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Review

The vascular endothelial growth factor family; proteins which guide the development of the vasculature

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Summary. The development of the vascular tree during embryogenesis involves vasculogenesis, angiogenesis and tissue-specific differentiation of endothelium which gives rise to many different vessel types. These processes are physiologically complex and are therefore difficult to study in vitro. However, the discovery of endothelial cell-specific receptors and cognate ligands has led to the generation of transgenic and knockout mouse models which have shed light on the molecular mechanisms that regulate the development of blood and lymphatic vessels during embryogenesis. Such mouse models have demonstrated that members of the vascular endothelial growth factor (VEGF) family of proteins and the VEGF receptors are critical regulators of vasculogenesis, angiogenesis and endothelial cell differentiation. The availability of purified VEGF family members and of inhibitors of these growth factors may provide a means to modulate blood vessel growth for the treatment of cancer, retinopathies and diseases of ischemia.

Keywords: angiogenesis, vasculogenesis, embryogenesis, tumour development, VEGF

Vascular endothelial growth factor (VEGF), a secreted glycoprotein, was purified and cloned in the 1980s based on its activities as an inducer of vascular permeability (Senger *et al.* 1983; Connolly *et al.* 1989; Keck *et al.* 1989) and as a mitogen for endothelial cells (Ferrara & Henzel 1989; Leung *et al.* 1989). Since then it has become clear that VEGF plays a crucial role in the blood vessel growth integral to embryogenesis and tumour development. More recently, numerous proteins have been identified which are closely related in structure to VEGF and which are also thought to be involved in vascular development. These proteins constitute the VEGF family. Here we summarize what is known about the structure and biological functions of VEGF family members. It is clear that the VEGF family of vascular growth and differentiation factors, and inhibitors thereof, offer great potential for manipulation of blood vessel growth in patients.

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Blood vessel growth in the embryo and adult

The formation of blood vessels in the embryo occurs by two distinct processes, vasculogenesis or angiogenesis (for review see Risau 1997). Vasculogenesis is the *in situ* differentiation, from mesoderm, of angioblasts (endothelial cell precursors which have not formed a lumen) and association of these cells to form blood vessels (Risau & Flamme 1995). The primary vascular plexus

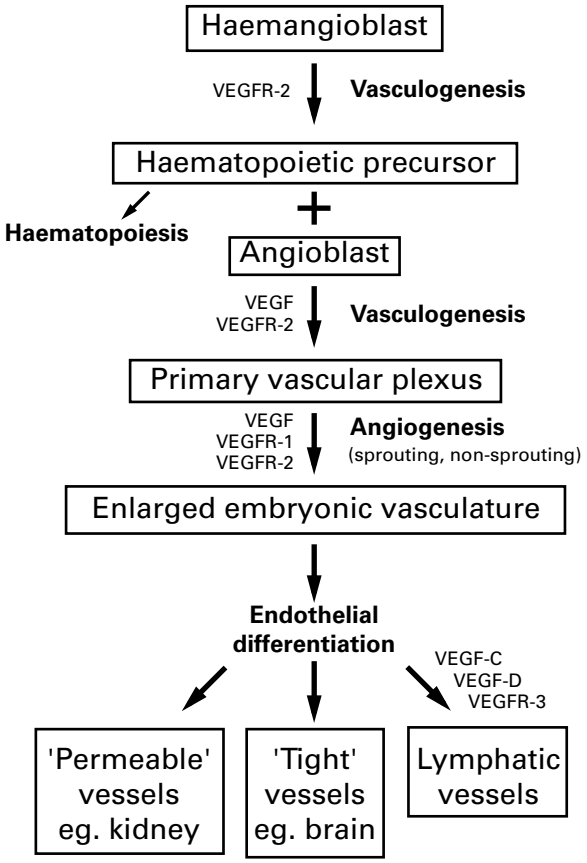

Figure 1. Schematic representation of some key processes in vascular development during embryogenesis. The order of events during embryogenesis proceeds from the top of the figure to the bottom. The steps of vasculogenesis shown are those which occur in the yolk sac, where both early haematopoiesis and endothelial cell differentiation occur side-by-side in the blood islands (Risau & Flamme 1995). The haemangioblast is a hypothesized cell type which gives rise to both haematopoietic and endothelial cell precursors (Choi *et al.* 1998). VEGF family members and receptors thought to be involved in steps of vascular development are shown. The scheme presented here is a simplification as vasculogenesis and angiogenesis occur simultaneously in some organs. The list of vessel types arising from endothelial cell differentiation shown here is far from complete.

of the early embryo is established by vasculogenesis (Figure 1). In contrast, angiogenesis is the formation of blood vessels from preexisting vessels and is primarily responsible for the development of blood vessels during later embryogenesis and adult life. Two types of angiogenesis have been described: sprouting of vessels from preexisting vessels and non-sprouting angiogenesis (intussusception) which involves splitting of vessels to generate greater numbers of vessels (Patan *et al.* 1996).

Once blood vessels have been established in the embryo, endothelial cells undergo tissue-specific changes to generate numerous types of functionally distinct vessels as organs differentiate (Risau 1995). This process gives rise to a variety of endothelial cell types with distinct morphologies and biochemical characteristics. The diversity of the endothelium is exemplified by lymphatic endothelial cells which have a discontinuous or even partially absent basement membrane (Leak 1970), the endothelial cells of the peritubular capillaries in the kidney which are highly fenestrated and brain endothelial cells which are linked by tight junctions to form the blood–brain barrier.

In the adult, angiogenesis is tightly controlled – under normal circumstances it occurs almost exclusively in the female reproductive system. However, angiogenesis can be activated in the adult in response to tissue damage and is critical in certain pathological conditions such as tumourigenesis, rheumatoid arthritis and diabetic retinopathy (for review see Folkman & Shing 1992). Recent findings suggest that angiogenesis is not the only mechanism responsible for blood vessel growth in adults, as circulating endothelial precursor cells have been isolated from human peripheral blood which can differentiate into endothelial cells and be incorporated into newly growing vessels at sites of angiogenesis (Asahara *et al.* 1997). Thus endothelial cell differentiation, a hallmark of vasculogenesis, can contribute to vessel growth in adults.

The processes essential for development of the vasculature are physiologically complex. For example, angiogenesis involves not only endothelial cell proliferation, but also degradation of the extracellular matrix, cell migration, cell-cell adhesion, lumen formation and recruitment of pericytes and smooth muscle cells (Risau 1997). Therefore blood vessel development must require endothelial cells to respond to a variety of extracellular signals that activate receptors responsible for growth and differentiation. Studies carried out over the past five years have conclusively demonstrated that members of the VEGF family of growth factors are prominent among the extracellular signalling molecules that guide vascular development (Carmeliet *et al.* 1996; Ferrara *et al.* 1996; Jeltsch *et al.* 1997).

Tumour angiogenesis

Many solid tumours are capable of inducing angiogenesis – this serves to provide the tumour with nutrients for growth and appears to be critical for the generation of metastases. The concept has emerged that tumour cells may produce factors which either induce or inhibit angiogenesis and that the onset of angiogenic activity is

determined by the balance of these factors (Folkman & Shing 1992). It is now known that VEGF is critical for supporting, and perhaps initiating, angiogenesis in many tumours (Kim *et al.* 1993; Saleh *et al.* 1996).

Numerous therapeutic approaches for treatment of cancer, which target tumour angiogenesis, are currently under development. The potential attractions of such approaches are (i) selective toxicity due to the paucity of angiogenesis in the adult; (ii) assured access of drugs to target endothelial cells; (iii) the genetic stability of the target endothelial cells which means that drug resistant variants are unlikely to arise.

The VEGF family: general structural features

The VEGF family, members of which were originally defined on the basis of similarity of primary structure to VEGF, consists of VEGF (Leung *et al.* 1989), VEGF-B (Olofsson *et al.* 1996a), VEGF-C (Joukov *et al.* 1996), VEGF-D (Achen *et al.* 1998) and placenta growth factor (PlGF) (Maglione *et al.* 1991). These glycoproteins are members of a structural superfamily of growth factors containing a cystine knot motif which also includes platelet-derived growth factor BB (PDGF-BB) and transforming growth factor β2 (TGFβ2) (McDonald & Hendrickson 1993). Crystallographic studies of numerous cystine knot growth factors, including VEGF (Muller *et al.* 1997a; Muller *et al.* 1997b), revealed that the six conserved cysteine residues of the motif contribute to a three-dimensional fold involving an unusual clustering of three cystine bridges. These bridges are intertwined in such a way as to resemble a knot. VEGF is the only member of the VEGF family for which the crystal structure has been determined. The crystal structure of VEGF is most similar to that of PDGF-BB (Muller *et al.* 1997a).

The VEGF family: specific characteristics and biological functions

VEGF

Structure. VEGF, also known as Vascular Permeability Factor (VPF), is a 34–46 kD homodimeric glycoprotein which is a highly specific mitogen for vascular endothelial cells, is capable of inducing angiogenesis (Leung *et al.* 1989), is a potent inducer of vascular permeability (Senger *et al.* 1983; Keck *et al.* 1989) and is a survival factor for newly formed blood vessels (Alon *et al.* 1995; Benjamin & Keshet 1997). VEGF monomers are linked together by two disulphide bridges to form the homodimer (Pötgens *et al.* 1994; Muller *et al.* 1997a). The known human VEGF isoforms are 121, 145, 165, 189 and 206 amino acids in length and are generated by alternative splicing of VEGF RNA derived from a single gene (Leung *et al.* 1989; Houck *et al.* 1991; Tischer *et al.* 1991; Poltorak *et al.* 1997). $VEGF_{165}$ is the predominant isoform secreted by many normal and transformed cells, however, transcripts for $VEGF_{121}$ and $VEGF_{189}$ are detected in most tissues that express the *VEGF* gene (Houck *et al.* 1991). In contrast, $VEGF_{145}$ and $VEGF_{206}$ are more restricted in expression (Houck *et al.* 1991; Poltorak *et al.* 1997). The four largest isoforms of VEGF bind to heparin and heparan sulphate proteoglycans whereas $VEGF_{121}$ does not (Houck *et al.* 1992; Poltorak *et al.* 1997). The affinities of the VEGF isoforms for heparin affect their bioavailability; $VEGF_{121}$ is secreted as a freely soluble protein, $VEGF_{165}$ is secreted, however, a proportion of the protein remains associated with the cell surface or extracellular matrix (ECM), and $VEGF_{189}$ and $VEGF_{206}$ are almost completely bound to the ECM (Houck *et al.* 1992; Park *et al.* 1993).

Function during embryogenesis. Embryonic vascular development is dependent on VEGF as the formation of vessels in mouse embryos heterozygous for a disrupted *VEGF* gene was aberrant and resulted in embryonic lethality (Carmeliet *et al.* 1996; Ferrara *et al.* 1996). VEGF deficiency impaired numerous steps of early vascular development including vasculogenesis, angiogenesis and formation of large vessels. A lethal phenotype for a heterozygous animal is highly unusual for inactivation of an autosomal gene and indicates stringent dose-dependent regulation of vascular development by VEGF.

Three high affinity receptors for VEGF have been identified, VEGFR-1 (also known as Flt1) (De Vries *et al.* 1992), VEGFR-2 (also known as Flk1 and KDR in mouse and man, respectively) (Figure 2) (Quinn *et al.* 1993) and neuropilin-1 (Soker *et al.* 1998). VEGFR-1 and VEGFR-2 are cell surface receptor tyrosine kinases which are localized on endothelial cells during embryonic development. Neuropilin-1, in addition to binding $VEGF_{165}$, is a receptor that mediates the chemorepulsive activity of the collapsin/semaphorins, a large family of transmembrane and secreted glycoproteins that function in repulsive growth cone and axon guidance in the developing embryo (He & Tessier-Lavigne 1997; Kolodkin *et al.* 1997). The mechanism of signalling by neuropilin-1 and the physiological significance of this protein as a VEGF receptor is at present unclear.

The co-ordinated patterns of expression of the genes for VEGF, VEGFR-1 and VEGFR-2 suggest that these proteins participate in paracrine systems which regulate vascular development during embryogenesis (Breier *et al.* 1992; Jakeman *et al.* 1993; Millauer *et al.* 1993;

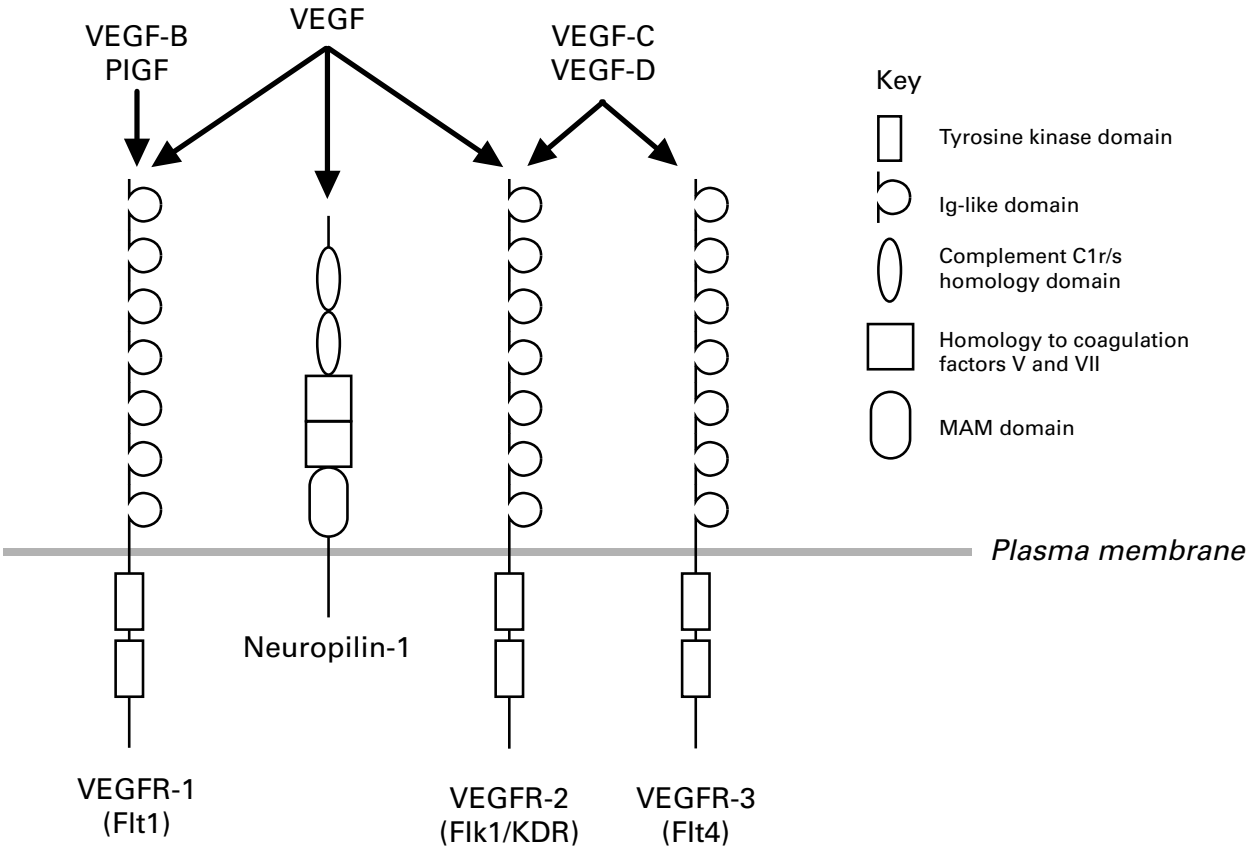

Figure 2. Interactions between VEGF family members and VEGF receptors. VEGF family members are shown at the top and arrows indicate the receptor specificities of these ligands. The similar structures of the extracellular domains of VEGFR-1, VEGFR-2 and VEGFR-3, each consisting of 7 immunoglobulin (Ig)-like domains, are apparent. The structures and proposed functions of the domains of the extracellular portion of neuropilin-1 are discussed by He & Tessier-Lavigne (1997). It is not known if neuropilin-1, a recently identified receptor for VEGF, binds to VEGF-B, PlGF, VEGF-C or VEGF-D.

Peters *et al.* 1993; Breier *et al.* 1995). Consistent with such a hypothesis were the findings that these two VEGF receptors are essential for vascular development. Embryos lacking VEGFR-2, which die at approximately embryonic day 9, have drastically reduced numbers of haematopoietic precursors and angioblasts, indicating that this receptor plays a crucial role in vasculogenesis (Shalaby *et al.* 1995). In contrast, VEGFR-1 is not required for vasculogenesis as embryos lacking this receptor produce both haematopoietic and endothelial cells, however, assembly of endothelial cells into functional blood vessels is abnormal leading to death at approximately embryonic day 8.5 (Fong *et al.* 1995). It has been proposed that VEGFR-1 signalling may regulate endothelial cell-cell or cell–matrix interactions during vascular development (Fong *et al.* 1995).

Interestingly, the delayed endothelial cell differentiation which results from VEGF deficiency was not as extreme as the aborted endothelial cell differentiation resulting from VEGFR-2 deficiency. This observation suggests that other ligands for VEGFR-2 may be essential for normal vasculogenesis. Alternative VEGFR-2 ligands are discussed in following sections.

Role in tumour formation. VEGF is an inducer of tumour angiogenesis (Kim *et al.* 1993; Saleh *et al.* 1996) and is thought to be crucial for the angiogenesis which supports the female reproductive system (Ferrara *et al.* 1998). *VEGF* gene expression is upregulated within solid tumours, in cells adjacent to necrotic regions, probably in response to hypoxia (Shweiki *et al.* 1992; Plate *et al.* 1993a, b). Secretion of VEGF in central, hypoxic regions of a tumour establishes a concentration gradient of this mitogen across the tumour which serves to attract the growth of blood vessels. Blood vessels in the vicinity of the tumour can respond to VEGF because tumours

induce expression of VEGFR-1 and VEGFR-2 in the endothelial cells of nearby vessels (Plate *et al.* 1993a). The paracrine system involving VEGF and its receptors has been blocked in tumour models by use of neutralizing VEGF antibodies (Kim *et al.* 1993), overexpression of a dominant negative mutant of VEGFR-2 to block receptor activation (Millauer *et al.* 1994; 1996) and expression of VEGF antisense RNA in tumour cells to block VEGF synthesis (Saleh *et al.* 1996). In each case, vascularization of most tumours tested was severely impaired leading to drastic reductions in the rate of tumour growth. However, some tumours did not respond to approaches designed to block the VEGF/VEGFR-2 system, suggesting that these tumours utilize other angiogenic factors and/or receptors to induce angiogenesis (Millauer *et al.* 1996). Clinical trials are currently underway to test numerous compounds which block VEGF action as antitumour agents.

The induction of *VEGF* gene expression by hypoxia in tumour cells involves both an increase in the rate of gene transcription, mediated by the transcription factor hypoxia-inducible factor-1 (Forsythe *et al.* 1996), and enhancement of the stability of VEGF mRNA (Ikeda *et al.* 1995). These mechanisms may serve to stimulate angiogenesis during embryogenesis as well as tumour development. Interestingly, VEGF mRNA is stabilized in the absence of hypoxia by inactivation of the von Hippel-Lindau protein (pVHL), a tumour surpressor protein (Gnarra *et al.* 1996; Iliopoulos *et al.* 1996). Inactivation of pVHL can lead to formation of haemangioblastomas.

Therapeutic angiogenesis. The capacity of VEGF to induce angiogenesis suggests that this protein could be used to augment collateral vessel formation as an alternative to reconstructive surgery for treatment of disorders involving inadequate tissue perfusion. This notion is supported by the finding that treatment with VEGF_{165} or DNA encoding VEGF_{165} augmented collateral vessel formation in a rabbit model of acute limb ischemia (Takeshita *et al.* 1994; Tsurumi *et al.* 1997).

PlGF

PlGF is a disulphide-linked, homodimeric glycoprotein which exhibits approximately 46% identity in amino acid sequence to VEGF (Maglione *et al.* 1991), binds and activates VEGFR-1 but not VEGFR-2 (Figure 2) (Park *et al.* 1994; Sawano *et al.* 1996) and can form heterodimers with VEGF (DiSalvo *et al.* 1995; Cao *et al.* 1996a). Three isoforms of PlGF have been characterized (Maglione *et al.* 1993; Cao *et al.* 1997), only one of which, PlGF-2, binds to heparin (Hauser & Weich 1993; Park *et al.* 1994; Cao *et al.* 1997). The multiple forms of PlGF are generated by alternative splicing of PlGF RNA derived from a single gene (Maglione *et al.* 1993; Cao *et al.* 1997). The *PlGF* gene is predominantly expressed in placenta (Maglione *et al.* 1993). PlGF homodimers are generally thought to be very poorly mitogenic for endothelial cells *in vitro* and extremely weak inducers of angiogenesis and vascular permeability in comparison to VEGF (Park *et al.* 1994; DiSalvo *et al.* 1995; Cao *et al.* 1996b; Kurz *et al.* 1998). However, PlGF-1 homodimers have been described as strongly angiogenic and mitogenic for endothelial cells in one report (Ziche *et al.* 1997).

In addition to forming homodimers, PlGF can form heterodimers with VEGF which are angiogenic and mitogenic for endothelial cells, although not as potently so as VEGF homodimers (DiSalvo *et al.* 1995; Cao *et al.* 1996a, b). Synthesis of VEGF/PlGF heterodimers *in vivo* would require colocalization of expression of the *VEGF* and *PlGF* genes. Such colocalization occurs in the trophoblastic giant cells associated with the parietal yolk sac at early stages of embryogenesis (Achen *et al.* 1997). Despite characterization of PlGF bioactivities and patterns of gene expression, the biological function of this VEGF family member is still unclear. Analysis of mutant mice deficient in PlGF may be required to define PlGF function.

VEGF-B

VEGF-B, also known as VEGF-related factor (VRF), is approximately 44% identical in amino acid sequence to VEGF, binds to VEGFR-1 (Dr B. Olofsson *et al.* unpublished observation) (Figure 2), forms disulphide-linked homodimers and exists as two isoforms consisting of 167 and 186 amino acids which arise due to alternative RNA splicing (Grimmond *et al.* 1996; Olofsson *et al.* 1996a, b). These isoforms differ in sequence only in the C-terminal region. VEGF-B_{167} binds to heparin and remains predominantly cell-associated whereas VEGF-B_{186} is freely secreted from cells (Olofsson *et al.* 1996a, b). It has been reported that VEGF-B_{167} is mitogenic for endothelial cells *in vitro* (Olofsson *et al.* 1996a).

The *VEGF-B* gene is strongly expressed in the developing heart during embryogenesis and in adult cardiac and skeletal muscle (Grimmond *et al.* 1996; Olofsson *et al.* 1996a, b). The pattern of *VEGF-B* gene expression overlaps with that for *VEGF* gene expression in numerous tissues, which is noteworthy because both isoforms of VEGF-B can form heterodimers with VEGF (Olofsson *et al.* 1996a, b). *VEGF-B* gene expression is

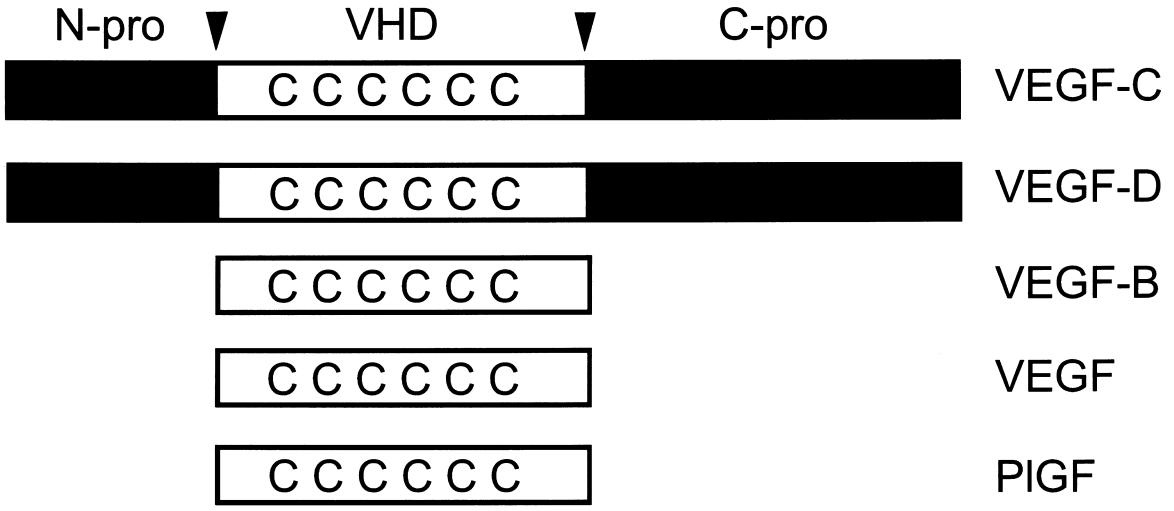

Figure 3. Schematic alignment of the primary structures of VEGF family members. VHD denotes the VEGF homology domain. N- and C-terminal regions of VEGF-C and VEGF-D, which are proteolytically cleaved from the VEGF homology domain, are designated N-pro and C-pro, respectively. Arrowheads denote the sites of proteolytic cleavage in VEGF-C and VEGF-D. Conserved cysteine residues in the VEGF homology domain, which constitute the cystine knot motif, are marked C. The positions of these residues are not shown accurately. The shortest isoforms of VEGF-B, VEGF and PlGF, which each consist essentially of a VEGF homology domain, are depicted here. Longer isoforms of each of these proteins exist which have extensions near their C-termini.

not induced by hypoxia (Enholm *et al.* 1997). It has been proposed that VEGF-B may play a role in regulating the vascularization of adult and embryonic tissues, in particular of muscle (Olofsson *et al.* 1996a).

VEGF-C

VEGF-C, also known as VEGF-related protein (VRP), was originally reported as a ligand for the tyrosine kinase VEGFR-3 (Flt4) (Joukov *et al.* 1996; Lee *et al.* 1996), a receptor similar in domain structure to VEGFR-1 and VEGFR-2 (Galland *et al.* 1993), but which does not bind VEGF or PlGF (Figure 2) (Pajusola *et al.* 1994; Lee *et al.* 1996). The amino acid sequence of VEGF-C has a central region, designated the VEGF homology domain, which is related to other members of the VEGF family, contains the cystine knot motif and exhibits approximately 30% identity to VEGF. In addition, the VEGF-C sequence has N-terminal and C-terminal extensions which are not present in VEGF, PlGF or VEGF-B (Figure 3) (Joukov *et al.* 1996; Lee *et al.* 1996). VEGF-C induces vascular permeability and is mitogenic for endothelial cells *in vitro*, although less potently so than VEGF (Joukov *et al.* 1997). *VEGF-C* gene expression is induced by numerous proinflammatory cytokines, but not by hypoxia (Enholm *et al.* 1997; Ristimaki *et al.* 1998).

The VEGF-C receptor VEGFR-3 is expressed in endothelial cell precursors in day 8.5 mouse embryos and later in development is expressed in venous and lymphatic endothelium (Kaipainen *et al.* 1995). The pattern of *VEGF-C* gene expression in relation to that for the *VEGFR-3* gene during the sprouting of the lymphatic endothelium suggests that these molecules constitute a paracrine system which regulates angiogenesis of the lymphatic vasculature during embryonic development (Kukk *et al.* 1996). This hypothesis was supported by the findings that VEGF-C is lymphangiogenic in the avian chorioallantoic membrane (CAM) model (Oh *et al.* 1997) and that overexpression of VEGF-C in the skin of transgenic mice caused lymphatic, but not vascular, endothelial proliferation and vessel enlargement (Jeltsch *et al.* 1997). The observations that VEGF-C is lymphangiogenic and that *VEGF-C* gene expression is induced by proinflammatory cytokines suggests that such cytokines can modulate lymphatic vessel growth via VEGF-C and thereby regulate the composition and pressure of interstitial fluid and facilitate lymphocyte trafficking (Ristimaki *et al.* 1998).

VEGF-C is also a ligand for VEGFR-2 (Figure 2) (Joukov *et al.* 1996), but the functional significance of this potential interaction *in vivo* is unknown. VEGF-C at high concentrations induces a very mild angiogenic response in the CAM, which may be mediated by VEGFR-2, however, the lymphangiogenic response to VEGF-C is far more striking (Oh *et al.* 1997). A role for

VEGF-C in vasculogenesis in the early embryo has been proposed based on the hypothesis that VEGF-C may be responsible for inducing the VEGFR-2-mediated endothelial cell differentiation which occurs in VEGF-deficient mice (Eichmann *et al.* 1998). VEGF-C does not bind to VEGFR-1 (Lee *et al.* 1996; Joukov *et al.* 1997).

The biosynthesis of VEGF-C involves proteolytic processing that gives rise to a mature, secreted protein which essentially consists of the VEGF-homology domain (Figure 3) (Joukov *et al.* 1997). Therefore VEGF-C is initially synthesized as a precursor protein with the N- and C-terminal amino acid extensions mentioned above being propeptides. Proteolytic processing regulates the receptor specificity of VEGF-C, as stepwise proteolytic processing generates several VEGF-C forms with increasing activity towards VEGFR-3, whereas only fully processed VEGF-C activates VEGFR-2 (Joukov *et al.* 1997). VEGF-C forms mostly noncovalent homodimers in contrast to VEGF, PlGF and VEGF-B which form disulphide-linked dimers (Joukov *et al.* 1997). This finding was surprising given that the two cysteine residues of VEGF, which are involved in the intersubunit disulphide bonds, are conserved in VEGF-C (Joukov *et al.* 1997; Muller *et al.* 1997b).

VEGF-D

VEGF-D was first reported as a 'c-fos-induced growth factor' (FIGF) (Orlandini *et al.* 1996), but was subsequently designated VEGF-D based on the functional characteristics of the protein (Achen *et al.* 1998). VEGF-D is closely related to VEGF-C in primary structure, having a central VEGF homology domain and long N-and C-terminal extensions which are not found in other VEGF family members (Figure 3). VEGF-D and VEGF-C share 31% amino acid identity. The VEGF homology domain of VEGF-D is more similar to that of VEGF-C (61% identity in amino acid sequence) than to the other VEGF family members. Intriguingly, the C-terminal region of VEGF-D is rich in cysteine residues, many of which are located such that they resemble the spacing of the repeat units ($CysX_{10}CysXCysXCys$) which are found in the Balbiani ring 3 protein (BR3P), a major cysteine-rich protein synthesized in the larval salivary glands of the midge *Chironomus tentans* (Dignam & Case 1990; Achen *et al.* 1998). VEGF-D may interact with membrane-bound proteins via the cysteine residues as such intermolecular interactions have been proposed for BR3P (Paulsson *et al.* 1990). BR3P-like cysteine repeats are also found in the C-terminal region of VEGF-C (Joukov *et al.* 1996; Lee *et al.* 1996).

VEGF-D exhibits similar receptor-binding specificities to VEGF-C, as it binds to and activates both VEGFR-2 and VEGFR-3 (Figure 2) (Achen *et al.* 1998). As is the case for VEGF-C, the capacity of VEGF-D to bind to these receptors is associated with the VEGF homology domain. The biosynthesis of VEGF-D involves similar proteolytic processing to that which generates mature VEGF-C (Stacker and Achen, manuscript in preparation). The similarities in structure, processing and receptor specificities between VEGF-C and VEGF-D demonstrate the existence of a subfamily of the vascular endothelial growth factors which has VEGF-C and VEGF-D as founding members.

The distributions of the VEGF-D receptors VEGFR-2 and VEGFR-3 on vascular and lymphatic endothelial cells during embryonic development suggest that VEGF-D may play a role in attracting the growth of these vessels into developing tissues. The finding that VEGF-D is mitogenic for endothelial cells *in vitro* is consistent with this hypothesis. In the light of these observations, it is noteworthy that the *VEGF-D* gene is strongly expressed in the developing lung during embryogenesis (Yamada *et al.* 1997). In the adult human, VEGF-D transcripts are found in many tissues but are most abundant in lung and heart (Yamada *et al.* 1997; Achen *et al.* 1998).

The expression of the gene for VEGF-D is induced by the transcription factor c-fos (Orlandini *et al.* 1996). Constitutive, ubiquitous expression of c-fos in transgenic mice induced formation of osteosarcomas (Grigoriadis *et al.* 1995) and tumours generated from c-fos-deficient cells failed to undergo malignant progression (Saez *et al.* 1995). Such experiments have indicated an essential role for c-fos in malignant tumour progression. It will be important to determine whether high levels of *VEGF-D* gene expression occur in tumours that overexpress c-fos and whether or not VEGF-D can contribute to the malignant tumour phenotype by promoting vascular and/or lymphatic angiogenesis.

Viral VEGFs

VEGF-like molecules have been described from the orf virus, a member of the Poxvirus family which infects sheep, goats and humans (Lyttle *et al.* 1994). Infection with orf virus causes a pustular dermitis which is characterized by capillary proliferation, dilation and swelling of the dermus (Groves *et al.* 1991). Two forms of VEGF-like molecules have been identified from orf viruses, OV-VEGF2 and OV-VEGF7, which are most closely related in primary structure to VEGF. OV-VEGF2 and OV-VEGF7 exhibit 29% and 23% amino

acid identity with human VEGF_{121}, respectively. These proteins possess a VEGF homology domain in which all six cysteine residues of the cystine-knot motif are conserved.

The pathology of orf virus infection may well be explained by the activity of the VEGF-like molecules, as the infection appears to involve both induction of endothelial cell proliferation and enhancement of vascular permeability, well-characterized activities of VEGF. This hypothesis is supported by the finding that lesions resulting from infection by orf virus mutants in which the VEGF-like gene has been deleted are substantially less vascularised than are lesions associated with wild type orf virus (Drs L. Savory, S. B. Fleming, D. J. Lyttle & A. A. Mercer, personal communication). In addition, it has been shown that the VEGF-like proteins activate at least one of the VEGF receptors (Drs S. A. Stacker, L. Savory, S. B. Fleming & A. A. Mercer, unpublished observation).

Conclusion

Over the past 20 years many of the molecular mechanisms which direct the development of the haematopoietic system have been characterized (Metcalf 1989). This work has led to a deep understanding of how the diversity of blood cell lineages is generated. We are beginning to see a similar situation emerge for the development of the endothelium as key regulators of vasculogenesis, angiogenesis and lymphangiogenesis are identified. Members of the VEGF family are prominent among these regulators. Much work remains to be done, in particular defining the differences between embryonic and adult angiogenesis and determining the mechanisms which guide the formation of large vessels as opposed to microvessels and the differentiation of tissue-specific forms of endothelial cells. Nevertheless, our current knowledge of the biological functions of VEGF family members may be sufficient to allow us to manipulate the growth of blood vessels in tissue to the benefit of patients in the clinic. To this end, VEGF family members and inhibitors thereof are now in phase I/II clinical trials.

The capacity to inhibit blood vessel growth in the clinic will be of importance for the treatment of cancer, rheumatoid disease and retinopathies. Stimulation of blood vessel growth will be useful for treatment of myocardial and lower limb ischemia and will facilitate wound healing, skin grafting and tissue engineering. The emerging understanding of the molecular mechanisms which regulate development of the vascular tree will have a major impact on modern medicine.

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