

Stratmann, R., Krieg, M., Haas, R. and Plate, K. H. (1997). Putative control of angiogenesis in hemangioblastomas by the von Hippel-Lindau tumor suppressor gene. *J. Neuropathol. Exp. Neurol.* **56**, 1242-1252.

Stringer, S. E. and Gallagher, J. T. (1997). Heparan sulphate. *Int. J. Biochem. Cell. Biol.* **29**, 709-714.

Sugihara, T., Wadhwa, R., Kaul, S. C. and Mitsui, Y. (1998). A novel alternatively spliced form of murine vascular endothelial growth factor, $VEGF_{115}$. *J. Biol. Chem.* **273**, 3033-3038.

Sun, P. D. and Davies, D. R. (1995). The cystine-knot growth-factor superfamily. *Annu. Rev. Biophys. Biomol. Struct.* **24**, 269-291.

Takagi, H., King, G. L., Robinson, G. S., Ferrara, N. and Aiello, L. P. (1996). Adenosine mediates hypoxic induction of vascular endothelial growth factor in retinal pericytes and endothelial cells. *Invest. Ophthalmol. Vis. Sci.* **37**, 2165-2176.

Takagi, S., Kasuya, Y., Shimizu, M., Matsuura, T., Tsuboi, M., Kawakami, A. and Fujisawa, H. (1995). Expression of a cell adhesion molecule, neuropilin, in the developing chick nervous system. *Dev. Biol.* **170**, 207-222.

Takahashi, T. and Shibuya, M. (1997). The 230 kDa mature form of KDR/Flk-1 (VEGF receptor-2) activates the PLC-gamma pathway and partially induces mitotic signals in NIH3T3 fibroblasts. *Oncogene* **14**, 2079-2089.

Terman, B. I., Carrion, M. E., Kovacs, E., Rasmussen, B. A., Eddy, R. L. and Shows, T. B. (1991). Identification of a new endothelial cell growth factor receptor tyrosine kinase. *Oncogene* **6**, 1677-1683.

Terman, B. I., Dougher-Vermazen, M., Carrion, M. E., Dimitrov, D., Armellino, D. C., Gospodarowicz, D. and Bohlen, P. (1992). Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem. Biophys. Res. Commun.* **187**, 1579-1586.

Terman, B., Khandke, L., Dougher-Vermazan, M., Maglione, D., Lassam, N. J., Gospodarowicz, D., Persico, M. G., Bohlen, P. and Eisinger, M. (1994). VEGF receptor subtypes KDR and FLT1 show different sensitivities to heparin and placenta growth factor. *Growth Factors* **11**, 187-195.

Tessler, S., Rockwell, P., Hicklin, D., Cohen, T., Levi, B. Z., Witte, L., Lemischka, I. R. and Neufeld, G. (1994). Heparin modulates the interaction of $VEGF_{165}$ with soluble and cell associated Flk-1 receptors. *J. Biol. Chem.* **269**, 12456-12461.

Thieme, H., Aiello, L. P., Takagi, H., Ferrara, N. and King, G. L. (1995). Comparative analysis of vascular endothelial growth factor receptors on retinal and aortic vascular endothelial cells. *Diabetes* **44**, 98-103.

Tischer, E., Mitchell, R., Hartman, T., Silva, M., Gospodarowicz, D., Fiddes, J. C. and Abraham, J. A. (1991). The human gene for vascular

endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J. Biol. Chem.* **266**, 11947-11954.

Toi, M., Hoshina, S., Takayanagi, T., Tominaga, T. (1994). Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. *Jpn J. Cancer Res.* **85**, 1045-1049.

Tordjman, R., Ortega, N., Coulombel, L., Plouet, J., Romeo, P. H. and Lemarchandel, V. (1999). Neuropilin-1 is expressed on bone marrow stromal cells: a novel interaction with hematopoietic cells? *Blood* **94**, 2301-2309.

Tuder, R. M., Flook, B. E. and Voelkel, N. F. (1995). Increased gene expression for VEGF and the VEGF receptors KDR/Flk and Flt in lungs exposed to acute or to chronic hypoxia. Modulation of gene expression by nitric oxide. *J. Clin. Invest.* **95**, 1798-1807.

Unemori, E. N., Ferrara, N., Bauer, E. A. and Amento, E. P. (1992). Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. *J. Cell. Physiol.* **153**, 557-562.

Vincenti, V., Cassano, C., Rocchi, M. and Persico, G. (1996). Assignment of the vascular endothelial growth factor gene to human chromosome 6p21. 3. *Circulation* **93**, 1493-1495.

Waltenberger, J., Claesson-Welsh, L., Siegbahn, A., Shibuya, M. and Heldin, C. H. (1994). Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J. Biol. Chem.* **269**, 26988-26995.

Waltenberger, J., Mayr, U., Pentz, S. and Hombach, V. (1996). Functional upregulation of the vascular endothelial growth factor receptor KDR by hypoxia. *Circulation* **94**, 1647-1654.

Wang, H. and Keiser, J. A. (1998). Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: role of Flt-1. *Circ. Res.* **83**, 832-840.

Wen, Y., Edelman, J. L., Kang, T., Zeng, N. and Sachs, G. (1998). Two functional forms of vascular endothelial growth factor receptor-2/Flk-1 mRNA are expressed in normal rat retina. *J. Biol. Chem.* **273**, 2090-2097.

Wiesmann, C., Fuh, G., Christinger, H. W., Eigenbrot, C., Wells, J. A. and de Vos, A. M. (1997). Crystal structure at 1.7 Å resolution of VEGF in complex with domain 2 of the Flt-1 receptor. *Cell* **91**, 695-704.

Wilting, J., Birkenhager, R., Eichmann, A., Kurz, H., Martiny-Baron, G., Marme, D., McCarthy, J. E., Christ, B. and Weich, H. A. (1996). $VEGF_{121}$ induces proliferation of vascular endothelial cells and expression of Flk-1 without affecting lymphatic vessels of chorioallantoic membrane. *Dev. Biol.* **176**, 76-85.

Wise, L. M., Veikkola, T., Mercer, A. A., Savory, L. J., Fleming, S. B., Caesar, C., Vitali, A., Makinen, T., Alitalo, K. and Stacker, S. A. (1999). Vascular endothelial growth factor (VEGF)-like protein from *orf* virus NZ2 binds to VEGFR2 and neuropilin-1. *Proc. Nat. Acad. Sci. USA* **96**, 3071-3076.

Witzenbichler, B., Asahara, T., Murohara, T., Silver, M., Spyridopoulos, I., Magner, M., Principe, N., Kearney, M., Hu, J. S. and Isner, J. M. (1998). Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. *Am. J. Pathol.* **153**, 381-394.

Yamada, Y., Nezu, J., Shimane, M. and Hirata, Y. (1997). Molecular cloning of a novel vascular endothelial growth factor, VEGF-D. *Genomics* **42**, 483-488.

Zachary, I. (1998). Vascular endothelial growth factor: how it transmits its signal. *Exp. Nephrol.* **6**, 480-487.