Mol Neurobiol (2011) 43:192–206
DOI 10.1007/s12035-011-8167-3

Evolution of the VEGF-Regulated Vascular Network from a Neural Guidance System

Sreenivasan Ponnambalam · Mario Alberghina

Received: 29 November 2010 / Accepted: 12 January 2011 / Published online: 28 January 2011

Abstract The vascular network is closely linked to the neural system, and an interdependence is displayed in healthy and in pathophysiological responses. How has close apposition of two such functionally different systems occurred? Here, we present a hypothesis for the evolution of the vascular network from an ancestral neural guidance system. Biological cornerstones of this hypothesis are the vascular endothelial growth factor (VEGF) protein family and cognate receptors. The primary sequences of such proteins are conserved from invertebrates, such as worms and flies that lack discernible vascular systems compared to mammals, but all these systems have sophisticated neuronal wiring involving such molecules. Ancestral VEGFs and receptors (VEGFRs) could have been used to develop and maintain the nervous system in primitive eukaryotes. During evolution, the demands of increased morphological complexity required systems for transporting molecules and cells, i.e., biological conductive tubes. We propose that the VEGF–VEGFR axis was subverted by evolution to mediate the formation of biological tubes necessary for transport of fluids, e.g., blood. Increasingly, there is evidence that aberrant VEGF-mediated responses are also linked to neuronal dysfunctions ranging from motor neuron disease, stroke, Parkinson's disease, Alzheimer's disease, ischemic brain disease, epilepsy, multiple sclerosis, and neuronal repair after injury, as well as common vascular diseases (e.g., retinal disease). Manipulation and correction of the VEGF response in different neural tissues could be an effective strategy to treat different neurological diseases.

Keywords Evolution · Endothelial · Neuronal · VEGF · Survival · Guidance · Disease · Signaling

S. Ponnambalam
Endothelial Cell Biology Unit, Institute of Molecular & Cellular Biology, LIGHT Laboratories, University of Leeds,
Clarendon Way,
Leeds LS2 9JT, UK
e-mail: s.ponnambalam@leeds.ac.uk

M. Alberghina (✉)
Department of Biochemistry and Molecular Biology,
University of Catania Medical School,
Viale Andrea Doria 6,
95125 Catania, Italy
e-mail: malber@unict.it

Introduction

Growth factors are soluble proteins that act as molecular cues to regulate animal development and its interaction with the environment. A classical model is the synthesis and secretion of soluble growth factors into the extracellular medium and subsequent binding to different high-affinity membrane-bound receptors. Such receptor–ligand complexes, through intracellular signaling, are capable of triggering a myriad of changes including metabolic pathways, gene expression, cell survival, migration, proliferation, and apoptosis. The vascular endothelial growth factor (VEGF) family and its cognate receptors define a class of proteins that regulate many aspects of vascular physiology, but they are being increasingly implicated in neural function. Here, we put forward the hypothesis that the VEGF receptor–ligand system was originally used to specify neural cell fate, wiring, and survival, but was evolved to regulate biological tube formation and to maintain the vascular network. The discovery that the nervous and vascular systems involve common growth factors suggests that they have evolved in an interconnected way.

Evolution of the VEGF System of Growth Factors and Receptors

The current idea is that the VEGF and receptor protein superfamily were evolved to mediate the formation and regulation of biological tubes (blood vessels) and used to transport fluids among different organs in a complex eukaryote system. However, increasing evidence suggests that these molecules have more than superficial links between the vascular system and the neural network; this has been extensively reviewed by Carmeliet et al. [1–4]. A key question is how the VEGF family of proteins and receptors have evolved to regulate vascular function. One approach towards formulating a conceptual framework in this understanding is to first analyze the sequence similarity of VEGF and receptor gene products among eukaryote orthologs. The VEGF gene family comprises five members, including placental growth factor (A, B, C, D, and PlGF). In addition, the parapoxvirus Orf virus genome also encodes a related protein termed VEGF-E, whereas certain vipers produce venoms containing a related protein called VEGF-F. These gene products show remarkable complexity with multiple splice variants encoded by single genes: the human VEGF-A gene alone encodes at least 6 different splice variants or protein isoforms of 121, 145, 165, 183, 189, and 206 residues in length. These VEGF-A isoforms have different degrees of pro-angiogenic activity; however, a detailed comparison has been lacking due to the need for functional recombinant protein isoforms for biochemical and cellular studies.

There is also evidence of additional complexity within the VEGF-A subfamily with the discovery of isoforms bearing anti-angiogenic activity. More recently, it has been shown by Bates et al. [5] that alternate splicing of the human VEGF-A primary RNA transcript can replace the terminal six amino acids to produce additional VEGF-A isoforms with different biochemical properties. Such isoforms have been termed VEGF-A_{xxx}b (i.e., VEGF-A_{165}b etc.). Although evidence for the existence of such isoforms has not as yet been forthcoming in other mammals including mice, recombinant human VEGF-A_{165}b can block angiogenesis in mouse models [6]. The complexity and range of human VEGF-A protein isoforms alone suggest that such isoforms may be preferentially expressed or selected during multicellular eukaryote evolution. Comparison of the human VEGF protein sequences suggest that VEGF-A is the primordial ancestor that was subverted and encoded within the Orf virus genome (Fig. 1a).

How conserved is VEGF during evolution? Examination of different eukaryote species is highly revealing (Fig. 1a). The jellyfish *Podocoryne carnea* is a member of the aquatic phylum *Cnidaria* and lacks a dedicated vascular bed, but has a gastrovascular system. It expresses VEGF and VEGF receptor (VEGFR)-related sequences [7], suggesting a role for these proteins in the formation and regulation of its biological tube system. The VEGF family is highly conserved in vertebrates including marine animals such as the zebrafish *Danio rerio*. However, in less complex multicellular eukaryotes, such as the worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, an ancestral VEGF- and platelet-derived growth factor (PDGF)-related protein called PDGF/VEGF-like factor (PVF) is found [8–10]. Interestingly, marine animals such as the Florida lancelet (amphiouxus) and jellyfish *P. carnea* contain equivalent proteins that are more closely related to the PVF orthologs from worms and flies (Fig. 1a). A common ancestor for the PDGF and VEGF families is further supported by the primary sequence similarity between these two sets of functionally distinct cytokines (Fig. 1a). Crystallographic analysis shows that VEGF and PDGF growth factors form homodimers with a "cystine knot" comprising four intrachain and interchain disulfide bonds involving eight different cysteine residues [11, 12]. Interestingly, sequence alignment of VEGF, PDGF, and fibroblast growth factor (FGF) proteins argue for a common evolutionary ancestor (Fig. 1a). However, although the VEGF, PDGF, and FGF receptor tyrosine kinases show significant sequence similarity and domain organization [13], the FGF family is structurally unique, with these growth factors existing as disulfide-linked monomers.

The VEGF, PDGF, and FGF receptor tyrosine kinases share significant similarity including an extracellular domain composed of immunoglobulin-like repeats and an intracellular split tyrosine kinase domain [14, 15]. Both VEGFR and platelet-derived growth factor receptors (PDGFR) protein family expression is largely found in the hematopoietic or vascular system and in blood vessel tissues such as the endothelium. VEGFRs were initially described as endothelial specific, but it has been clearly established that these receptors are expressed in a multitude of non-vascular cells such as the ependymal cells, neuronal stem and adult neuronal cells, retinal pigment epithelium, lens epithelial cells, skeletal muscle progenitor cells, bone cells, follicular cells and kidney podocytes. For example, VEGFR1 has been shown to regulate the epithelial–mesenchymal transition [16], proliferation of retinal progenitor cells [17] and cardiomyocyte function [18]. In contrast, VEGFR2 is more narrowly expressed in endothelial tissues, although it appears to be an increasingly important marker for stem cell differentiation and development [19–21]. Both VEGFR1 and VEGFR2 appear to be expressed in different subsets of neural cells and functionality in such tissues is less clear (see later). The VEGFR3 protein is found on both endothelial and lymphoid cells and is also increasingly detected in other tissues such as the brain where it may have a role in glial function [22].

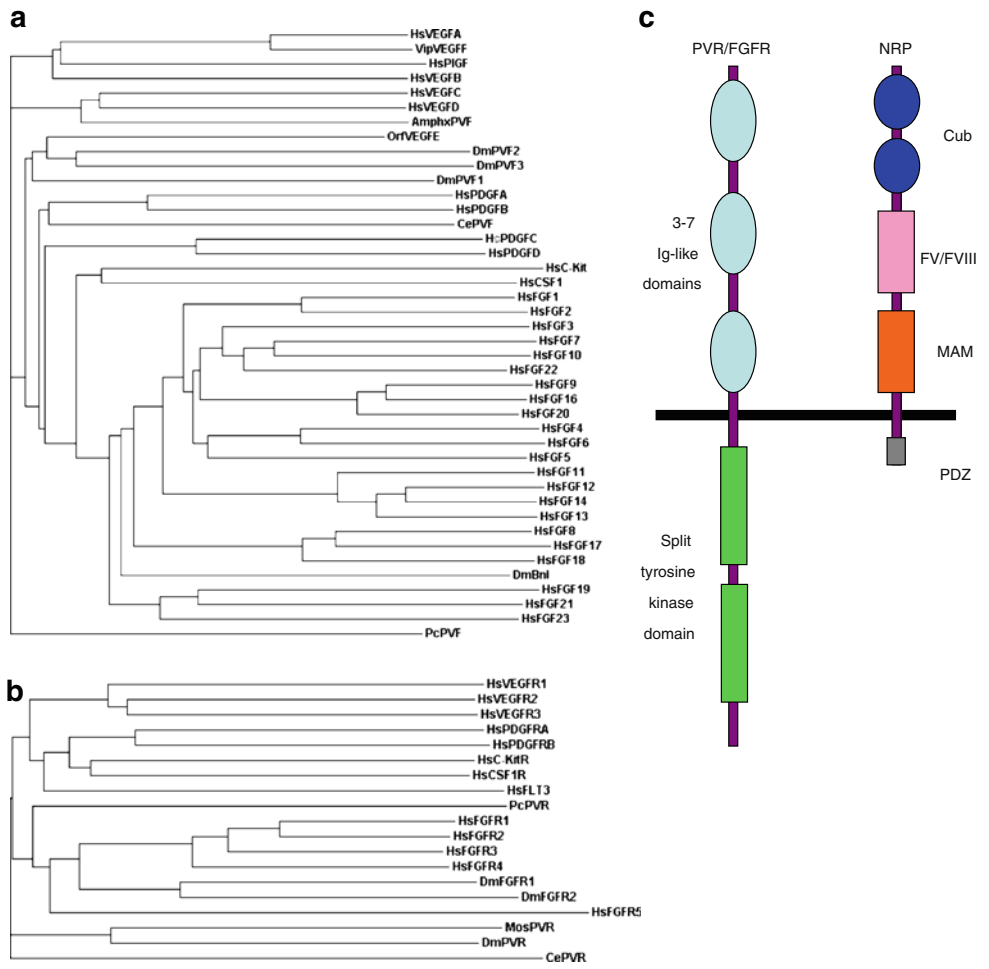

Fig. 1 Sequence similarity of the VEGF, PDGF, and FGF protein families. **a** A phylogram tree showing homology between VEGF, PDGF, and FGF family members derived using the ClustalW2 program using default parameters. Sequences used were *Homo sapiens* (*Hs*), *D. melanogaster* (*Dm*), *C. elegans* (*Ce*), jellyfish *P. carnea* (*Pc*), habu snake viper (*Vip*), Orf virus (*Orf*), and amphioxus (*Amphx*). PVF denotes PDGF–VEGF ancestral gene ortholog. The *D. melanogaster* ligand branchless (*Bnl*) shares distant similarity to FGFs. **b** A phylogram tree showing homology between VEGFR, PDGFR, and FGFR family members. Sequences compared were as previously described and the yellowfever mosquito *Aedes aegypti* (Mos). PVR denotes PDGFR–VEGF receptor ancestral gene ortholog. **c** Schematic representation of an ancestral PDGF/VEGFR/FGFR molecule. In comparison, the vertebrate neuropilins contain a Cub (or CUB) domain, coagulation factor FV/FVIII repeat, MAM domain for homodimerization and an intracellular PDZ domain for cytosolic protein–protein interactions

Fibroblast growth factor receptors (FGFR) expression is more widely distributed including skin, connective tissues, peripheral, and central nervous system. The sequence similarity between these receptors would thus argue that the VEGFR system was derived from an ancestral system resembling that found in *D. melanogaster* and *C. elegans* (Fig. 1b). Although an archetypal receptor tyrosine kinase can be used to depict the VEGFR–PDGFR–FGFR molecule, there are other VEGF receptors such as neuropilins that are structurally different, lack tyrosine kinase activity and use different VEGF binding sites (Fig. 1c). These neuropilins appear to function as co-receptors that modulate endothelial and neuronal guidance by forming a VEGF–VEGFR–neuropilin signaling complex at the plasma membrane [23]. The neuropilins also bind secreted, extracellular soluble ligands called "semaphorins" that regulate axon guidance, neuronal patterning, and synaptogenesis [24]. Evolutionary conserved transmembrane semaphorin–plexin signaling systems govern various aspects of axonal guidance in invertebrates such as *D. melanogaster* [25] and vertebrates, and epidermal morphogenesis in *C. elegans* [26]. In addition, a recent study describes that the semaphorin family member, secreted Sema3E, directly binds to VEGFR2, is expressed by neurons of the subiculum in developing mouse brain and associates with the PlexinD1/Neuropilin-1 receptor complex to control axon growth [27]. These findings implicate VEGFR2 in axonal wiring through a mechanism that is dependent on Sema3E and independent of VEGF ligands.

An analysis of neuropilin-related sequences revealed orthologs in many vertebrates including marine animals

such as zebrafish and pufferfish. However, comparisons to invertebrate genomes reveal the presence of other proteins containing modules such as the CUB domain, but it is unclear as to whether these are orthologs or functionally unrelated. However, vertebrate neuropilin displays similarity to tolloid-related proteins found in organisms such as *D. melanogaster* which also have metalloprotease-like domains, but its relevance to the PVF system is unknown.

VEGF Function in Higher Eukaryotes

The function of the VEGF family of growth factors and receptors has been reviewed extensively [15, 28, 29]. These proteins regulate many aspects of vascular physiology, but their roles in other tissues are only gradually emerging. One problem is that mouse knockout models for VEGF-A [30, 31], VEGFR1 [32], or VEGFR2 [33] display early embryonic lethality making it difficult to assess gene function in different adult tissues. However, the advent of tissue-specific gene manipulation in mouse models has enabled better understanding of VEGF and receptor function. Depletion of VEGF in skeletal muscle [34], lung [35], and retina [36] can produce a variety of pathological phenotypes associated with perturbation of vascular function in these tissues. A mechanistic framework towards understanding VEGF function is that high affinity homo- and heteromeric receptor complexes bind different VEGFs and transduce signals that regulate cellular physiology including cell survival, migration, proliferation, angiogenesis, and vasculogenesis. Such receptor–ligand complexes undergo post-translational modifications (phosphorylation, ubiquitination) that determine turnover and degradation which further influence cellular outputs [37].

There is increasing interest in understanding VEGFR and neuropilin function in neural tissues. The exact function of these membrane-bound receptors in the nervous system is unclear, but evidence suggests that their function is linked to neural guidance, replenishment and/or survival in response to soluble ligands such as VEGF. One hypothesis is that VEGF receptor–ligand complexes were part of an ancestral neural guidance system used to set up and maintain complex circuits during multicellular eukaryote development. The VEGF-A gene and its expression profile alone reveals substantial complexity with between six and 12 possible splice isoforms and both pro- and anti-angiogenic capability associated with different VEGF-A isoforms [5]. The complex range of protein isoforms generated by alternate RNA splicing of the carboxy-terminal exon 8 in the VEGF-A gene on chromosome 6 raises many interesting but unanswered questions. Certain VEGF-A splice variants produced by normal tissues can inhibit growth of microvessels or capillaries. In many human tissues (vitreous fluid, renal cortex, normal colonic epithelium, bladder smooth muscle, lung and pancreatic islets) and primary cultured cells (podocytes, retinal pigment epithelium, colonic epithelial cells), the VEGF-A_{165}b isoform generated by RNA splicing of exon 8b, constitutes more than or close to half of the total VEGF-A detected in circulatory fluids. VEGF-A_{165}b is characterized by replacement of the …CDKPRR–COOH sequence with …SLTRKR–COOH. These differences have profound implications for receptor–ligand interactions and cellular function(s). The anti-angiogenic properties of VEGF-A_{xxx}b have thus opened an avenue for targeting regulators of alternative RNA splicing (e.g., splicing factors) as potential therapeutic agents in angiogenic pathologies [38].

Increasing length of VEGF-A gene products is associated with increasing insolubility and association with the extracellular matrix, including retention of heparin-binding ability for interaction with complex polysaccharides found on both soluble and membrane-associated factors. One likelihood is that some forms of VEGF-A are better retained by the extracellular matrix whereas other (shorter) isoforms are poorly retained, but circulate freely in the extracellular medium (Fig. 2). Recent findings using primary cultured endothelial cells further support this idea with differences in VEGFR2-linked intracellular signaling depending on VEGF-A solubility or extracellular matrix-associated VEGF-A [39]. Retinal development is regulated by VEGF-dependent blood capillary formation and a balance between soluble and ECM-bound VEGF-A isoforms appears to be essential for maintenance of visual acuity [40].

One hypothesis is that a “molecular trail” of VEGF can guide endothelial cells towards their targets, including tissues and solid tumors. For example, insoluble VEGF associated with the ECM can be recognized by endothelial VEGFRs to mediate cell guidance, sprouting, and new blood vessel formation towards vascular patterning and growth in the developing organism (Fig. 2). The balance between soluble and insoluble VEGFs could thus mediate different biological outputs such as cell growth, migration, and guidance towards target tissues [1]. In support of this hypothesis, the different VEGF-A isoforms display differential solubility and association with the ECM, with longer isoforms being more insoluble, a property that is associated with the carboxy-proximal heparin-binding domain. In addition, the abundant VEGF-A_{165} isoform can itself undergo cleavage by proteases such as plasmin to generate smaller, more soluble and diffusible species such as VEGF-A_{111} [41, 42]. This has pathological significance as such processes are elevated in chronic wounds in human [43] and mouse [44] models. Mutational and functional analysis of VEGF-A suggest that such proteolysis is required for VEGF-A-stimulated cell proliferation [45]. Intriguingly, ultraviolet radiation and genotoxic drugs can stimulate production of proteolysis-resistant VEGF-A_{111} in a human–mouse xeno-

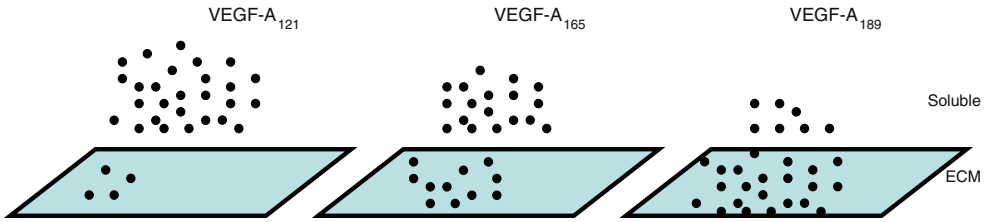

Fig. 2 Differential VEGF solubility mediates cell guidance. Cartoon depicting differential VEGF solubility exemplified by VEGF-A_{121} (highly soluble), VEGF-A_{165} (intermediate solubility), and VEGF-A_{189} (highly insoluble) resulting in different concentrations of VEGF-A found associated with the ECM. Adapted from [1]

graft tumor model [46], suggesting that VEGF-A cleavage could be a post-translational mechanism to control bioavailability for tissue remodeling.

What factor(s) could govern the balance of VEGF-A isoforms in a particular niche or environment? This is unclear, but recent work by Bates et al. [5] indicates that intracellular signaling can influence VEGF-A splice site selection. In epithelial cells, insulin-like growth factor-1 and tumor necrosis factor favored increasing VEGF-A_{xxx} families, whereas transforming growth factor β1 favored increasing VEGF-A_{xxx}b splice isoform levels involving the p38 MAPK pathway and phosphorylation of splicesome subunits [47]. More recent work in this system also implicates roles for the spliceosome protein kinase and protein kinase C in production of VEGF-A splice isoforms (VEGF-A_{165} vs. VEGF-A_{165}b) that have differing effects on angiogenesis [38]. One hypothesis is that in a vascular–neural niche, neural VEGFRs respond to such VEGF "trails" as cues to guide neuronal and/or neural cells towards targets or modulate neural function [1]. For example, there is evidence that neuropilins regulate neuronal patterning in response to VEGF-A [48].

VEGF-C and VEGF-D, which bind and activate VEGFR3 as well as VEGFR2, are mitogenic for cultured endothelial cells and occur also in the developing brain. Furthermore, VEGF-C and VEGF-D are paracrine factors essential for lymphangiogenesis [22], and have been implicated in tumor spread to the lymphatic system. In several human cancers, increased expression in primary tumors of VEGF-C is correlated with formation of peritumor lymphatics vessels, tumor cell chemotaxis, intralymphatic intravasation, and regional lymph node metastasis [49]. The balance of VEGF-A and VEGF-C can also dictate cell survival such as kidney podocytes [50]. Intriguingly, additional VEGF-C isoforms produced by mouse podocytes have recently been discovered [51] suggesting additional complexity in VEGFR2 and VEGFR3 regulated responses. However, the cellular mechanism of de novo lymphangiogenesis in human diseases is currently controversial. It could involve division of local pre-existing endothelial cells or incorporation of circulating progenitors.

The VEGFR3 gene is highly transcribed in postnatal brain both in a subset of retinal neurons and in glial precursor cells [52], whereas VEGF-C and VEGF-D are variably produced by different neuronal and glial cells [22]. In addition, VEGF-C receptor VEGFR3 is expressed in neural progenitor cells in *Xenopus laevis* and mouse embryos [53].

VEGF Function in Lower Eukaryotes

The conservation of the PVF–PVR (PDGF/VEGF receptor) system in the mosquito, fruit fly and nematode indicates an essential role in invertebrate development and physiology. How can we reconcile VEGF function in vertebrate such as mammals in comparison to invertebrates that lack a recognizable vascular system? One likelihood is that an ancestral VEGF-VEGFR complex was the PVF–PVR system (Fig. 1) which was used to promote formation of multicellular network such as neural circuits. In the nematode *C. elegans*, the PVF gene product still retains the capacity to bind to the vertebrate VEGFR1 and VEGFR2 membrane proteins [8]. However, in the fruit fly *D. melanogaster*, the PVF–PVR system acts to guide border cells towards the developing oocyte using long cellular extensions in "pathfinding" [9, 54]. This system is also required to guide embryonic hemocyte precursor cells and for cell survival [55, 56]. Intriguingly, the PVF–PVR system mediates the movement of cells needed for the formation of adult male terminalia [57], suggesting that PVF–PVR-coded molecules can mediate tissue "sculpting" needed for complex membrane and tube systems. Age-related changes in *D. melanogaster* also increases expression of soluble PVF2, and potentially modulating the formation of intestinal stem cells and progenitor cells in midgut tissues [58]. Recent work shows that PVR functionality is dependent on both the Rab11 GTPase and an E3 ubiquitin ligase, suggesting that receptor trafficking, ubiquitination and degradation regulate PVF–PVR function [59]. Other invertebrates, such as the yellow fever mosquito *Aedes aegypti* and Florida lancelet (amphiouxus), also express VEGF-related orthologs, again suggesting potential roles in cellular network formation in different tissues.

During angiogenesis, in addition to VEGF signaling, angiopoietin/Tie signaling is also involved. Lymphatic and blood vascular endothelial cells are regulated by two endothelial specific receptor tyrosine kinase systems, VEGFRs and the Tie receptors, activated by the VEGF and angiopoietin ligands, respectively. The angiopoietin/Tie system controls endothelial cell survival, vascular maturation and integrity, and quiescence. The angiopoietin family includes four ligands (Ang-1, Ang-2, and Angi-3/4) and two corresponding tyrosine kinase receptors (Tie1 and Tie2). Ang-1 and Ang-2 are specific ligands of Tie2, binding the receptor with similar affinity. Tie2 activation promotes vessel assembly and maturation by mediating survival, antiapoptotic signals for endothelial cells and regulating the recruitment of mural cells. Ang-1 (secreted by smooth cells and pericytes) acts in a paracrine, obligate, agonistic manner inducing Tie2 autophosphorylation, activates PI3-K and Akt pathway, and subsequent vessel stabilization. Ang-1 induces lymphatic vessel enlargement, sprouting and proliferation in a VEGFR-3-dependent manner. In contrast, Ang-2 is produced almost exclusively by endothelial cells (stored in Weibel–Palade bodies), functions as a switch of vascular responsiveness to exogenous stimuli (VEGF, FGF-2, hypoxia), and acts rapidly as an autocrine antagonist of Ang-1-mediated Tie2 activation signaling. Ang-2 thereby primes the vascular endothelium to exogenous cytokines and induces vascular destabilization at higher concentrations. Ang-2 is strongly expressed in the vasculature of many tumors and it has been suggested that Ang-2 may act synergistically with other cytokines such as VEGF to promote tumor-associated angiogenesis and tumor progression [60].

In contrast to the VEGF lineage, the existence of angiopoietin-like molecules and their receptors in simple invertebrates without a vascular system has not been demonstrated. Members of angiopoietin family and receptor tyrosine kinase Tie-2 may be considered a later highly conserved evolutionary achievement needed for complexity in endothelial cell migration, patterning, and maturation of vascular network [61].

Evolutionary Perspectives on Vascular System Development

In invertebrates, such as insect (avascular CNS in *D. melanogaster*) and crustacean, interaction between neurons and perineural glial cells, through septate and tight junctions, is central in establishing a functional blood–brain barrier (BBB). An evolutionary perspective suggests that glia–barrier system in lower invertebrates evolved into an endothelial barrier system in higher invertebrates beyond elasmobranch [62]. It was likely due to evolutionary selective pressure needed to preserve ionic homeostasis from any fluctuation around the synaptic active zones and to restrict substances that could damage the neuronal cells. Phylogenetically, true microcirculatory vessels appeared in some anellids (nemerteans). Invertebrates, such as cephalopod mollusc cuttlefish *Sepia officinalis*, which has a closed circulatory system, already have a BBB formed by perivascular glia cell processes in the microvessels lined with an endothelium, and venous vessels. This also reveals the existence of pericytes in arterial vessels, which may be considered a later development in BBB structure [63].

There is no phylogenetic theory about the origin of endothelial cells in vertebrates. It has been proposed that proto-endothelial cells originated from a type of specialized migratory blood cells, called amoebocytes (coelomic origin, i.e., derived from coelomic epithelium during development) which acquired an epithelial phenotype [64]. The presence of the VEGFR-like ortholog (PVR) on hemocytes from *D. melanogaster* supports this hypothesis.

An intriguing aspect of the molecular mechanisms that have been conserved during evolution is the vessel tubular-sprouting capacity of the open colonial circulatory system of ascidian *Botryllus schlosseri*, resembling that occurring during vertebrate angiogenesis. The interzooidal vessels are limited by a simple, flat epithelium, originated by epidermal extrusion, with the basal lamina lining the lumen. The regenerating structures of the extracorporeal circulatory system were found to be under the control of the VEGF pathway [65, 66].

Evolution of Primary Signaling Events in VEGF-Regulated Physiology

Based on our knowledge described above, it may be instructive to take an evolutionary glance on one part of the complex intracellular signaling events immediately downstream of the VEGFR multiprotein complexes. Interaction of VEGF with particular receptor subtypes possessing an internal tyrosine kinase domain activates different pathways, e.g., phosphoinositide 3-kinase (PI3K)/PTEN/Akt, Ras/Raf-MEK/ERK, endothelial nitric oxide synthase/nitric oxide and inositol triphosphate/calcium ion signaling events. The PTEN gene encodes a phosphatase that antagonizes PI3K signaling by removing the 3′ phosphate from phosphatidylinositol 3,4,5-trisphosphate. PI3K, c-Akt and PTEN are involved in a very ancient signaling system, and are highly conserved proteins among organisms as diverse as amoeba *Dictyostelium discoideum*, platyhelminthes (planarian *Schmidtea mediterranea;* trematode *Schistosoma mansoni*), nematodes (*C. elegans*), and vertebrates [67–73]. The PTEN gene is conserved in the invertebrate *D. melanogaster* and undergoes alternative RNA splicing [74] (Fig. 3).

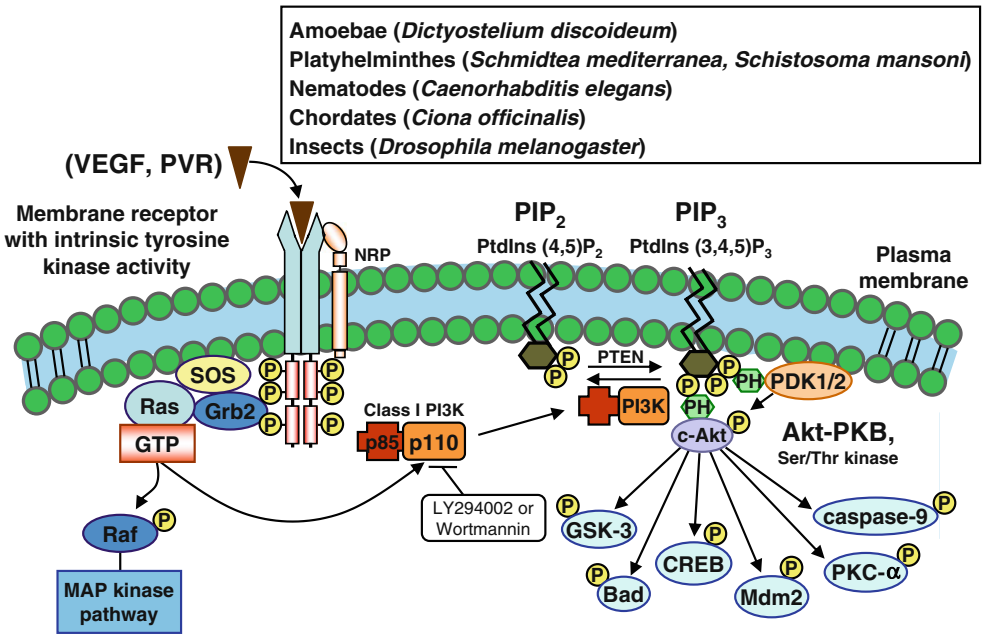

Fig. 3 Model showing the primary steps for VEGFR and PVR signaling. Growth factor binding to a membrane receptor tyrosine kinase activates signaling cascade via the MAP kinases. In parallel, the PI3K/Akt pathway can be activated by the receptor tyrosine kinase or inactivation of the tumor suppressor, PTEN. PI3K activation causes synthesis of $PI(3,4,5)P_3$, resulting in recruitment of the serine/threonine kinase c-Akt (protein kinase B) to the plasma membrane. The PDK1/2 kinase phosphorylates and activates membrane-bound c-Akt, which in turn phosphorylates multiple targets including glycogen synthase kinase 3 (GSK-3), the transcriptional activator CREB, mdm2 forming a complex with the p53 tumor suppressor, and regulators of apoptosis such as Bad or caspase-9. PI3K and PTEN encoding genes have been also found in invertebrates

Cell growth and cell divisions are two fundamental biological processes for cells in multicellular organisms. Thus, it is not surprising that molecules involved in these processes are highly conserved within eukaryotes, including plants and unicellular organisms such as yeast. In the genome of sea squirt *Ciona intestinalis*, the genes encoding components of the PI3K pathway, that have importance for cell growth, differentiation, survival, adhesion and chemoattractant gradient sensing, have been examined [75]. It was found that the genome of this invertebrate encodes all the essential constituents of the PI3K pathway. In addition, genes encoding the class IB PI3K catalytic and regulatory subunits, which had not previously been known in animals other than mammals, were found to be present in this genome. Since this ascidian is regarded as one of the most primitive extant chordates, such an analysis gives insight into how fundamentally important genes were conserved during chordate evolution [75]. Clearly, a great deal remains to be learned about how this leading signaling pathway started to function in primordial cells.

Evolution of Biological Tubes

There are only two cell types that form the lumen of tubular systems: either endothelial cells in the vascular system or epithelial cells in all other organs. The sprouting of the tracheal system in the fruit fly *D. melanogaster* results in an interconnected network of gas-filled epithelial tubes that develop during embryogenesis and functions as the main gas exchange organ in the larva. This process is driven by an FGF-like ortholog called branchless that is secreted by non-tracheal cells and whose function is inhibited by Sprouty/Notch [76]. Furthermore, larval tracheal cells respond to hypoxia by activating a program of branching and growth that is driven by hypoxia-inducible factor (HIF-1α, *sima*) dependent expression of the *breathless* gene which encodes an FGF receptor orthologue [77]. Thus, a single growth factor is reiteratively used to pattern each level of tracheal branching, and the change in branch patterning results from a switch from developmental to physiological control of its expression. This reactive respiratory (tracheal) system seems to have features in common with the mammalian circulatory system due to an angiogenesis-like, hypoxic response. A rather astonishing number of similarities between the developing tracheal system and the formation of blood vessels via angiogenesis have recently emerged. The vertebrate vasculature is first assembled from scattered endothelial precursor cells, and is then enlarged and remodeled by the sprouting, splitting, and regression of branches. Gerhardt et al. [78] have shown that VEGF-A regulates angiogenic sprouting by guiding filopo-

dial extensions from specialized endothelial cells situated at the tip of vascular sprouts. There is thus much similarity to how neural growth cones and tracheal cells navigate through the developing embryo.

A key feature in evolution is the development of biological tubes to mediate the movement of molecules and cells over relatively long distances within a multicellular organism. Within a relatively simple nematode such as *C. elegans*, there is a single long alimentary canal in addition to organs such as oviducts, ovaries, uterus, and vulva. How does the PVF–PVR system regulate tissue and organ development within this organism? That is currently unknown, but one speculation is that the PVF–PVR system may be used to regulate the neuronal network found in the adult animal. In addition, this system could be used to regulate the formation of the gut. The *D. melanogaster* ortholog is also implicated in diverse events ranging from cell migration to cell size [79].

How have endothelial-based vascular tubes evolved from simple multicellular organisms? One postulate is that a primitive PVF–PVR system used to form multicellular networks in tissues such as neurons or the gut was evolved towards forming long-range vascular tubes to enable movement of molecules and nutrients (e.g., blood) and circulatory cells (e.g., hemocytes, leukocytes). This system needed cells to be initially aligned in a linear manner, followed by accumulation and membrane fusion of intracellular vesicles and vacuoles (Fig. 4). Subsequent fusion of these large vacuoles with the endothelial plasma membrane enables formation of a central lumen linking the aligned endothelial cells. Live cell imaging studies in the zebrafish *D. rerio* has provided evidence for such a model for new blood vessel formation [80]. How does this mechanism compare to other "biological tube" networks found in multicellular organisms? In mammals, epithelial and neural tissues have been noted to also undergo regulated programs of cellular alignment such as the formation of epithelial tubes (e.g., renal tubules in the kidney) and the embryonic neural tube which is the precursor for the brain and spinal cord. One speculation is that conserved mechanisms resembling the VEGF–VEGFR system exists in these other tissues to specify epithelial or neural cells to align and promote the formation of the central lumen. This raises the question whether the VEGF–VEGFR system can be responsible for such phenomena in these other tissues. In the case of the epithelium, it has been observed that VEGFR1 is expressed in a wide range of primary and transformed epithelial cells, suggesting a functional role for this receptor in different epithelial tissues. The VEGFR1 mouse knockout, although showing that this gene is essential for vascular and mammalian development [32], does not shed any light on its potential role in other tissues. Intriguingly, an in vitro model for human colonic differentiation shows that the VEGF-A–VEGFR1 axis is required for cell survival and the epithelial–mesenchymal transition [16]. In contrast, the VEGFR2 gene product is essential for embryonic and vascular development [33], but its expression is largely restricted to cells of hematopoietic and endothelial origin.

What is the molecular mechanism underlying biological tube formation within a collection of endothelial cells? In the case of the mammalian endothelium, remodeling of actin and microtubule networks is essential for mediating membrane and vesicle movements needed for the formation of a central lumen. The endothelial $\alpha 2\beta 1$ integrin mediates interaction with extracellular matrix components such as collagen leading to activation of the Cdc42 GTPase and

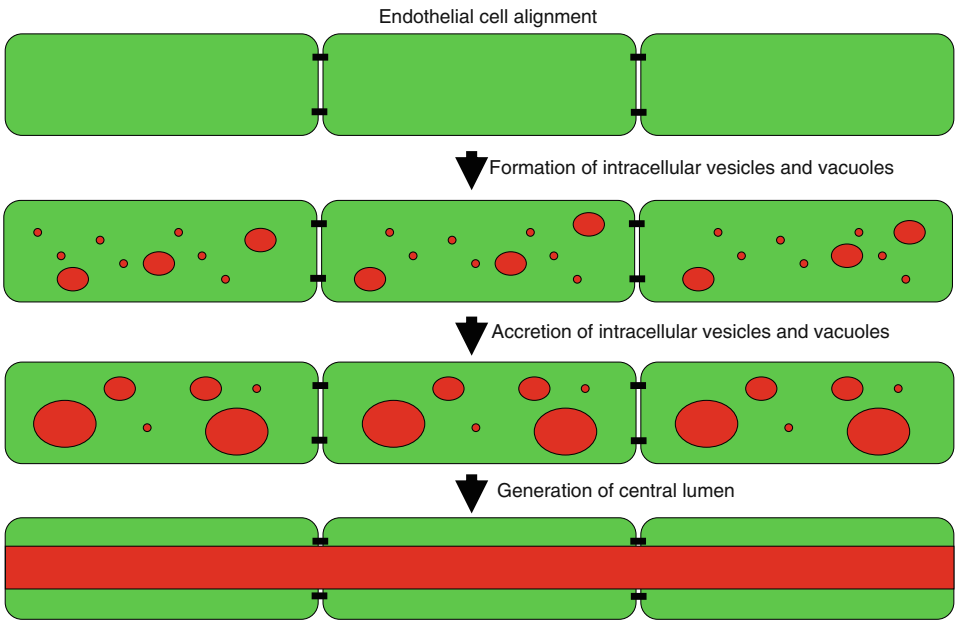

Fig. 4 A model for formation of the vascular lumen by endothelial cells (ECs). ECs become aligned end-to-end in response to a vasculogenesis and angiogenesis cues such as VEGF-A. These endothelial cells are anchored through homophilic cell–cell interactions at the plasma membrane using membrane proteins such as VE–cadherin. The increasing accumulation of endothelial vesicles and vacuoles drives fusion and formation of large intracellular vacuoles. This results in the formation of a central lumen around which subsequent newly formed endothelial cells are organized to form the endothelium. Adapted from [80]

actin remodeling; this also involves other factors such as Par6b, Par3, and junction adhesion molecules [81]. The VE-cadherin adhesion molecule also modulates angiogenesis involving Rho GTPases, Rho kinases, and myosin light chain kinase [82]. The phosphorylation of myosin-II is thus implicated in modulating actin dynamics necessary for angiogenesis and blood vessel formation.

Neural Guidance and Survival

How does VEGF-A regulate neuronal function? The majority of evidence suggests neural requirement for VEGF-A but the extent of this role is a matter for much debate. Neurons generally appear to express VEGF-A, VEGFR2, VEGFR3, and neuropilins, but lack VEGFR1 [83]. The balance between guidance molecules and angiogenic factors working through neuropilins and VEGFRs could modulate neuronal and neural progenitor cell proliferation, migration, apoptosis, or survival [84]. Increasing evidence suggests that VEGF-A-mediated activation of neuronal VEGFR2 stimulates intracellular signaling pathways that control cell survival and apoptosis [85]. One study suggests that VEGF-A is a survival factor for retinal neurons and provides critical neuroprotective functions during ischemia [86]. As VEGF-A was originally identified as a hypoxia-inducible factor that stimulates endothelial function and neovascularization, it is interesting to evaluate the effects of hypoxia in the nervous system. The nervous system is exquisitely sensitive to reduced oxygen conditions such as hypoxia which can result in impaired function and cell death. Hypoxia triggers a highly conserved cellular circuit which stimulates expression of the VEGF-A gene products that regulate many aspects of vascular physiology. A similar mechanism is activated in neural tissues in response to ischemia resulting in increased VEGF-A mRNA synthesis, expression and secretion [87].

In neurons, protection from hypoxic ischemia leading to increased VEGF-A synthesis, VEGFR2 activation and MAP kinase signaling is linked to phosphorylation and activation of a global transcriptional activator, i.e., cAMP-responsive element-binding protein (CREB). VEGF-A-stimulated VEGFR2 activation and signaling is protective against hypoxia in both hippocampal and cortical neurons with reduced cell death and increased survival [88]. In an animal spinal cord injury model, VEGF-A administration led to decreased apoptosis and improved neuronal function [89]. Thus, one hypothesis is that VEGF-A acts as an autocrine survival signal in neural tissues to protect against the damaging effects of stress conditions such as hypoxia. This becomes especially important in terminally differentiated tissues that lack capacity for self-regeneration and renewal, e.g. adult neurons.

Intriguingly, there is evidence that VEGF-A regulates neural stem cell renewal and interaction with the surrounding vascular network including the endothelium [90, 91]. In one study, VEGF-A acts as a chemoattractant that guides neural cell progenitors and involves intracellular signaling through VEGFR2 [92]. On pluripotent embryonic stem cells, VEGF-A displayed a marked ability to act as an autocrine survival factor in contrast to other growth factors including PDGF and FGF [85]. Studies on cells derived from embryonic mouse knockouts suggest that VEGF directly inhibits primitive neural stem cell survival but promotes definitive neural stem cell survival [93]. Human embryonic stem cells respond to VEGF by directly elevating expression of genes such as Sox1 and nestin that are implicated in neurogenesis [94]. It has been also suggested that cerebral microvascular endothelial cells can mediate recruitment of neural stem cells to the neurovascular niche through a VEGF-A-regulated mechanism [95, 96].

Interestingly, other neural cells, such as astrocytes and glial cells, also display VEGF-regulated responses but with subtle differences. For example, astrocytes express VEGF-A and VEGFR1 but not VEGFR2 [97]. In different neural cells, the role of VEGF-A may be to support neuroprotection and survival [98]. One likelihood is that different neural populations of progenitors and terminally differentiated cells respond to VEGF cues using different receptor combinations to modulate cell proliferation, apoptosis or survival to control both central and peripheral nervous system function. However, there must be a note of caution with more work needed to assess the requirement and role of VEGFs in neural function: one view is that VEGF-A expression is an effect rather than a cause of dysfunctional states [99].

VEGF and Neural Pathophysiology

An increasingly important aspect of the many properties associated with the VEGF is the role played by such growth factors in neural function and pathophysiology. In principle, better understanding of such phenomena raises possibilities for VEGF-linked therapy in a variety of neurological diseases. Such diseases generally appear to fall into two categories: those in which VEGF-A expression is lowered in neural tissues, and those in which VEGF-A expression is elevated in both neural cells and surrounding tissues. In both cases, perturbation in VEGF-A-stimulated signaling pathways appears to trigger significant CNS and/or PNS dysfunction, highlighting a key role for VEGF receptor–ligand complexes in regulating neural function.

A striking example of VEGF-linked pathophysiology is motor neuron disease or amylotrophic lateral sclerosis (ALS) [100]. Patients with ALS display reduced VEGF-A

levels in the brain and in circulatory fluids [101], a condition consistent with lowered responses to VEGF-A by motor neurons. The mouse VEGF-A δ/δ model, where hypoxia-stimulated VEGF-A expression is ablated, shows adult-onset motor neuron degeneration and an altered profile of gene expression [102]. Multiple factors likely contribute to this disease pathology: this is illustrated by a mouse model of ALS based on the superoxide dismutase SOD1-G93A allele where VEGF-A administration prevents motor neuron degeneration [103]. A likely mechanism to explain the effects of SOD1 alleles on ALS involves an RNA stabilization factor that binds to the 3′ non-translated region of the VEGF-A mRNA and is redistributed from the nucleus to the cytoplasm upon expression of mutant SOD1 [104]. Expression of this mutant human SOD1 in zebrafish *D. rerio* embryos also caused motor neuron death [105]. Overexpression of VEGF-A in neurons of a double-transgenic ALS mouse model [106] or a zebrafish model [105] appears to alleviate axonopathic disease symptoms. However, more evidence is still needed for conclusive proof that VEGF deficiency causes ALS [107] as there are genetic studies that shown no association between genetic polymorphisms in VEGF-A and the incidence of ALS [108].

In a rat Parkinson's disease model, the loss of dopaminergic neurons could be ameliorated by VEGF-A infusion into the striatum [109] or VEGF-A viral gene therapy [110]. Furthermore, in a rat Huntington's disease model, motor impairment was improved by VEGF-A administration into the brain striatum [111]. Intriguingly, VEGF-A dysfunction is also associated with Alzheimer's disease (AD) [4]. One possibility is that non-coding polymorphisms within the vicinity of the VEGF-A locus influence protein expression and/or alternate RNA splicing, leading to an altered expression profile of VEGF-A isoforms that modulate neural function. Low levels of serum VEGF-A are associated with AD predisposition [112], but high intrathecal VEGF-A levels can be found in AD cerebrospinal fluid [113]. The β-amyloid peptide, implicated in causing AD, can bind VEGF-A and thus modulates intracellular signaling in endothelial cells and blood-brain barrier permeability [114]. This is further supported by evidence for increased VEGF-A accumulation in plaque deposits from AD patients [115]. There is evidence for genetic polymorphisms in VEGF-A correlating with increased risk of AD [116], but there are also opposing conclusions from similar studies on different populations [117].

Cerebral ischemia such as stroke can be linked to VEGFR2 allelic variants [118]. There are elevated VEGF-A levels in the human brain after a stroke incident: this is likely to be a physiological and neuroprotective response to counter the damaging effects of hypoxia [119]. It has been noted that increased VEGF levels is associated with altered BBB permeability and temporal lobe epilepsy [120]; such phenomena may be age-related [121]. There is also increasing interest in the VEGF-B gene product which can have neuroprotective and anti-apoptotic effects in stroke models [122]. Intriguingly, the neuroprotective effects of VEGF-B appear to be mediated through VEGFR1 and by suppression of expression of pro-apoptotic regulatory factors [122]. A key switch in this pathway is likely to be the c-Akt serine/threonine protein kinase which can phosphorylate multiple targets associated with cellular homeostasis and survival [123] (see Fig. 3). There is thus a critical balance between VEGF-induced neural survival and repair and increased blood–brain barrier permeability that could trigger hemorrhage or stroke.

VEGF-A and VEGFR1 levels are elevated in lumbar motor neurons after sciatic nerve crush injury [124]. Local administration of two different molecules, VEGF-A or angiostatin, to a spinal cord injury model showed reduced cellular apoptosis consistent with improved blood vessel homeostasis and neurotrophic effects on neurons and/or glial cells [89]. In a rat spinal cord injury model, ex vivo administration of virally transduced NSCs overexpressing VEGF-A also enhanced proliferation of glial progenitors [125]. Increased neural VEGF-A levels in multiple sclerosis (MS) may be a contributory factor in myelin sheath degeneration. VEGF levels were higher in MS patients with disease or relapse compared to controls or MS patients in remission [126]. A condition resembling MS can be induced in rat models by simultaneous immunization with myelin oligodendrocyte protein and intraspinal injection of VEGF-A [127]. Increased VEGF-A may thus "prime" an autoimmune reaction resulting in myelin sheath breakdown leading to disease pathology.

Altered VEGF levels are also associated with a number of retinal diseases, including altered angiogenesis mediated by the retinal vasculature [4]. Eye tissues can encounter both hypoxia and hyperoxia conditions which markedly alter local VEGF-A levels [128]. VEGF-A regulates retinal progenitor cell proliferation and commitment towards formation of postmitotic neurons [129]. Again, VEGF-A appears to act as an autocrine survival factor for retinal neurons, Müller cells [130], and protects against ischemia [86].

In a branch retinal vein occlusion model, rapid and transient VEGF-A expression is associated with modulated potassium channels, aquaporin function and osmotic swelling in Müller cells [131]. Intriguingly, another regulatory factor is the group IV phospholipase A_2 (PLA_2) enzyme which undergoes calcium-stimulated activation to produce arachidonic acid. It has been noted that during retinal angiogenesis, neovascularization and hypoxia, this group IV PLA_2 enzyme is activated [132], whereas inhibition of the downstream cyclooxygenase-2 enzyme diminishes VEGF-A production in Müller cells in response to hypoxia [133].

One intriguing correlate between brain dysfunction and angiogenesis is cerebral cavernous malformation (CCM). This pathology, which exhibits lesion in the brain or spinal cord, also shows a cluster of enlarged or irregular capillaries which are thinner, less elastic and leaky. At least three different genes associated with this disease have been designated CCM1, CCM2, and CCM3. Genetic analysis of *D. rerio* CCM1 and CCM2 genes shows functional requirement for blood vessel development: loss of either CCM1 or CCM2 causes dilated and leaky blood vessels [134]. Functional analysis of the Rap1b GTPase, associated with CCM1 gene product in *D. rerio*, also disrupts endothelial cell–cell junctions further pointing to functional cooperativity between these two proteins to maintain blood vessel integrity in different tissues [135]. Recent studies have suggested that CCM1, Rap1b, and VE-cadherin form a multiprotein complex that regulates endothelial cell polarity and lumen formation [136].

Concluding Remarks and Future Perspectives

An emerging pattern is that different VEGFs have the capacity to bind cognate receptors expressed by cells of the PNS and CNS includes neurons, glia, astrocytes, neural progenitors, and stem cells to modulate cellular and animal physiology. In contrast to the VEGFRs, the neuropilins have capacity to bind both semaphorin and VEGF ligands to trigger intracellular signaling and neural "pathfinding", suggesting complex temporal and spatial mechanisms to ensure correct wiring and maintenance of neural circuits. Polymorphisms or altered levels in VEGFs, membrane receptors and associated signaling factors can not only affect vascular physiology but also neural function and homeostasis. It is also likely that some of these factors have unique functions that have yet to be revealed: for example, some of the VEGF splice variants could trigger neural-specific responses. Neural dysfunction such as decreased VEGF-A levels causing motor neuron death (e.g., ALS) could potentially be reversed by administration of the growth factor VEGF-A. Due to the potent effects of the most commonly used isoform (VEGF-A_{165}), it may be worthwhile evaluating different VEGF isoforms for their capacity to rescue motor neuron survival. For example, it has been reported that VEGF-B may have unique neuro-protective activity in this context [122, 137]. Alternatively, under conditions where excessive VEGF levels are a problem, e.g., MS, a different strategy may be needed. One way would be to use humanized blocking antibodies to interfere with receptor–ligand complex formation; however, side effects are again an issue with potential multiple role(s) for VEGF-A alone in vascular and non-vascular tissues. A different approach may be to use VEGFs, soluble receptor isoforms or engineered hybrids that compete for or interfere with key brain- and disease-specific pathway(s) but allow other pathways to proceed normally.

The development of VEGFR tyrosine kinase inhibitors for blocking angiogenesis in aggressive diseases such as metastatic colorectal carcinoma (e.g., Sutent) raises the possibility of using such therapies to inhibit the effects of elevated VEGF-A levels in MS patients. Finally, the use of gene therapy to express specific VEGFs, receptors or engineered proteins locally in neural tissues offers promise. One approach may be to inject viral vectors carrying specific transgenes for expression of proteins in different tissues. Alternatively, it may be better to take primary cells from the patient and generate stable transgene expression in vitro before transplantation into the appropriate neural tissue. In this context, the discovery that neural stem cells and progenitors respond to VEGFs suggests that cellular manipulation in vitro using gene therapy and/or VEGF-linked treatments could be a prelude for transplantation back into the patient to replenish or repair damaged tissues.

In conclusion, our view is that the vascular network is an ancestral neural guidance system that was co-opted by evolution towards the development of biological tubes to move molecules and cells around a complex multicellular animal. Much work needs to be done to test this idea: the use of genetically tractable vertebrate models (e.g., zebrafish), invertebrates (fruit fly, nematode) can test aspects of this evolutionary hypothesis. As in life, the road forwards in understanding complex biological mechanisms is better informed by our view of history, i.e., the evolution of this unique system in complex multicellular eukaryotes.

Acknowledgments Work in our laboratories was supported by grants from the British Heart Foundation (S.P.), The Wellcome Trust (S.P.), Heart Research UK (S.P.), and Ministry of Scientific Research of Italy, PRIN 2009 (M.A.).

References

1. Carmeliet P, Tessier-Lavigne M (2005) Common mechanisms of nerve and blood vessel wiring. Nature 436:193–200
2. Eichmann A, Le Noble F, Autiero M, Carmeliet P (2005) Guidance of vascular and neural network formation. Curr Opin Neurobiol 15:108–115
3. Zacchigna S, Ruiz de Almodovar C, Carmeliet P (2008) Similarities between angiogenesis and neural development: what small animal models can tell us. Curr Top Dev Biol 80:1–55
4. Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P (2009) Role and therapeutic potential of VEGF in the nervous system. Physiol Rev 89:607–648
5. Harper SJ, Bates DO (2008) VEGF-A splicing: the key to anti-angiogenic therapeutics? Nat Rev Cancer 8:880–887
6. Hua J, Spee C, Kase S, Rennel ES, Magnussen AL, Qiu Y, Varey A, Dhayade S, Churchill AJ, Harper SJ, Bates DO, Hinton DR (2010) Recombinant human VEGF165b inhibits experimental

choroidal neovascularization. Invest Ophthalmol Vis Sci 51:4282–4288
7. Seipel K, Eberhardt M, Muller P, Pescia E, Yanze N, Schmid V (2004) Homologs of vascular endothelial growth factor and receptor, VEGF and VEGFR, in the jellyfish *Podocoryne carnea*. Dev Dyn 231:303–312
8. Tarsitano M, De Falco S, Colonna V, McGhee JD, Persico MG (2006) The *C. elegans* pvf-1 gene encodes a PDGF/VEGF-like factor able to bind mammalian VEGF receptors and to induce angiogenesis. FASEB J 20:227–233
9. Duchek P, Somogyi K, Jekely G, Beccari S, Rorth P (2001) Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. Cell 107:17–26
10. Cho NK, Keyes L, Johnson E, Heller J, Ryner L, Karim F, Krasnow MA (2002) Developmental control of blood cell migration by the *Drosophila* VEGF pathway. Cell 108:865–876
11. Muller YA, Li B, Christinger HW, Wells JA, Cunningham BC, de Vos AM (1997) Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor-binding site. Proc Natl Acad Sci USA 94:7192–7197
12. Reigstad LJ, Sande HM, Fluge O, Bruland O, Muga A, Varhaug JE, Martinez A, Lillehaug JR (2003) Platelet-derived growth factor (PDGF)-C, a PDGF family member with a vascular endothelial growth factor-like structure. J Biol Chem 278:17114–17120
13. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141:1117–1134
14. Robinson DR, Wu YM, Lin SF (2000) The protein tyrosine kinase family of the human genome. Oncogene 19:5548–5557
15. Shibuya M, Claesson-Welsh L (2006) Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res 312:549–560
16. Bates RC, Goldsmith JD, Bachelder RE, Brown C, Shibuya M, Oettgen P, Mercurio AM (2003) Flt-1-dependent survival characterizes the epithelial–mesenchymal transition of colonic organoids. Curr Biol 13:1721–1727
17. Nishiguchi KM, Nakamura M, Kaneko H, Kachi S, Terasaki H (2007) The role of VEGF and VEGFR2/Flk1 in proliferation of retinal progenitor cells in murine retinal degeneration. Invest Ophthalmol Vis Sci 48:4315–4320
18. Zentilin L, Puligadda U, Lionetti V, Zacchigna S, Collesi C, Pattarini L, Ruozi G, Camporesi S, Sinagra G, Pepe M, Recchia FA, Giacca M (2010) Cardiomyocyte VEGFR-1 activation by VEGF-B induces compensatory hypertrophy and preserves cardiac function after myocardial infarction. FASEB J 24:1467–1478
19. Kennedy M, D'Souza SL, Lynch-Kattman M, Schwantz S, Keller G (2007) Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. Blood 109:2679–2687
20. Ferreira LS, Gerecht S, Shieh HF, Watson N, Rupnick MA, Dallabrida SM, Vunjak-Novakovic G, Langer R (2007) Vascular progenitor cells isolated from human embryonic stem cells give rise to endothelial and smooth muscle like cells and form vascular networks in vivo. Circ Res 101:286–294
21. Bearzi C, Leri A, Lo Monaco F, Rota M, Gonzalez A, Hosoda T, Pepe M, Qanud K, Ojaimi C, Bardelli S, D'Amario D, D'Alessandro DA, Michler RE, Dimmeler S, Zeiher AM, Urbanek K, Hintze TH, Kajstura J, Anversa P (2009) Identification of a coronary vascular progenitor cell in the human heart. Proc Natl Acad Sci USA 106:15885–15890
22. Kranich S, Hattermann K, Specht A, Lucius R, Mentlein R (2009) VEGFR-3/Flt-4 mediates proliferation and chemotaxis in glial precursor cells. Neurochem Int 55:747–753
23. Fantin A, Maden CH, Ruhrberg C (2009) Neuropilin ligands in vascular and neuronal patterning. Biochem Soc Trans 37:1228–1232
24. Pasterkamp RJ, Giger RJ (2009) Semaphorin function in neural plasticity and disease. Curr Opin Neurobiol 19:263–274
25. Yu L, Zhou Y, Cheng S, Rao Y (2010) Plexin a-semaphorin-1a reverse signaling regulates photoreceptor axon guidance in *Drosophila*. J Neurosci 30:12151–12156
26. Nukazuka A, Fujisawa H, Inada T, Oda Y, Takagi S (2008) Semaphorin controls epidermal morphogenesis by stimulating mRNA translation via eIF2alpha in *Caenorhabditis elegans*. Genes Dev 22:1025–1036
27. Bellon A, Luchino J, Haigh K, Rougon G, Haigh J, Chauvet S, Mann F (2010) VEGFR2 (KDR/Flk1) signaling mediates axon growth in response to semaphorin 3E in the developing brain. Neuron 66:205–219
28. Ferrara N (2009) Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 29:789–791
29. Carmeliet P, De Smet F, Loges S, Mazzone M (2009) Branching morphogenesis and antiangiogenesis candidates: tip cells lead the way. Nat Rev Clin Oncol 6:315–326
30. 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, Nagy A (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 380:435–439
31. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW (1996) Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 380:439–442
32. Fong GH, Rossant J, Gertsenstein M, Breitman ML (1995) Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 376:66–70
33. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC (1995) Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62–66
34. Olfert IM, Howlett RA, Tang K, Dalton ND, Gu Y, Peterson KL, Wagner PD, Breen EC (2009) Muscle-specific VEGF deficiency greatly reduces exercise endurance in mice. J Physiol 587:1755–1767
35. Tang K, Rossiter HB, Wagner PD, Breen EC (2004) Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. J Appl Physiol 97:1559–1566
36. Le YZ, Bai Y, Zhu M, Zheng L (2010) Temporal requirement of RPE-derived VEGF in the development of choroidal vasculature. J Neurochem 112:1584–1592
37. Bruns AF, Bao L, Walker JH, Ponnambalam S (2009) VEGF-A-stimulated signalling in endothelial cells via a dual receptor tyrosine kinase system is dependent on co-ordinated trafficking and proteolysis. Biochem Soc Trans 37:1193–1197
38. Nowak DG, Amin EM, Rennel ES, Hoareau-Aveilla C, Gammons M, Damodoran G, Hagiwara M, Harper SJ, Woolard J, Ladomery MR, Bates DO (2010) Regulation of vascular endothelial growth factor (VEGF) splicing from pro-angiogenic to anti-angiogenic isoforms: a novel therapeutic strategy for angiogenesis. J Biol Chem 285:5532–5540
39. Chen TT, Luque A, Lee S, Anderson SM, Segura T, Iruela-Arispe ML (2010) Anchorage of VEGF to the extracellular matrix conveys differential signaling responses to endothelial cells. J Cell Biol 188:595–609
40. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA (2009) An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. Proc Natl Acad Sci USA 106:18751–18756
41. Houck KA, Leung DW, Rowland AM, Winer J, Ferrara N (1992) Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. J Biol Chem 267:26031–26037

42. Keyt BA, Berleau LT, Nguyen HV, Chen H, Heinsohn H, Vandlen R, Ferrara N (1996) The carboxyl-terminal domain (111–165) of vascular endothelial growth factor is critical for its mitogenic potency. J Biol Chem 271:7788–77895
43. Lauer G, Sollberg S, Cole M, Flamme I, Sturzebecher J, Mann K, Krieg T, Eming SA (2000) Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. J Invest Dermatol 115:12–18
44. Roth D, Piekarek M, Paulsson M, Christ H, Bloch W, Krieg T, Davidson JM, Eming SA (2006) Plasmin modulates vascular endothelial growth factor-A-mediated angiogenesis during wound repair. Am J Pathol 168:670–684
45. Lauer G, Sollberg S, Cole M, Krieg T, Eming SA (2002) Generation of a novel proteolysis resistant vascular endothelial growth factor165 variant by a site-directed mutation at the plasmin sensitive cleavage site. FEBS Lett 531:309–313
46. Mineur P, Colige AC, Deroanne CF, Dubail J, Kesteloot F, Habraken Y, Noel A, Voo S, Waltenberger J, Lapiere CM, Nusgens BV, Lambert CA (2007) Newly identified biologically active and proteolysis-resistant VEGF-A isoform VEGF111 is induced by genotoxic agents. J Cell Biol 179:1261–1273
47. Nowak DG, Woolard J, Amin EM, Konopatskaya O, Saleem MA, Churchill AJ, Ladomery MR, Harper SJ, Bates DO (2008) Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. J Cell Sci 121:3487–3495
48. Schwarz Q, Gu C, Fujisawa H, Sabelko K, Gertsenstein M, Nagy A, Taniguchi M, Kolodkin AL, Ginty DD, Shima DT, Ruhrberg C (2004) Vascular endothelial growth factor controls neuronal migration and cooperates with Sema3A to pattern distinct compartments of the facial nerve. Genes Dev 18:2822–2834
49. Zhang Z, Helman JI, Li LJ (2010) Lymphangiogenesis, lymphatic endothelial cells and lymphatic metastasis in head and neck cancer—a review of mechanisms. Int J Oral Sci 2:5–14
50. Muller-Deile J, Worthmann K, Saleem M, Tossidou I, Haller H, Schiffer M (2009) The balance of autocrine VEGF-A and VEGF-C determines podocyte survival. Am J Physiol Renal Physiol 297:F1656–F1667
51. Wang ZG, Puri TS, Quigg RJ (2010) Characterization of novel VEGF (vascular endothelial growth factor)-C splicing isoforms from mouse. Biochem J 428:347–354
52. Choi JS, Shin YJ, Lee JY, Yun H, Cha JH, Choi JY, Chun MH, Lee MY (2010) Expression of vascular endothelial growth factor receptor-3 mRNA in the rat developing forebrain and retina. J Comp Neurol 518:1064–1081
53. Le Bras B, Barallobre MJ, Homman-Ludiye J, Ny A, Wyns S, Tammela T, Haiko P, Karkkainen MJ, Yuan L, Muriel MP, Chatzopoulou E, Breant C, Zalc B, Carmeliet P, Alitalo K, Eichmann A, Thomas JL (2006) VEGF-C is a trophic factor for neural progenitors in the vertebrate embryonic brain. Nat Neurosci 9:340–348
54. Fulga TA, Rorth P (2002) Invasive cell migration is initiated by guided growth of long cellular extensions. Nat Cell Biol 4:715–719
55. Heino TI, Karpanen T, Wahlstrom G, Pulkkinen M, Eriksson U, Alitalo K, Roos C (2001) The *Drosophila* VEGF receptor homolog is expressed in hemocytes. Mech Dev 109:69–77
56. Bruckner K, Kockel L, Duchek P, Luque CM, Rorth P, Perrimon N (2004) The PDGF/VEGF receptor controls blood cell survival in *Drosophila*. Dev Cell 7:73–84
57. Macias A, Romero NM, Martin F, Suarez L, Rosa AL, Morata G (2004) PVF1/PVR signaling and apoptosis promotes the rotation and dorsal closure of the *Drosophila* male terminalia. Int J Dev Biol 48:1087–1094
58. Choi NH, Kim JG, Yang DJ, Kim YS, Yoo MA (2008) Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor. Aging Cell 7:318–334
59. Janssens K, Sung HH, Rorth P (2010) Direct detection of guidance receptor activity during border cell migration. Proc Natl Acad Sci USA 107:7323–7328
60. Wu X, Liu N (2010) The role of Ang/Tie signaling in lymphangiogenesis. Lymphology 43:59–72
61. Dormer A, Beck G (2005) Evolutionary analysis of human vascular endothelial growth factor, angiopoietin, and tyrosine endothelial kinase involved in angiogenesis and immunity. In Silico Biol 5:323–339
62. Banerjee S, Bhat MA (2007) Neuron-glial interactions in blood–brain barrier formation. Annu Rev Neurosci 30:235–258
63. Abbott NJ, Bundgaard M (1992) Electron-dense tracer evidence for a blood–brain barrier in the cuttlefish *Sepia officinalis*. J Neurocytol 21:276–294
64. Munoz-Chapuli R, Carmona R, Guadix JA, Macias D, Perez-Pomares JM (2005) The origin of the endothelial cells: an evo–devo approach for the invertebrate/vertebrate transition of the circulatory system. Evol Dev 7:351–358
65. Gasparini F, Burighel P, Manni L, Zaniolo G (2008) Vascular regeneration and angiogenic-like sprouting mechanism in a compound ascidian is similar to vertebrates. Evol Dev 10:591–605
66. Tiozzo S, Voskoboynik A, Brown FD, De Tomaso AW (2008) A conserved role of the VEGF pathway in angiogenesis of an ectodermally-derived vasculature. Dev Biol 315:243–255
67. Wymann MP, Pirola L, Katanaev VL, Bulgarelli-Leva G (1999) Phosphoinositide 3-kinase signalling: no lipids. Biochem Soc Trans 27:629–634
68. Scanga SE, Ruel L, Binari RC, Snow B, Stambolic V, Bouchard D, Peters M, Calvieri B, Mak TW, Woodgett JR, Manoukian AS (2000) The conserved PI3′K/PTEN/Akt signaling pathway regulates both cell size and survival in *Drosophila*. Oncogene 19:3971–3977
69. Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, Hemmings BA, Hafen E (2002) Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. Science 295:2088–2091
70. Dormann D, Weijer CJ (2006) Chemotactic cell movement during *Dictyostelium* development and gastrulation. Curr Opin Genet Dev 16:367–373
71. Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606–619
72. Oviedo NJ, Pearson BJ, Levin M, Sanchez Alvarado A (2008) Planarian PTEN homologs regulate stem cells and regeneration through TOR signaling. Dis Model Mech 1:131–143
73. Zhang D, Aravind L (2010) Identification of novel families and classification of the C2 domain superfamily elucidate the origin and evolution of membrane targeting activities in eukaryotes. Gene 469:18–30
74. Smith A, Alrubaie S, Coehlo C, Leevers SJ, Ashworth A (1999) Alternative splicing of the *Drosophila* PTEN gene. Biochim Biophys Acta 1447:313–317
75. Kawashima T, Tokuoka M, Awazu S, Satoh N, Satou Y (2003) A genomewide survey of developmentally relevant genes in *Ciona intestinalis*. VIII. Genes for PI3K signaling and cell cycle. Dev Genes Evol 213:284–290
76. Horowitz A, Simons M (2008) Branching morphogenesis. Circ Res 103:784–795
77. Centanin L, Dekanty A, Romero N, Irisarri M, Gorr TA, Wappner P (2008) Cell autonomy of HIF effects in *Drosophila*: tracheal cells sense hypoxia and induce terminal branch sprouting. Dev Cell 14:547–558
78. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C (2003) VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161:1163–1177

79. Sims D, Duchek P, Baum B (2009) PDGF/VEGF signaling controls cell size in *Drosophila*. Genome Biol 10:R20
80. Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM (2006) Endothelial tubes assemble from intracellular vacuoles in vivo. Nature 442:453–456
81. Sacharidou A, Koh W, Stratman AN, Mayo AM, Fisher KE, Davis GE (2010) Endothelial lumen signaling complexes control 3D matrix-specific tubulogenesis through interdependent Cdc42- and MT1-MMP-mediated events. Blood 115:5259–5269
82. Abraham S, Yeo M, Montero-Balaguer M, Paterson H, Dejana E, Marshall CJ, Mavria G (2009) VE-Cadherin-mediated cell-cell interaction suppresses sprouting via signaling to MLC2 phosphorylation. Curr Biol 19:668–674
83. Maurer MH, Tripps WK, Feldmann RE Jr, Kuschinsky W (2003) Expression of vascular endothelial growth factor and its receptors in rat neural stem cells. Neurosci Lett 344:165–168
84. Bagnard D, Vaillant C, Khuth ST, Dufay N, Lohrum M, Puschel AW, Belin MF, Bolz J, Thomasset N (2001) Semaphorin 3A-vascular endothelial growth factor-165 balance mediates migration and apoptosis of neural progenitor cells by the recruitment of shared receptor. J Neurosci 21:3332–3341
85. Brusselmans K, Bono F, Collen D, Herbert JM, Carmeliet P, Dewerchin M (2005) A novel role for vascular endothelial growth factor as an autocrine survival factor for embryonic stem cells during hypoxia. J Biol Chem 280:3493–3499
86. Nishijima K, Ng YS, Zhong L, Bradley J, Schubert W, Jo N, Akita J, Samuelsson SJ, Robinson GS, Adamis AP, Shima DT (2007) Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. Am J Pathol 171:53–67
87. Jin KL, Mao XO, Nagayama T, Goldsmith PC, Greenberg DA (2000) Induction of vascular endothelial growth factor and hypoxia-inducible factor-1alpha by global ischemia in rat brain. Neuroscience 99:577–585
88. Jin KL, Mao XO, Greenberg DA (2000) Vascular endothelial growth factor: direct neuroprotective effect in in vitro ischemia. Proc Natl Acad Sci USA 97:10242–10247
89. Widenfalk J, Lipson A, Jubran M, Hofstetter C, Ebendal T, Cao Y, Olson L (2003) Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury. Neuroscience 120:951–960
90. Li Q, Ford MC, Lavik EB, Madri JA (2006) Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells and endothelial cells: an in vitro study. J Neurosci Res 84:1656–1668
91. Jung KH, Chu K, Lee ST, Kim SJ, Sinn DI, Kim SU, Kim M, Roh JK (2006) Granulocyte colony-stimulating factor stimulates neurogenesis via vascular endothelial growth factor with STAT activation. Brain Res 1073–1074:190–201
92. Zhang H, Vutskits L, Pepper MS, Kiss JZ (2003) VEGF is a chemoattractant for FGF-2-stimulated neural progenitors. J Cell Biol 163:1375–1384
93. Wada T, Haigh JJ, Ema M, Hitoshi S, Chaddah R, Rossant J, Nagy A, van der Kooy D (2006) Vascular endothelial growth factor directly inhibits primitive neural stem cell survival but promotes definitive neural stem cell survival. J Neurosci 26:6803–6812
94. Kim BK, Kim SE, Shim JH, Woo DH, Gil JE, Kim SK, Kim JH (2006) Neurogenic effect of vascular endothelial growth factor during germ layer formation of human embryonic stem cells. FEBS Lett 580:5869–5874
95. Schmidt NO, Koeder D, Messing M, Mueller FJ, Aboody KS, Kim SU, Black PM, Carroll RS, Westphal M, Lamszus K (2009) Vascular endothelial growth factor-stimulated cerebral microvascular endothelial cells mediate the recruitment of neural stem cells to the neurovascular niche. Brain Res 1268:24–37
96. Guo Y, Shi D, Li W, Liang C, Wang H, Ye Z, Hu L, Li Y (2010) Effects of cerebral microvascular endothelial cells and vascular endothelial growth factor on the proliferation and differentiation of NSCs: a comparative study. Br J Neurosurg 24:62–68
97. Mani N, Khaibullina A, Krum JM, Rosenstein JM (2005) Astrocyte growth effects of vascular endothelial growth factor (VEGF) application to perinatal neocortical explants: receptor mediation and signal transduction pathways. Exp Neurol 192:394–406
98. Sun FY, Guo X (2005) Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. J Neurosci Res 79:180–184
99. Merrill MJ, Oldfield EH (2005) A reassessment of vascular endothelial growth factor in central nervous system pathology. J Neurosurg 103:853–868
100. Lambrechts D, Carmeliet P (2006) VEGF at the neurovascular interface: therapeutic implications for motor neuron disease. Biochim Biophys Acta 1762:1109–1121
101. Devos D, Moreau C, Lassalle P, Perez T, De Seze J, Brunaud-Danel V, Destee A, Tonnel AB, Just N (2004) Low levels of the vascular endothelial growth factor in CSF from early ALS patients. Neurology 62:2127–2129
102. Brockington A, Heath PR, Holden H, Kasher P, Bender FL, Claes F, Lambrechts D, Sendtner M, Carmeliet P, Shaw PJ (2010) Downregulation of genes with a function in axon outgrowth and synapse formation in motor neurones of the VEGFdelta/delta mouse model of amyotrophic lateral sclerosis. BMC Genomics 11:203
103. Lunn JS, Sakowski SA, Kim B, Rosenberg AA, Feldman EL (2009) Vascular endothelial growth factor prevents G93A-SOD1-induced motor neuron degeneration. Dev Neurobiol 69:871–884
104. Lu L, Zheng L, Viera L, Suswam E, Li Y, Li X, Estevez AG, King PH (2007) Mutant Cu/Zn-superoxide dismutase associated with amyotrophic lateral sclerosis destabilizes vascular endothelial growth factor mRNA and downregulates its expression. J Neurosci 27:7929–7938
105. Lemmens R, Van Hoecke A, Hersmus N, Geelen V, D'Hollander I, Thijs V, Van Den Bosch L, Carmeliet P, Robberecht W (2007) Overexpression of mutant superoxide dismutase 1 causes a motor axonopathy in the zebrafish. Hum Mol Genet 16:2359–2365
106. Wang Y, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA, Jin K (2007) Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. J Neurosci 27:304–307
107. Sathasivam S (2008) VEGF and ALS. Neurosci Res 62:71–77
108. Chen W, Saeed M, Mao H, Siddique N, Dellefave L, Hung WY, Deng HX, Sufit RL, Heller SL, Haines JL, Pericak-Vance M, Siddique T (2006) Lack of association of VEGF promoter polymorphisms with sporadic ALS. Neurology 67:508–510
109. Yasuhara T, Shingo T, Muraoka K, Kameda M, Agari T, Wen Ji Y, Hayase H, Hamada H, Borlongan CV, Date I (2005) Neurorescue effects of VEGF on a rat model of Parkinson's disease. Brain Res 1053:10–18
110. Tian YY, Tang CJ, Wang JN, Feng Y, Chen XW, Wang L, Qiao X, Sun SG (2007) Favorable effects of VEGF gene transfer on a rat model of Parkinson disease using adeno-associated viral vectors. Neurosci Lett 421:239–244
111. Emerich DF, Mooney DJ, Storrie H, Babu RS, Kordower JH (2010) Injectable hydrogels providing sustained delivery of vascular endothelial growth factor are neuroprotective in a rat model of Huntington's disease. Neurotox Res 17:66–74

112. Mateo I, Llorca J, Infante J, Rodriguez-Rodriguez E, Fernandez-Viadero C, Pena N, Berciano J, Combarros O (2007) Low serum VEGF levels are associated with Alzheimer's disease. Acta Neurol Scand 116:56–58
113. Tarkowski E, Issa R, Sjogren M, Wallin A, Blennow K, Tarkowski A, Kumar P (2002) Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. Neurobiol Aging 23:237–243
114. Patel NS, Mathura VS, Bachmeier C, Beaulieu-Abdelahad D, Laporte V, Weeks O, Mullan M, Paris D (2010) Alzheimer's beta-amyloid peptide blocks vascular endothelial growth factor mediated signaling via direct interaction with VEGFR-2. J Neurochem 112:66–76
115. Yang SP, Bae DG, Kang HJ, Gwag BJ, Gho YS, Chae CB (2004) Co-accumulation of vascular endothelial growth factor with beta-amyloid in the brain of patients with Alzheimer's disease. Neurobiol Aging 25:283–290
116. Del Bo R, Ghezzi S, Scarpini E, Bresolin N, Comi GP (2009) VEGF genetic variability is associated with increased risk of developing Alzheimer's disease. J Neurol Sci 283:66–68
117. Landgren S, Palmer MS, Skoog I, Minthon L, Wallin A, Andreasen N, Zetterberg M, Blennow K, Zetterberg H (2010) No association of VEGF polymorphims with Alzheimer's disease. Neuromolecular Med 12:224–228
118. Zhang W, Sun K, Zhen Y, Wang D, Wang Y, Chen J, Xu J, Hu FB, Hui R (2009) VEGF receptor-2 variants are associated with susceptibility to stroke and recurrence. Stroke 40:2720–2726
119. Issa R, Krupinski J, Bujny T, Kumar S, Kaluza J, Kumar P (1999) Vascular endothelial growth factor and its receptor, KDR, in human brain tissue after ischemic stroke. Lab Invest 79:417–425
120. Rigau V, Morin M, Rousset MC, de Bock F, Lebrun A, Coubes P, Picot MC, Baldy-Moulinier M, Bockaert J, Crespel A, Lerner-Natoli M (2007) Angiogenesis is associated with blood–brain barrier permeability in temporal lobe epilepsy. Brain 130:1942–1956
121. Marcon J, Gagliardi B, Balosso S, Maroso M, Noe F, Morin M, Lerner-Natoli M, Vezzani A, Ravizza T (2009) Age-dependent vascular changes induced by status epilepticus in rat forebrain: implications for epileptogenesis. Neurobiol Dis 34:121–132
122. Li Y, Zhang F, Nagai N, Tang Z, Zhang S, Scotney P, Lennartsson J, Zhu C, Qu Y, Fang C, Hua J, Matsuo O, Fong GH, Ding H, Cao Y, Becker KG, Nash A, Heldin CH, Li X (2008) VEGF-B inhibits apoptosis via VEGFR-1-mediated suppression of the expression of BH3-only protein genes in mice and rats. J Clin Invest 118:913–923
123. Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH, Hermann DM (2006) The phosphatidylinositol-3 kinase/Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. FASEB J 20:1185–1187
124. Islamov RR, Chintalgattu V, Pak ES, Katwa LC, Murashov AK (2004) Induction of VEGF and its Flt-1 receptor after sciatic nerve crush injury. Neuroreport 15:2117–2121
125. Kim HM, Hwang DH, Lee JE, Kim SU, Kim BG (2009) Ex vivo VEGF delivery by neural stem cells enhances proliferation of glial progenitors, angiogenesis, and tissue sparing after spinal cord injury. PLoS One 4:e4987
126. Su JJ, Osoegawa M, Matsuoka T, Minohara M, Tanaka M, Ishizu T, Mihara F, Taniwaki T, Kira J (2006) Upregulation of vascular growth factors in multiple sclerosis: correlation with MRI findings. J Neurol Sci 243:21–30
127. Sasaki M, Lankford KL, Brown RJ, Ruddle NH, Kocsis JD (2010) Focal experimental autoimmune encephalomyelitis in the lewis rat induced by immunization with myelin oligodendrocyte glycoprotein and intraspinal injection of vascular endothelial growth factor. Glia 58:1523–1531
128. Yamada H, Yamada E, Hackett SF, Ozaki H, Okamoto N, Campochiaro PA (1999) Hyperoxia causes decreased expression of vascular endothelial growth factor and endothelial cell apoptosis in adult retina. J Cell Physiol 179:149–156
129. Hashimoto T, Zhang XM, Chen BY, Yang XJ (2006) VEGF activates divergent intracellular signaling components to regulate retinal progenitor cell proliferation and neuronal differentiation. Development 133:2201–2210
130. Saint-Geniez M, Maharaj AS, Walshe TE, Tucker BA, Sekiyama E, Kurihara T, Darland DC, Young MJ, D'Amore PA (2008) Endogenous VEGF is required for visual function: evidence for a survival role on muller cells and photoreceptors. PLoS One 3: e3554
131. Rehak M, Hollborn M, Iandiev I, Pannicke T, Karl A, Wurm A, Kohen L, Reichenbach A, Wiedemann P, Bringmann A (2009) Retinal gene expression and Muller cell responses after branch retinal vein occlusion in the rat. Invest Ophthalmol Vis Sci 50:2359–2367
132. Barnett JM, McCollum GW, Penn JS (2010) Role of cytosolic phospholipase A(2) in retinal neovascularization. Invest Ophthalmol Vis Sci 51:1136–1142
133. Yanni SE, McCollum GW, Penn JS (2010) Genetic deletion of COX-2 diminishes VEGF production in mouse retinal Muller cells. Exp Eye Res 91:34–41
134. Hogan BM, Bussmann J, Wolburg H, Schulte-Merker S (2008) ccm1 cell autonomously regulates endothelial cellular morphogenesis and vascular tubulogenesis in zebrafish. Hum Mol Genet 17:2424–2432
135. Gore AV, Lampugnani MG, Dye L, Dejana E, Weinstein BM (2008) Combinatorial interaction between CCM pathway genes precipitates hemorrhagic stroke. Dis Model Mech 1:275–281
136. Lampugnani MG, Orsenigo F, Rudini N, Maddaluno L, Boulday G, Chapon F, Dejana E (2010) CCM1 regulates vascular-lumen organization by inducing endothelial polarity. J Cell Sci 123:1073–1080
137. Falk T, Zhang S, Sherman SJ (2009) Vascular endothelial growth factor B (VEGF-B) is up-regulated and exogenous VEGF-B is neuroprotective in a culture model of Parkinson's disease. Mol Neurodegener 4:49