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Protein family review

The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease
David IR Holmes*[†] and Ian Zachary*

Addresses: *BHF Laboratories and The Rayne Institute, Department of Medicine, University College London, 5 University Street, London WC1E 6JJ, UK. [†]Ark Therapeutics Ltd, 1 Fitzroy Mews, London W1T 6DE, UK.

Correspondence: Ian Zachary. E-mail: I.Zachary@ucl.ac.uk

Summary

Vascular endothelial growth factors (VEGFs) are a family of secreted polypeptides with a highly conserved receptor-binding cystine-knot structure similar to that of the platelet-derived growth factors. VEGF-A, the founding member of the family, is highly conserved between animals as evolutionarily distant as fish and mammals. In vertebrates, VEGFs act through a family of cognate receptor tyrosine kinases in endothelial cells to stimulate blood-vessel formation. VEGF-A has important roles in mammalian vascular development and in diseases involving abnormal growth of blood vessels; other VEGFs are also involved in the development of lymphatic vessels and disease-related angiogenesis. Invertebrate homologs of VEGFs and VEGF receptors have been identified in fly, nematode and jellyfish, where they function in developmental cell migration and neurogenesis. The existence of VEGF-like molecules and their receptors in simple invertebrates without a vascular system indicates that this family of growth factors emerged at a very early stage in the evolution of multicellular organisms to mediate primordial developmental functions.

The formation of a vascular system is a prerequisite for vertebrate embryogenesis and involves two fundamental processes: vasculogenesis, defined as the differentiation of endothelial cell progenitors and their assembly into the primary capillary plexus, and angiogenesis, the sprouting of new capillaries from pre-existing vessels [1]. In the adult, angiogenesis is also essential during pregnancy and in tissue growth and repair, and is a key underlying process in the pathogenesis of several major human diseases, including cancer. Since its discovery in 1983 [2] and the subsequent cloning of the gene in 1989 [3,4], vascular endothelial growth factor (VEGF-A, also called VEGF or vascular permeability factor) has emerged as the single most important regulator of blood vessel formation in health and disease; it is essential for embryonic vasculogenesis and angiogenesis, and is a key mediator of neovascularization in cancer and other diseases [1]. VEGF-A is the prototypical member of a family of related growth factors that includes placental growth factor (PLGF), VEGF-B, VEGF-C, and VEGF-D (also

known as c-Fos-induced growth factor, FIGF), and the viral VEGF-Es encoded by strains *D1701*, *NZ2* and *NZ7* of the parapoxvirus *Orf* (which causes pustular dermatitis) [5,6]. The biological functions of the VEGFs are mediated by a family of cognate protein tyrosine kinase receptors (VEGFRs) [7-9]. VEGF-A binds to VEGFR2 (also called KDR/Flk-1) and VEGFR1 (Flt-1); VEGF-C and VEGF-D bind VEGFR2 and VEGFR3 (Flt4); PLGF and VEGF-B bind only to VEGFR1; and VEGF-E binds only to VEGFR2. In addition, certain VEGF family isoforms bind to non-tyrosine kinase receptors called neuropilins (NRPs) [10,11].

Gene organization and evolutionary history
Evolution
VEGFs belong to the VEGF/PDGF (platelet-derived growth factor) group of the cystine-knot superfamily of hormones and extracellular signaling molecules [12], which are all characterized by the presence of eight conserved cysteine

residues forming the typical cystine-knot structure (named after cystine, a dimer of two cysteines linked by a disulfide bond). The VEGF/PDGF group is evolutionarily related to other groups within the cystine-knot superfamily, notably the glycoprotein hormone and mucin-like protein families and, more distantly, the transforming growth factor-β (TGF-β) family. The absence of any of these proteins in unicellular eukaryotes such as yeast suggests that the cystine-knot structure evolved to perform hormonal and extracellular-signaling functions in multicellular organisms with tissue-level organization.

The known members of the human VEGF family are shown in Table 1. VEGFs have been found in all vertebrate species so far examined and are highly conserved between species. VEGF-A has been found in teleost fish (the zebrafish *Danio*

rerio and the pufferfish *Fugu rubripes*), frogs (*Xenopus laevis*), birds (*Gallus gallus*), and mammals (Table 1). The sequence and genomic organization of the vertebrate *VEGF-A* genes is highly conserved between teleost fish and mammals, even though separation of these two groups from their common ancestor occurred around 450 million years ago: pufferfish VEGF-A shows 68% and 69.7% amino-acid identity with human and mouse VEGF-A, respectively [13].

VEGF-like proteins emerged relatively early in the evolution of multicellular animal life, as indicated by their presence in several invertebrate species. Invertebrate VEGF/VEGFR systems have been identified in fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*) and, most recently, jellyfish (*Podocoryne carnea*). *Drosophila* has three PDGF/VEGF-like factors (PVFs), which act through a

Table 1

The human VEGF family and related proteins from *Drosophila* and *Orf* virus

Species (strain) and gene name	Number of exons	Chromosomal location*	Accession number†	References	Homologs‡ found in other species
Human *VEGF-A*	8	6p12	NM_003376	[59]	*Mus musculus*
					Rattus norvegicus
					Sus scrofa
					Bos taurus
					Canis familiaris
					Gallus gallus
					Xenopus laevis
					Danio rerio
					Fugu rubripes
Human *VEGF-B*	7	11q13	NM_003377	[23]	*M. musculus*
					R. norvegicus
					B. taurus
Human *VEGF-C*	7	4q34.1-q34.3	NM_005429	[60]	*M. musculus*
					R. norvegicus
					B. taurus
					D. rerio
Human *VEGF-D*	7	Xp22.31	NM_004469	[61]	*M. musculus*
					R. norvegicus
					B. taurus
Human *PLGF*	7	14q24-q31	NM_002632	[22]	*M. musculus*
					R. norvegicus
					B. taurus
Orf virus (D1701) *VEGF-E*	-	-	AF106020		-
Orf virus (NZ2) *VEGF-E*	-	-	S67520		-
Orf virus (NZ7) *VEGF-E*	-	-	S67522		-
D. melanogaster PVF1	6	X 17E1-17E6	NM_078683	[14-16]	*Caenorhabditis elegans*§
					Podocoryne carnea¶
D. melanogaster PVF2	5	2L 27E1	NM_078775	[16]	-
D. melanogaster PVF3	6	2L 27E1-27E2	NM_078776	[16]	-

*Chromosome locations of human and *Drosophila* genes are from Entrez Gene and FlyBase. †Accession numbers are from RefSeq and GenBank.
‡Homolog data are from HomoloGene, Entrez Gene and [13]. §Putative homolog identified by survey of *C. elegans* genome [17]. ¶Possible homolog [18].

single receptor, PVR [14-16]. In *C. elegans*, four VEGFRs, VERs (vascular endothelial growth factor receptor related) 1, 2, 3 and 4, have been identified [17]. Definitive identification of a VER ligand is awaited, although a putative homolog of *Drosophila* PVF1 was revealed by a survey of the *C. elegans* genome [17]. A single VEGF/VEGFR system has been found in *P. carnea* [18], with the VEGF being a possible homolog of *Drosophila* PVF1. In all cases, the invertebrate ligands appear to be more closely related to the VEGFs than to the PDGFs.

Alignment of the VEGF/PDGF homology domains (VHD) of VEGFs, PDGFs and PVFs, encompassing the residues making up the cystine-knot structure, reveals a high degree of regional conservation (Figure 1a). The eight cysteine residues of the cystine-knot structure are highly conserved, except in *Drosophila* PVF2, which lacks cysteine 2, and human PDGF-C and PDGF-D, which both lack cysteine 4. Phylogenetic analysis of these sequences reveals that the VEGF/PDGF family tree is essentially composed of two branches evolved from a putative common ancestor, a VEGF branch comprising VEGFs A-D, PLGF, *Orf* virus encoded VEGF-Es and *Drosophila* PVFs 1-3, and a PDGF branch, comprising PDGFs A-D (Figure 1b). Within the human VEGF family, VEGF-A is most closely related to PLGF (53% amino-acid identity within the VHD [19]). The *Orf* virus-encoded VEGF-Es segregate into two groups, with VEGF-$E_{(D1701)}$ and VEGF-$E_{(NZ2)}$ most closely related to VEGF-A and PLGF, and VEGF-$E_{(NZ7)}$ more similar to VEGF-C and VEGF-D. The *Drosophila* PVFs are more closely related to the VEGFs than the PDGFs, albeit distantly, with PVF1 most closely related to VEGF-C and VEGF-D (Figure 1b).

Gene structure and alternative splicing
The gene structures and encoded functional domains of human and *Drosophila* VEGFs are shown in Figure 2. The human VEGF genes are characterized by a highly conserved seven exon structure, with the exception of *VEGF-A*, which has eight exons. Alternative splicing of the human *VEGF-A* gene gives rise to at least six different transcripts (Table 2), encoding isoforms of the following lengths (in amino acids, excluding the signal peptide): 121 (120 in mouse), 145, 165 (164 in mouse), 183, 189 and 206 [20]. All transcripts contain exons 1-5 and 8, with diversity generated through the alternative splicing of exons 6 and 7. A hydrophobic signal sequence essential for secretion of VEGF-A is encoded within exon 1 and a small region of exon 2, and the VHD is encoded by exons 3 and 4. Human VEGF-A_{121} and VEGF-A_{165} and their equivalents in other species are the two major isoforms in mammals; VEGF-A_{121} lacks exons 6 and 7, and VEGF-A_{165} lacks exon 6 (Table 2). Exon 6 encodes a heparin-binding domain, while exons 7 and 8 encode a NRP1/heparin-binding domain; with the exception of VEGF-A_{121}, all isoforms are thought to bind the polysaccharide heparin. VEGF-A_{165} binds to NRP1 and NRP2, whereas VEGF-A_{145} binds only to NRP2 [10,11]. Recently, another splice variant of human VEGF-A was identified, VEGF-A_{165b},

which lacks exon 6 and contains an alternative exon 8 encoding a novel carboxy-terminal sequence, thereby raising the possibility of the existence of a family of sister isoforms containing this novel carboxyl terminus [21].

Human PLGF exists in four isoforms, PLGF-1 to PLGF-4, with PLGF-1 and PLGF-2 believed to be the major isoforms. The *PLGF-1* and *PLGF-2* transcripts encode isoforms (excluding signal peptide) of 131 and 152 amino acid residues, respectively. PLGF-2 is able to bind heparin and NRP1 through an exon 6 encoded heparin-binding domain [22]; PLGF-1 lacks exon 6 and is thus unable to bind heparin [19]. PLGF-3 also lacks exon 6 but additionally contains a 216-nucleotide insertion between exons 4 and 5. PLGF-4 consists of the same sequence as PLGF-3, plus the heparin-binding domain encoded by exon 6. PLGF-3 and PLGF-4 may function similarly to the larger VEGF-A isoforms, VEGF-A_{189} and VEGF-A_{206}. In mice, PLGF-2 is the only PLGF isoform identified so far.

Alternative splicing of the human *VEGF-B* gene gives rise to two transcripts, encoding isoforms (excluding signal peptide) of 167 and 186 amino acid residues, differing only in their carboxy-terminal domains [23,24]. VEGF-B_{186} transcripts contain the entire exon 6 and encode a soluble isoform. In VEGF-B_{167} transcripts, the use of an alternative splice acceptor site in exon 6 introduces a frameshift, resulting in an alternative exon 6 (referred to as exon 6b in [23]), encoding an NRP1/heparin-binding domain similar to that encoded by exons 7 and 8 in VEGF-A_{165}.

Little is known about alternative splicing of human VEGF-C and VEGF-D, although multiple isoforms of mouse VEGF-D have been described [25]. VEGF-C and VEGF-D are closely related, both structurally and functionally. Both are ligands for VEGFR2 and VEGFR3 and are initially synthesized as disulfide-linked polypeptides containing amino- and carboxy-terminal propeptide extensions not found in other VEGF proteins, flanking a central receptor-binding VHD. The unprocessed full-length forms preferentially bind VEGFR3 and have low affinity for VEGFR2, whereas the fully processed forms have increased affinity for VEGFR2 [26,27]. VEGF-C and VEGF-D lack the NRP/heparin-binding domain found in some VEGF isoforms and appear to be unable to bind NRPs.

Characteristic structural features
The crystal structure of VEGF-A_{8-109}, comprising the VHD, has been determined [28] and subsequently refined to a resolution of 1.93 Å. These studies show that VEGF-A consists of two monomers, each containing a core cystine-knot structure held together by three intrachain disulphide bonds as in the structure of PDGF; the monomers are arranged head-to-tail in a homodimer with two interchain disulphide bridges. Mutational analysis has revealed that symmetrical binding
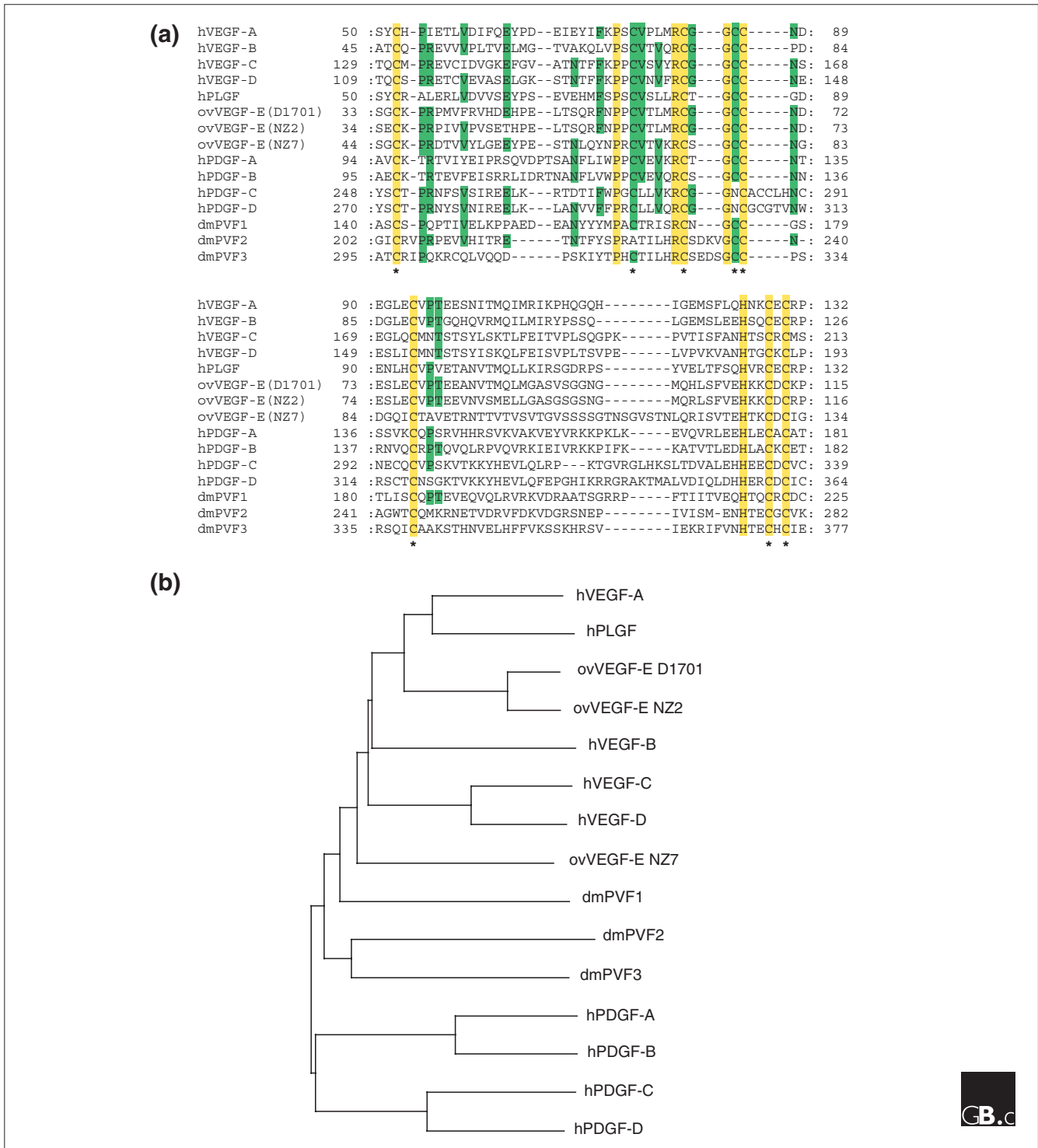
Figure 1
Comparison of human VEGFs with PDGFs and related sequences from *Drosophila* and *Orf* virus. Abbreviations: h, human; dm, *Drosophila melanogaster*; ov, *Orf* virus. **(a)** An alignment of the deduced amino-acid sequences of the VEGF/PDGF homology domain (VHD) from various human, *Drosophila* and *Orf* virus VEGFs and PGDFs. Sequence data were obtained from the GenBank and SwissProt databases; the multiple alignment was generated using MultAlin and further optimized manually. Residues that are conserved in at least 50% of the aligned sequences are shaded in green; those fully conserved are in yellow. The eight cysteine residues that constitute the cystine-knot structure [12] are denoted by asterisks below the sequences. **(b)** Predicted evolutionary relationships between human, *Drosophila* and *Orf* virus VEGFs and PDGFs. VHD sequences from (a) were aligned using ClustalW and the neighbor-joining method was used to construct a phylogenetic tree with TreeView. Branch lengths are proportional to the estimated evolutionary distance between protein sequences.
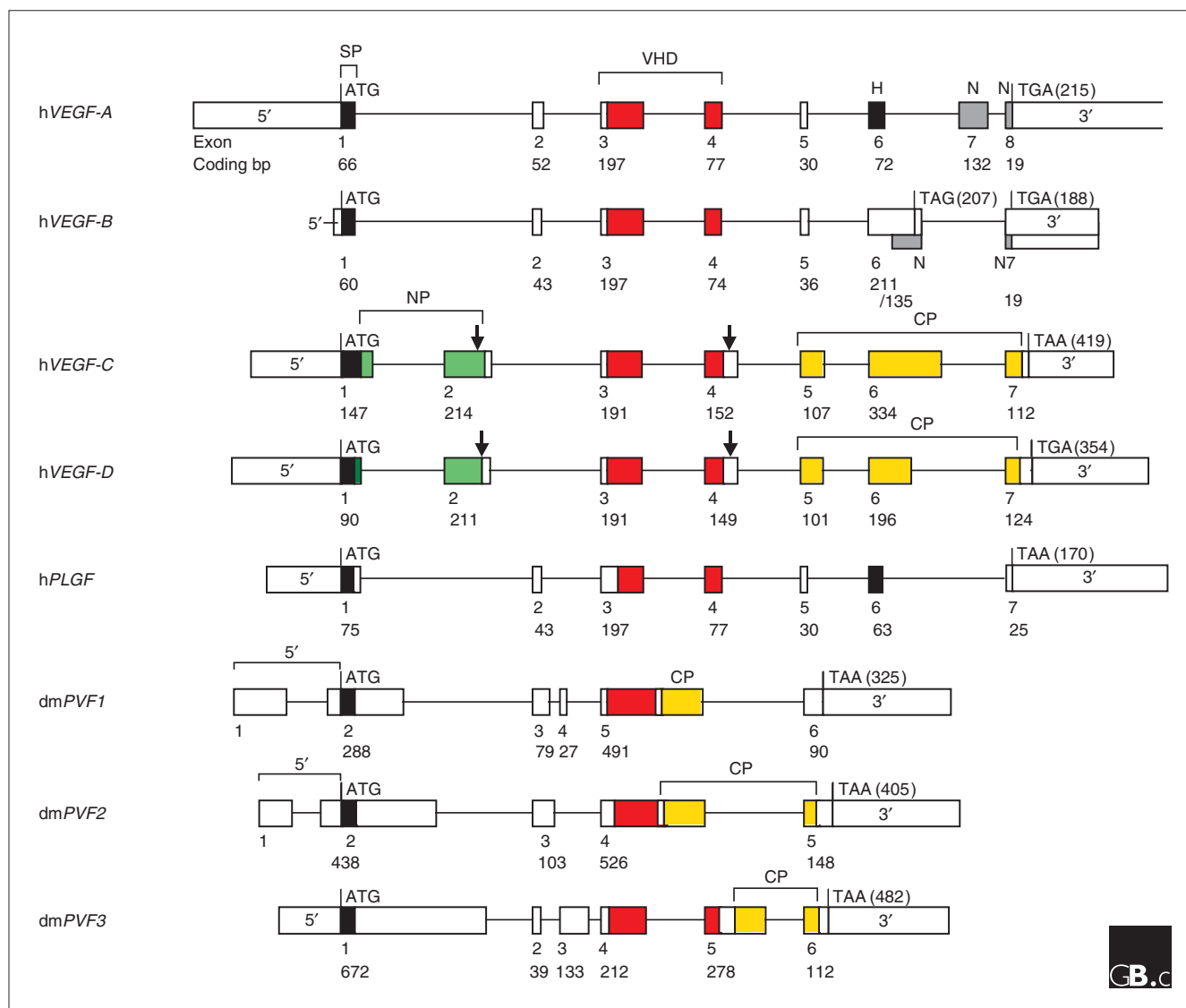
Figure 2
Gene organization and encoded functional domains of the human VEGF genes and related genes from *Drosophila*. Exons, represented by boxes, are numbered and the length of coding sequence in each is marked below in base-pairs. Start (ATG) and stop (TAA, TAG, TGA) codons are marked, and the length of each encoded unprocessed polypeptide including the signal peptide (in amino-acid residues) is indicated in parentheses. Exons are drawn to scale, except for the last exon of *hVEGF-A*, which is longer than 1 kilobase (kb). Introns, represented by horizontal lines, are not drawn to scale. Alternative exons and splicing patterns are not shown, with the exception of h*VEGF-B*, in which isoforms result from alternative splicing of exon 6 [23]. Arrows represent proteolytic cleavage sites. Abbreviations: 3', 3' untranslated region (UTR); 5', 5' UTR; CP, region encoding the carboxy-terminal propeptide domain; H, encodes the heparin-binding domain; N, encodes the NRP1/heparin-binding domain; NP, encodes the amino-terminal propeptide domain; SP, signal peptide; VHD, encodes the VEGF/PDGF homology domain. Information was compiled from published literature [14-16,22,23,59-61] and the Entrez Gene, RefSeq, GenBank and SwissProt databases.

sites for VEGFR2 are located at each pole of the homodimer and has identified key residues in each site involved in ligand-receptor interactions [28]. The crystal structure of $PLGF_{19-116}$, comprising the VHD, bound to the second immunoglobulin-like loop of VEGFR1 reveals that PLGF and VEGF-A bind to the same region of VEGFR1 in a very similar manner [29], despite only modest sequence conservation (50%) between the two ligands.

The binding of VEGFs to NRP1 appears to be mediated by two distinct domains. In VEGF-A, these correspond to the basic heparin-binding domain encoded by exon 6 and the NRP1/heparin-binding domain encoded by exons 7 and 8 [10]. The nuclear magnetic resonance (NMR) structure of the 55 carboxy-terminal residues of $VEGF-A_{165}$, containing the NRP1/heparin-binding domain encoded by exons 7 and 8, reveals this region to be composed of two subdomains,

Table 2

Isoforms of human VEGF-A

Isoform	Size (amino acids)	Coding exons*	Features
VEGF-A$_{121}$	121	1-5, 8	Secreted
VEGF-A$_{145}$	145	1-6, 8	Binds NRP2 but not NRP1; secreted
VEGF-A$_{165}$	165	1-5, 7, 8	The most abundant and biologically active isoform; secreted; binds NRP1 and NRP2
VEGF-A$_{165b}$	165	1-5, 7, alternative exon 8	Secreted, endogenous inhibitory form of VEGF-A$_{165}$
VEGF-A$_{183}$	183	1-5, short exon 6, 7, 8	Sequestered in ECM but released by cleavage
VEGF-A$_{189}$	189	1-8	Sequestered in ECM but released by cleavage
VEGF-A$_{206}$	206	1-8 plus additional exon 6-encoded sequence	Sequestered in ECM but released by cleavage

*All isoforms contain exons 1-5 and 8, except VEGF-A$_{165b}$, which contains an alternative exon 8. Abbreviations: ECM, extracellular matrix; NRP, neuropilin.

each containing two disulphide bridges and a short two-stranded antiparallel β sheet, with the carboxy-terminal sub-domain additionally containing a short α helix [30]. VEGF-B$_{167}$ also binds NRP1 via an NRP1/heparin-binding domain [31], encoded by an alternative exon 6 and part of exon 7; this has strong similarity to the domain encoded by exons 7 and 8 in VEGF-A$_{165}$ (Figure 2). PLGF-2 binds NRP1 through its exon-6-encoded basic domain, which is similar to that encoded by exon 6 of VEGF-A. The VEGF-A$_{145}$ isoform, which lacks exon 7, binds NRP2, presumably through its exon-6-encoded domain [11].

Localization and function
Cellular localization, expression patterns and regulation

The VEGFs are all secreted proteins. VEGF-A$_{121}$ and VEGF-A$_{165}$ are secreted as covalently linked homodimeric proteins, whereas the larger isoforms, VEGF-A$_{189}$ and VEGF-A$_{206}$, although believed to be secreted, are not readily diffusible and may remain sequestered in the extracellular matrix (Table 2). VEGF bioavailability may be regulated by plasmin-mediated proteolysis in the carboxy-terminal domains of the larger matrix-bound VEGF isoforms, such as VEGF-A$_{189}$, to release more diffusible, biologically active species [32]. Human VEGF-A$_{165}$, the most abundant and biologically active form, is glycosylated at Asn74 and is typically expressed as a 46 kDa homodimer of 23 kDa subunits. VEGF-A$_{121}$ has biological activity in endothelial cells, but has lower potency than VEGF-A$_{165}$. The amino- and carboxy-terminal propeptide domains of VEGF-C and VEGF-D are proteolytically cleaved, possibly by plasmin, releasing the VHD during or after secretion to generate a fully processed mature form, which forms noncovalent homodimers of approximately 21 kDa that bind VEGFR2 with greatly increased affinity [26,27].

Most information on the localization and expression of VEGFs has been derived from studies on VEGF-A. During embryogenesis in the mouse, VEGF-A can be detected from embryonic day 7 (E7) in the extra-embryonic and embryonic endoderm, and by E8.5 it is present at high levels in the trophoblast surrounding the embryo and in the embryonic myocardium, gut endoderm, embryonic mesenchyme and amniotic ectoderm. Later in development, VEGF-A is expressed in the mesenchyme and neuroectoderm of the head [33]. VEGF-A expression declines in most tissues in the weeks after birth and is relatively low in most adult organs, except in a few vascular beds, including those of the brain choroid plexus, lung alveoli, kidney glomeruli and heart. VEGF-A expression is also upregulated during specific physiological processes such as development of the endocrine corpus luteum in pregnancy, wound healing and tissue repair, and in diseases associated with neovascularization (formation of new blood vessels). VEGF-A is produced by diverse cell types, including aortic vascular smooth muscle cells, keratinocytes, macrophages and many tumor cells [34].

Oxygen tension is a key physiological regulator of *VEGF-A* gene expression [35]. The *VEGF-A* gene contains hypoxia-responsive enhancer elements (HREs) in its 5′ and 3′ UTRs [36,37], the 3′ enhancer being similar to sequences within the HRE of the gene encoding the hormone erythropoietin. Transcriptional regulation of the VEGF-A gene by hypoxia is mediated by binding of the transcription factor HIF-1 (hypoxia-inducible transcription factor 1) to the HRE. HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits, both of which are members of the basic helix-loop-helix-PAS family [38]. HIF-1α is normally very labile, but under hypoxic conditions, it accumulates because proteasomal degradation is inhibited: at normal oxygen tension, proline hydroxylation targets HIF-1α for proteasomal degradation, but is inhibited by hypoxia because of the requirement of the responsible prolyl hydroxylases for molecular dioxygen. The product of the Von Hippel-Lindau (VHL) tumor-suppressor gene is also required for proteasomal proteolysis: a genetic deficiency of this protein causes VHL disease, a condition characterized by retinal and cerebellar capillary hemangioblastomas (small, highly vascular tumors). In addition,

VEGF-A mRNA is stabilized under conditions of low oxygen tension as a result of binding of unidentified factors to its 3′ UTR. *VEGF-A* gene expression is also upregulated by a variety of growth factors and cytokines, including PDGF-BB, TGF-β, basic fibroblast growth factor (FGF-2), interleukin-1β and interleukin-6, some of which can act synergistically with hypoxia [1].

Function

All of the vertebrate VEGFs and their cognate receptors studied so far are able to regulate angiogenesis, and several have key biological roles in the formation of vascular structures either during development or in the adult. VEGFR function and signaling is reviewed extensively elsewhere [1,39,40] and is not discussed in this article. The pivotal role of VEGF-A in embryonic vascular development was demonstrated by the remarkable discovery that targeted inactivation of a single VEGF-A allele in mice caused a lethal impairment of angiogenesis, resulting in death between E11 and E12 [41,42]. The importance of larger VEGF-A isoforms, including VEGF-A$_{165}$, was confirmed by the finding that mice expressing only VEGF-A$_{120}$ - and lacking the longer heparin-binding isoforms - die within 2 weeks of birth owing to haemorrhage and ischemic cardiomyopathy (heart failure due to lack of blood supply to the heart muscle) [43]. A cardiomyocyte-specific *VEGF-A* gene knockout generated using *Cre-lox* technology results in reduced body weight and thin-walled, dilated, poorly vascularized hearts [1].

Studies involving inducible *VEGF-A* gene inactivation or administration of soluble (s) forms of the receptor Flt-1 to inhibit VEGF-A function have established that VEGF-A continues to be critically important during post-natal growth and organ development [1]. Inducible *Cre-lox*-mediated disruption of the *VEGF-A* gene in early post-natal life causes increased mortality, reduced body growth, and impaired organ development, particularly of the liver. Inhibition of VEGF-A by treatment of mice with sFlt-1 between 1 and 8 days after birth results in a more severe effect, characterized by growth arrest and lethality, but the effect of VEGF-A inhibition became less drastic if initiated at progressively later times in post-natal life. Inhibition of VEGF-A with sFlt-1 shows that VEGF-A-driven vascularization is also essential for endochondral bone formation and development of the corpus luteum during pregnancy [1].

VEGF-A-driven angiogenesis has a major role in the pathogenesis of diverse human diseases, including cancer, eye disorders and rheumatoid arthritis [44]. Recognition of the importance of VEGF-A for the development of several important classes of cancer recently culminated in the approval of Avastin, a humanized monoclonal antibody to VEGF-A, for the treatment of metastatic colorectal cancer [45]. There has also been great interest in using VEGF-A for the treatment of ischemic heart disease, where the aim is to promote blood-vessel formation and thereby provide a 'bio-

logical bypass' for diseased arteries. Despite abundant pre-clinical data suggesting that VEGF-A protein or gene therapy could be effective in treating ischemic heart disease, clinical trials have not so far yielded definitive evidence in support of this approach [1].

VEGF-A was originally identified as vascular permeability factor (VPF) as a result of its potent ability to increase vascular permeability, resulting in leakage of proteins and other molecules out of blood vessels [2,34]. The physiological significance of the permeability-increasing effect of VEGF-A remains unclear, but it is important in mediating some pathogenic consequences of VEGF-A overexpression in disease, an example being brain edema (swelling and build-up of fluid) following cerebral ischemia [1].

In addition to its major role in angiogenesis, VEGF-A probably has functions that are independent of both endothelial cells and blood-vessel formation. A growing body of evidence indicates that VEGF-A has neurotrophic and neuroprotective activities *in vitro* and *in vivo* [46,47]. It has also been implicated in amyotrophic lateral sclerosis (ALS), an incurable degenerative disorder of motor neurons. Reduced VEGF-A expression resulting from deletion of the HRE from the *VEGF-A* promoter predisposes mice to ALS-like motor-neuron degeneration, and mice can be protected against ALS by treatment with VEGF-A [48]. Furthermore, humans with particular *VEGF-A* promoter haplotypes have an increased risk of ALS associated with lower circulating levels of VEGF-A [49].

The VEGFR1-specific ligand, PLGF-1, appears to be weakly angiogenic when acting alone, but VEGF-A-PLGF heterodimers can bind to VEGFR2, are mitogenic for endothelial cells, and stimulate angiogenesis *in vivo* [50]. Though mice lacking PLGF are viable and develop normally, they have reduced angiogenesis in pathophysiological situations such as ischemia. PLGF-deficient mice also have delayed collateral artery growth following blockage of an artery, and PLGF stimulates collateral vessel growth. PLGF stimulates monocyte chemotaxis through VEGFR1, and there is increasing evidence that the biological effects of PLGF are mediated by mobilization of bone-marrow-derived haematopoietic progenitors.

A biological role for VEGF-B has not yet been clearly established. VEGF-B knockout mice are viable, healthy and fertile, but whereas Bellomo *et al.* [51] reported that VEGF-B-null mice have smaller hearts and recover more slowly from cardiac ischemia than wild-type littermates, Aase *et al.* [52] observed no effect of loss of VEGF-B on cardiac size or development and instead found a specific defect in atrial conduction in the adult. VEGF-B-deficient mice also have impaired development of pathophysiology when arthritis or hypoxic pulmonary hypertension are experimentally induced [53].

VEGF-C and its receptor, VEGFR3 (Flt-4), are strongly implicated in the formation of the lymphatic endothelium (lymphangiogenesis). Transgenic mice overexpressing VEGF-C in keratinocytes of the skin epidermis develop enlarged lymphatic vessels, while mice overexpressing VEGF-A$_{164}$ in the same location show only blood-vessel hyperplasia [54]. VEGF-C also stimulates angiogenesis in the mouse cornea [55], however, and also in rabbit models of ischemia in the hindlimb. VEGF-D is mitogenic in endothelial cells and promotes angiogenesis *in vitro* and in several models of angiogenesis *in vivo* [56]. VEGF-D also stimulates lymphangiogenesis in mice when overexpressed in skin keratinocytes and tumors [57], and it induces the survival and migration of lymphatic endothelial cells.

The viral VEGF-Es encoded by different strains of the parapoxvirus *Orf* appear to be important for viral infection and its associated pathology. Viruses of the *Orf* genus cause a contagious pustular dermatitis in sheep and goats, which is transmissible to humans, and produces lesions characterized by extensive neovascularization, vascular dilation, and epidermal proliferation. VEGF-E$_{(NZ2)}$ induces dermal vascularization and epidermal proliferation in sheep, and disruption of the *VEGF-E$_{(NZ2)}$* gene resulted in a marked decrease in the vascularization of viral lesions without impairing viral replication in the early stages of infection [58].

Drosophila PVFs and their receptor, PVR, have key roles in cell migration during two developmental processes [14-16]. Firstly, PVR is expressed by the border cells, a cluster of somatic follicle cells that migrate towards the oocyte during oogenesis; PVF1 is produced by oocytes and acts as a guidance cue for the PVR-expressing border cells during their migration [14]. Secondly, though devoid of endothelial cells or blood vessels, *Drosophila* does possess blood cells or hemocytes, and the PVF/PVR system is involved in the migration of these cells. PVR is expressed in the developing hemocytes during *Drosophila* embryogenesis, whereas PVF1, PVF2 and PVF3 are expressed along the hemocyte migratory route; inactivating mutations in either PVR or all three PVFs arrests hemocyte movement [16].

In *C. elegans*, which lacks a vascular system, the VEGFR-like VER proteins are localized to cells of neural origin, suggesting a role in neurogenesis [17]. The recently identified VEGF and VEGFR homologs in the jellyfish *P. carnea* [18] are expressed in tubular structures of the gastrovascular system and in the endoderm during development at the stage when undifferentiated cells migrate and differentiate into plate cells. In this process, the differentiating plate cells interact with matrix and smooth muscle cells, a process analogous to the interaction of endothelial and vascular smooth muscle cells in angiogenesis. As nematodes and jellyfish lack both a vascular circulatory system and blood cells, the discovery of VEGF and VEGFR-like molecules in these species suggests that these proteins performed primordial functions in tubu-

logenesis and neurogenesis at an early evolutionary stage and only later developed more specialized roles in hematopoiesis and vascular development in more complex organisms. The role of VEGFs and VEGFRs in cell migration appears to be fundamental to their biological functions in invertebrate and vertebrate species.

Frontiers

Although significant progress has been made towards elucidating the mechanisms mediating the angiogenic effects of VEGF-A, several formidable challenges lie ahead. The biological and signaling roles of the VEGF receptors, particularly VEGFR1 and neuropilin-1, have not yet been fully defined. Another key goal is the identification of the mechanisms underlying the role of VEGF-A in endothelial cell differentiation and early vascular development. An emergent area of interest is the study of VEGF and VEGFR homologs in invertebrates. A better understanding of how VEGF ligand-receptor systems function in *Drosophila* and *C. elegans* will shed light on the ancestral function of this family of molecules and may also generate novel insights into their biological roles in vertebrates. Another major goal in the future will be to clarify the distinct biological functions of different members of the VEGF family.

A key area of ongoing research will be the role of VEGFs in human disease. As recent work on ALS demonstrates [48,49], it is likely that new insights into the importance of VEGFs for disease will continue to be generated. Consequently, the scope for using anti-VEGF approaches therapeutically will grow, and the challenge will be to develop more effective and economic ways to prevent VEGF-driven pathophysiological angiogenesis or to correct VEGF deficits. The future use of VEGF therapy for cardiovascular disease remains an enticing prospect but awaits confirmatory data from clinical studies.

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