Published in final edited form as:

Lymphat Res Biol. 2008 ; 6(3-4): 173–180. doi:10.1089/lrb.2008.1014.

Developmental Angiogenesis of the Central Nervous System

Michael R. Mancuso, B.S., Frank Kuhnert, Ph.D., and Calvin J. Kuo, M.D., Ph.D.

Stanford University School of Medicine. Division of Hematology, Stanford, CA.

Abstract

The vasculature of the central nervous system (CNS) is highly specialized with a blood-brain-barrier, reciprocal neuroepithelial-endothelial cell interactions and extensive pericyte coverage. Developmentally, numerous important signaling pathways participate in CNS angiogenesis to orchestrate the precise timing and spatial arrangement of the complex CNS vascular network. From a therapeutic standpoint, the CNS vasculature has attracted increased attention since many human ailments, such as stroke, retinopathy, cancer and autoimmune disease are intimately associated with the biology of CNS blood vessels. This review focuses on growth factor pathways that have been shown to be important in developmental CNS vascularization through studies of mouse genetic models and human diseases.

Introduction

Amidst the glia and neurons in the CNS lies a network of blood vessels. During development, the vasculature of the CNS develops exclusively via angiogenesis—the growth of new blood vessels from pre-existing ones—as opposed to de novo vasculogenesis—the formation of vascular tubes from migrating angioblasts—as seen in other tissues.^{1–3}

In the absence of proper CNS vascularization, the neuroepithelium undergoes apoptosis followed by progressive tissue destruction, especially at the subventricular zones leading to embryonic lethality.⁴ In addition to providing nutrients and gas exchange to the underlying neural tissue, the vasculature of the CNS is specialized, with a blood-brain barrier, a striking degree of pericyte coverage, reciprocal interactions with neurons and glia, and the recent concept of a vascular niche for neural stem cells.^{5–10} Furthermore, the CNS vasculature plays a vital role in many common human disorders such as hereditary vascular malformations, stroke, retinopathy, cancer and autoimmune disease. Against this background, angiogenesis and vascular homeostasis of the CNS is gaining ever-increasing attention towards understanding the etiology of multiple inherited syndromes and also as a therapeutic target to treating many human diseases. Mouse and human genetic studies over the past twenty years have yielded significant progress towards understanding the molecular and cellular mechanisms underlying the growth and maturation of the CNS vasculature. The current review focuses on cell surface receptors and cognate ligands that are important for CNS angiogenesis emphasizing mouse and human genetic studies which have phenotypes exhibiting relative restriction to CNS angiogenesis as opposed to global angiogenesis deficits.

© Mary Ann Liebert, Inc.

Address reprint requests to: Dr. C. J. Kuo Stanford University School of Medicine Division of Hematology CCSR 1155 269 Campus Dr. Stanford, CA 94305 *E-mail:* cjkuo@stanford.edu.

Disclosures

Mr. Mancuso and Drs. Kuhnert and Kuo have no conflicts of interest or financial ties to report.

Embryonic Development of the CNS Vasculature

Grossly, vascular development of the CNS in mice commences with vasculogenic formation of the perineural plexus (the leptomeningeal arteries and veins). This vasculogenesis occurs at the ventral region of the neural tube around E7.5—E8.5.¹¹ Subsequently, around E9.5, capillary sprouts invade into the neuroepithelium by angiogenesis in a caudal to cranial direction. This is followed by extensive branching and arborization as capillary sprouts migrate from the pial surface to periventricular areas where angiogenic growth factors such as vascular endothelial growth factor (VEGF) are highly expressed and secreted by cell in the subventricular zone.^{12,13} This process continues through the remainder of embryonic development in all CNS tissues except for the retina proper as described below.¹⁴ Once the vascular plexus has migrated into the brain, the blood vessels of the CNS undergo vascular remodeling and pruning coupled with endothelial cell recruitment of vascular smooth muscle cells (VSMCs) to form the mature functional CNS vasculature via molecular pathways that are described below.

Developmental Retinal Angiogenesis

The retina is embryologically contiguous with the central nervous system, and develops as bilateral exvaginations of the forebrain neuroectoderm.^{14–16} At birth, the retina proper is avascular, although nutritional support is provided by the hyaloid vessels, an arterial network coursing within the vitreous humor, and by the choroidal vessels completely encircling the optic cup. Within this hyaloid network, efferent blood enters via a central hyaloid artery within the optic nerve, ramifies through hyaloid vessels in the vitreous, finally exiting via a venous choroidal net on the external eye. After birth, the hyaloid vasculature regresses, followed by definitive angiogenesis of the retina. In this second process, angiogenesis commences with initial vessel entry from the optic nerve head, with formation of a primitive vascular plexus extending within the nerve fiber layer of the inner retinal surface. This angiogenesis is guided by astrocyte secretion of angiogenic factors such as VEGF, and the primary plexus reaches the periphery of the retina by P8.¹⁷ This superficial plexus undergoes both significant remodeling—with some vessels regressing and others strengthening—as well as extension to deeper retinal layers with eventual formation of three parallel but interconnected networks in the nerve fiber layer and the synaptic plexiform layers via molecular pathways that are described below. The mature adult neural retina is supported by two distinct vascular systems: proper retinal vessels with barrier properties similar to those in the blood-brain barrier, and highly fenestrated choroidal vessels separated from the neural retina by the basement membrane (Bruch's membrane) of the retinal pigment epithelium (RPE).^{15,16,18} The development of the retinal vasculature is frequently used as a model for the mechanisms involved in vascular remodeling and maturation due to this stereotypical growth pattern and feasibility as a model system. At a molecular level, the regulation of embryonic CNS angiogenesis and subsequent vascular remodeling is regulated by numerous receptor-ligand interactions that are described below.

Pathways Important for Endothelial Cell Migration into the CNS

Migration of endothelial cells from the surrounding perineural plexus into the neuroepithelium marks the induction of CNS vascularization. In the brain, endothelial cells migrate from the pial surface towards the subventricular zones while in the retina endothelial cells migrate from the optic nerve head to the outer edges of the retina over the first 8 days of life. In both cases, endothelial cells take on one of two phenotypes as the vascular plexuses migrate into their respective avascular regions.^{17,19} Tip cells, at the forefront of the migrating plexus form filipodia, which are plasma membrane protrusions with an actin

core that sense growth factor gradients.^{17,20} Stalk cells, located behind the tip cells, form the vascular lumen and proliferate, pushing the plexus forward.^{17,19}

VEGF/VEGFR/Neuropilin-1

The VEGF family has multiple members including VEGF-A, B, C, D, and PlGF which bind to the receptors VEGFR-1 (FLT-1), VEGFR-2 (FLK, KDR) or VEGFR-3 (FLT-4) and the Neuropilin-1 and Neuropilin-2 co-receptors.²¹ VEGF-A (VEGF) signaling through VEGFR-2/Neuropilin-1 are thought to be the main players from the VEGF family in CNS angiogenesis.^{4,19} VEGF-A is alternatively spliced to yield several isoforms, with the larger isoforms having large basic domains leading to the protein to have high affinity to heparin and other matrix proteins,²¹ hence the terms: “soluble” versus “heparin-bound” VEGF. Previous reports have implicated heparin-bound VEGF as important for guiding migrating endothelial cells into avascular regions of the CNS during development.¹⁷

In endothelial tip cells of the vascular plexus, activation of VEGFR-2 activates both PI3K with subsequent activation of Rac/Rho and Grb2 with subsequent Cdc42 activation, both of which stimulate actin reorganization, filopodia formation, and cell migration.²² In the stalk cells, activation of VEGFR-2 leads to phospholipase activation of the MAP Kinase pathway to induce cellular proliferation. At early stages of vascular development in the embryo, VEGF-A is expressed in the subventricular zone,^{12,13} while VEGFR-2 is expressed by the endothelial cells of the perineural capillary plexus and the emerging capillary sprouts.^{23,24} As the cortex develops, neurons become the predominant source of VEGF. Once vascular remodeling is complete, glial cells become the predominant producers of VEGF in the CNS.²⁵

Genetic evidence showing an essential role for VEGF during vascularization of the CNS in mice originates from genetic studies in which Nestin-Cre was crossed into a floxed VEGF background. In these studies, loss of VEGF production by the neuroepithelium leads in failure of the forebrain to be vascularized⁴ and migratory arrest of endothelial cells at the periphery of the neuroepithelium. This process of VEGF-mediated endothelial migration into the CNS has been further shown to be dependent on the levels of VEGF being produced by the neuroepithelium and is endothelial cell autonomous.¹⁹ The importance of VEGF binding to heparin during CNS angiogenesis has been demonstrated by reduced vascular branching complexity, increased microvessel caliber and decreased endothelial filopodia in mice lacking the VEGF heparin binding isoforms VEGF₁₆₄ and VEGF₁₈₈, and solely producing VEGF₁₂₀.²⁶

Similarly, Neuropilin-1 knockout mice also demonstrate impairment of CNS vascularization. Neuropilin-1 knockout mice manifested a severe embryonic lethal phenotype at E12.5 in which endothelial cells could not penetrate the neuroepithelium in telencephalon and spinal cord, accompanied by CNS hemorrhage.^{27,28} A less severe angiogenic defect with intracranial vascular malformations was observed in diencephalon, midbrain and hindbrain, accompanied by lack of tip cells and hemorrhage.^{27,28,29} Relative to the null allele of Neuropilin-1, endothelial-specific Neuropilin-1 deletion produced less severe phenotypes with forebrain vascular malformations in telencephalon and diencephalon.²⁸

NOTCH/DII4

The “fine-tuning” of the number of tip cells that lead the vascular plexus into the CNS has recently been shown to be controlled via DII4/Notch signaling.³⁰ The Notch family proteins consist of four members (Notch 1–4)³¹ that are important for cell fate determination in multi-cellular organisms. The Notch receptors are transmembrane proteins with unique

extracellular and intracellular regions. The extracellular region contains EGF-like repeats and 3-lin-12/Notch motifs, while the intracellular region contains a RAM domain, multiple cdc10/ankyrin repeats, nuclear localization signal(s), and a PEST (proline, glutamate, serine, threonine-rich) protein instability domain.³² The Notch receptors bind several membrane-bound ligands, Jagged 1, Jagged 2, Delta-like (Dll) 1, Dll3 and Dll4, which all contain extracellular regions with a DSL motif and a variable number of EGF-like repeats.³¹ Endothelial cells express Notch-1 and Notch-4 receptors and Jagged 1, Dll1, and Dll4 (with VSMC's expressing Notch-3 as described below).^{33,34} Of these, Dll4 is exclusively expressed in endothelial cells. When Notch and Dll4 interact, the extracellular domain of Notch is processed by a disintegrin metalloproteinase. This processing leads to proteolytic cleavage of the Notch intracellular domain by a gamma-secretase which is dependent on the presence of presenillins.³⁵ This intracellular cleavage event releases the Notch intracellular domain (NICD) which then translocates to the cell nucleus to serve as a cofactor for transcribing target genes.^{34,36} In endothelial cells, the Notch target gene *Hey1* leads to activation of the cell cycle inhibitor p21.³⁷ There is also accumulating evidence that interaction of Notch with Delta or Jagged induces a reverse signaling pathway where these membrane bound ligands are also ubiquitinated and internalized; however, the function of this internalization on the endothelial cell remains to be investigated.

Dll4/Notch signaling has been shown to be vitally important to embryonic development as loss of single copy of the *Dll4* gene results in embryonic lethality.³⁸ Interestingly, a similar haploinsufficiency is seen with VEGF^{39,40} and *Dll4* and VEGF are the only two genes where haploinsufficiency results in embryonic lethality due to a vascular deficit. In the postnatal mouse retina, Notch is expressed by the endothelial cells in the stalk cell region while *Dll4* is expressed in the endothelial tip cell region of the migrating vascular plexus.³⁰ Pharmacological inhibition of Dll4/Notch signaling in endothelial cells causes an increase in filopodia formation.^{30,41–43} A similar phenomenon is observed in the postnatal mouse retina, where inactivation of the Dll4/Notch pathway either by pharmacologic blockade, genetic inactivation of one allele of *Dll4*, or endothelial-specific deletion of Notch all result in increased filopodia formation.³⁰ These findings highlight the notion that Dll4/Notch signaling dampens endothelial cell responsiveness to VEGF. Activation of the VEGF pathway has been shown to increase expression of *Dll4* in endothelial cells.⁴¹ Alternatively, Dll4/Notch signaling leads to a decreased response to VEGF, most likely by reducing expression of VEGFR-2^{43–45} and VEGFR-3. These studies are consistent with endothelial tip cells expressing *Dll4* to signal to stalk cells via Notch to inhibit their transformation into a migratory tip cell phenotype. Hence, Dll4/Notch signaling acts as a feedback mechanism to control endothelial cell responsiveness to VEGF within certain regions of the migrating vascular plexus, namely the stalk cell region. This phenomenon is vital to proper formation of the CNS vasculature as failure to regulate endothelial cell hypersensitivity to VEGF leads to non-productive angiogenesis, resulting in tissue ischemia and failure of the underlying tissue to thrive.⁴¹

Slit2/Robo4

Roundabout (Robo) proteins are guidance receptors that control axonal migration^{46,47} and, to date, four Robo proteins have been identified in vertebrates with Robo4 being specifically expressed by endothelial cells.^{48,49} When activated by Slit2, Robo4 inhibits the downstream effects of VEGF/VEGFR-2 signaling.⁵⁰ In the postnatal mouse retina, Robo4 is expressed mainly by the endothelial stalk cells of the migrating vascular plexus.⁵⁰ Similar to Dll4/Notch, Slit2/Robo4 signaling appears to prevent endothelial stalk cells from assuming the migratory tip cell phenotype, thus establishing endothelial cell-type hierarchy as the vasculature of the CNS is formed.

Integrins

As the vascular plexus migrates into the CNS, endothelial cell communication with the neuroepithelium is essential for proper vascular development and eventual function.^{51,52} Integrins, which are heterodimeric proteins composed of α and β subunits, are cell adhesion receptors involved in cell-to-cell or cell-to-extracellular matrix interactions, undergo bi-directional signaling, and serve as adaptor molecules. Approximately 20 and 35% of mice with global deletion of α_V or β_8 respectively, die with cerebral hemorrhage with vascular malformations.^{53,54} Surprisingly, conditional genetic deletion studies indicate that this effect is due to loss of integrin $\alpha_V\beta_8$, in the neuroepithelium and not the endothelium.^{51,52} With the possibility of CNS hemorrhage being attributed to loss of integrin α_V causing failure of newly formed blood vessels to recruit VSMC's formally excluded,⁵⁵ these studies highlight the importance of endothelial cell communication with the underlying neuroepithelium during CNS vascularization.

Wnt signaling

Wnts represent a family of 19 related secreted polypeptides having broad functions in embryogenesis and organ homeostasis and cancer amongst other roles. Wnts have now been recently described to be critical regulators of developmental CNS angiogenesis. McMahon and colleagues described mice doubly deficient in both Wnt7a and Wnt7b, which exhibit severe impairment of vascularization of the neural tube and forebrain, the latter apparently associated with glomeruloid malformations. Wnt7a and Wnt7b appear to be required in the neuroepithelium to regulate CNS angiogenesis, on the basis of their expression patterns, as well as deletion with neural-specific Cre strains. The Wnt7a/Wnt7b vascular deficits were phenocopied by endothelial deletion of the downstream transcription factor β -catenin, further emphasizing the role of canonical Wnt signaling in this process. Interestingly, the Wnt7a/Wnt7b/ β -catenin axis appears to be an upstream regulator of the blood-brain barrier marker Glut-1, which is deficient in CNS vasculature of both the Wnt7a/Wnt7b double knockout mice as well as the endothelial β -catenin deletion mutants.¹⁰² Essentially similar results have been obtained by Richard Daneman and Ben Barres (personal communication), while Liebner et al. demonstrate that Wnt/ β -catenin signaling induces blood-brain barrier features in cultured mouse endothelial cells.¹⁰³

Pathways Important for Migration of VSMC into the CNS and for Vascular Remodeling

As new blood vessels grow into the CNS, endothelial cells recruit VSMCs (pericytes or mural cells depending on context) during the process of vessel maturation. Absence of CNS VSMCs can lead to endothelial hyperplasia, abnormal junctions, and excessive luminal membrane folds⁵⁶ which can lead to vascular stabilization and lethal aneurismal hemorrhaging.⁵⁷

Pdgfb/Pdgfr β

The Pdgfb and Pdgfr- β pathway plays a critical role in the recruitment of VSMCs to newly formed vessels.⁵⁸ Initially, VSMCs are recruited independent of PDGFR- β signaling;⁵⁹ however, as angiogenesis commences, sprouting endothelial cells secrete PDGFB, which signals through PDGFR- β expressed by VSMCs, resulting in their proliferation and migration during vessel maturation. In the postnatal mouse retina, PDGFB is expressed by the tip cells of the migrating vascular plexus, which generates a PDGFB gradient driving VSMC recruitment to stabilize the newly formed blood vessels as they penetrate into the CNS.¹⁷ This PDGFB gradient is essential for maturation and stabilization of the CNS vascular as evidenced by the *pdgfb* and *pdgfrb* knockout mice where the CNS is the most

affected vascular tissue.^{57,59} The high severity of vascular defect observed in the CNS—compared to other tissues—is most likely due to the inability of the CNS to undergo PDGF-independent de novo induction of VSMCs due to its lack of vasculogenesis-competent mesenchyme.⁶⁰

TGF β /endoglin/ALK1

The TGF β superfamily contains close to thirty members—including the bone morphogenetic proteins (BMPs), activins, inhibins, and myostatin. During angiogenesis, TGF β signaling is important for endothelial cell proliferation, differentiation, and recruitment of mural cells to neovessels. There is also evidence that TGF β modulates VEGF and Notch signaling through regulating expression of VEGFR-261 and Dll4,⁶² respectively, or through cross-talk TGF β downstream effectors with the Notch ICD.^{63,64}

Three isoforms of TGF β (TGF β 1-3) signal through two classes of serine-threonine kinase receptors. Upon ligand binding, type II receptors undergo a conformational change to activate their type I counterparts leading to activation of members of the smad protein family. Endothelial cells express the type I receptors Activin-like-receptor-1 (Alk1) and Alk5, the type II receptor TGF β RII, and the type III co-receptor Endoglin. Alk1 appears to be endothelial cell specific while Alk5 is expressed on many cell types including endothelial cells and perivascular support cells (VSMCs).^{60,65} Alk1 activates smad1, 5, and 8, which are important for proliferation while Alk5 activates smad2 and smad3, which are important for differentiation. Endoglin has been shown to potentiate TGF β signaling.^{66,67} Mice lacking *tgb1* or genes encoding its receptors *alk1*,^{68,69} *alk5*,⁷⁰ TGF β Receptor II (*tbrII*),⁷¹ and *endoglin*⁷² die from vascular defects in the yolk sac and elsewhere.

TGF β signaling is currently one of the more poorly understood pathways in the context of neuroangiogenesis; however, this pathway is thought to be important for vascularization of the CNS as human mutations in *endoglin* and *alk1* cause hereditary hemorrhagic telangiectasia (HHT) types 1 and 2, respectively.^{73,74} HHT is an autosomal dominant disorder affecting 1:8000 individuals and is characterized by dilated, tortuous blood vessels with thin walls that bleed easily. Cerebral arteriovenous malformations are a serious complication of HHT that can lead to severe hemorrhage, stroke, and brain abscess formation in patients.⁷⁵ Lesions in the brain seem to predominate in patients with HHT1 over HHT2. The majority of patients with HHT1 have mutation in *endoglin* that generate null alleles which lead to reduced levels of Endoglin protein on the surface of endothelial cells⁷⁵ while patients with HHT2 have either insertions, deletions, splice site mutations, or nonsense mutations that frequently lead to truncated ALK-1 proteins or missense mutations in the *alk1* gene.⁷⁶

Insight into the underlying etiology of HHT has been gained from Endoglin and Alk-1 deficient mice. Both of these mice lose structural, molecular, and functional distinction between arteries and veins.^{69,77} Endoglin deficient mice die at midgestation from defects in angiogenesis, mural cell recruitment, and vascular remodeling⁷² and heterozygous adult mice generally exhibit greater number of dilated venules and a large number of irregular vessels with reduced numbers of VSMCs.^{69,78} Endoglin signaling is important for autoinducible TGF β production by endothelial cells,⁷⁹ which induces differentiation of VSMCs from the surrounding mesenchyme and reinforces TGF β expression in the endothelial cells in an autoregulatory loop.^{60,75,80} Thus, the effects of *endoglin* gene deletion on development of hemorrhagic telangiectasias are two fold. First, loss of Endoglin leads to failure of the endothelium to recruit smooth muscle cells or pericytes, which leads to vascular instability. Failure to recruit pericytes can also lead to secondary defects of the vascular basement membrane,⁸¹ which also compromises the integrity of the vascular wall. Second, disruption of TGF β signaling alters endothelial cell biology and impedes their

ability to form durable vascular structures. Endothelial cells from HHT1 patients exhibit pronounced cytoskeletal defects that are thought to weaken the structural integrity of the vascular wall.^{75,82} Further, these cells do not form vascular tube structures in culture, indicative of a failure in the eNOS/hsp90 pathway.^{75,83} However, it still remains to be determined how reductions in Endoglin or ALK-1 leads to vascular lesions in the brain. The high degree of variability in the clinical courses of HHT patients amongst different strains⁷⁵ and differences in the severity of phenotype amongst different strains of Endoglin^{+/-} mice indicate the genetic factors or epigenetic modifiers that modulate disease severity that have yet to be discovered.⁷⁸

Notch-3 signaling

Within the CNS vasculature, Notch-3 is expressed by VSMCs. While Notch-3 is not required for vascularization of the CNS per se,⁸⁴ mice lacking Notch-3 have irregularly enlarged arteries that exhibit a less festooned elastica lamina and impaired cerebral blood flow.⁸⁵ In humans, mutations in the *Notch3* gene cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), an inherited cerebrovascular disease characterized by recurrent transient ischemic attacks, strokes, and vascular dementia amongst other neurological defects.⁸⁶ CADASIL patients show accumulation of electron dense granules in the media of the small arteries and extensive cerebral white matter lesions and subcortical infarcts attributed to progressive destruction of vascular smooth muscle cells.⁸⁷ While the molecular mechanism underlying how Notch-3 signaling regulates vascular smooth muscle cells on the arteries has not been determined, these findings implicate Notch-3 as important for vascular homeostasis in the CNS.

Angiopoietin/Tie2

The Tie2 receptor and its ligands Angiopoietin-1 (Ang1) and Angiopoietin-2 (Ang2) are important for angiogenesis and vascular remodeling. Ang1 stimulates signaling of Tie2 while Ang2 is antagonistic to Tie2 in endothelial cells.⁸⁸ Mice deficient in Tie2 die around E10.5 with blood vessels that are immature, lacking branching networks and proper organization into large and small vessels, and fail to recruit VSMCs.^{89,90} There is also an absence of the angiogenesis that vascularizes the neuroectoderm by capillary sprouting from the primitive vascular network.^{89,90} Mice lacking Ang1 have a similar phenotype.⁹¹ In the developing mouse retina, Ang1 message is increased at birth and decreases throughout retinal development,⁹² indicating that Ang1/Tie2 signaling contributes to retinal angiogenesis but is not required for maintenance of the CNS vasculature. Temporal inactivation of Tie2 and/or Ang1 during CNS vascularization will better determine the direct role Ang1/Tie2 signaling plays in CNS angiogenesis. Alternatively, Ang2 has been shown to be required for angiogenesis and proper vascular remodeling of the postnatal mouse retina.⁹³ The dual function of Ang2 to act as an inducer of both blood vessel growth and regression highlights the importance of context in the functioning of this molecule during retinal vascularization.

Conclusions and Future Directions

Vascularization of the CNS occurs via angiogenesis and is a process that coordinates the precise timing and location of multiple cell types to form a hierarchical vascular network with CNS-specific properties including blood-brain barrier formation, a high degree of VSMC coverage, reciprocal interactions with neurons and glia, and vascular niche for neural stem cells. Emphasis is now being placed on crosstalk amongst above described pathways⁶⁴ that converge to activate downstream effectors that are crucial for CNS angiogenesis such as the Id1/Id3 transcription factors, which demonstrate a forebrain hemorrhage phenotype when deleted in mice.⁹⁴

While the cellular and molecular mechanisms underlying neurangiogenesis are being well characterized, there is great potential to identify novel regulators of CNS angiogenesis and vascular specialization. For example, several genes—including *krit1*, *mgc4607*, and *pdc10* (programmed cell death-10)—have been identified as genetic causes of cerebral cavernous malformations (CCM); however, very little is known about the functions of these genes during developmental neuroangiogenesis.⁹⁵ Additionally, the *ndp* gene encoding norrin, which is a secreted protein that binds the wnt-receptor Frizzled-4, has been found to be associated with retinal telangiectasis.⁹⁶

Endothelial- or pericyte-specific G-Protein-Couple-Receptors (GPCRs) represent a novel class of molecules that has not been studied under the context of CNS angiogenesis despite intriguing vascular-associated functions seen in Edg-1,⁹⁷ PAR-1, and the GPR73a and GPR73b, which have been identified as receptors for the angiogenic growth factors endocrine-gland-derived VEGF (EG-VEGF) and Bv8.^{21,98,99} The relevance of orphan GPCRs to developmental CNS angiogenesis is under intensive investigation in our own laboratory. Additionally, microRNAs also represent a novel class of molecules that likely exert novel and as-yet unappreciated functions during CNS angiogenesis.^{100,101}

Another area of interest outside the scope of the current review pertains to the vascular biology of the CNS in the adult organism. For example, are CNS angiogenesis and development or regulation of the blood-brain barrier functionally coupled? How are the developmental neuroangiogenesis pathways observed in the embryonic brain and postnatal retina relevant to CNS vascular homeostasis in the adult? Answers to these and other questions pertaining to the CNS angiogenesis will provide great insight in helping develop therapies to treat many human diseases that are intimately tied to the CNS vasculature.

Coda

This review is dedicated to Dr. Judah Folkman, whose tragic passing away has saddened all in the vascular biology field for which he was a founding pioneer. It was Dr. Folkman's lecture at the 1996 Whitehead Symposium which inspired one of us (CJK) to study angiogenesis and, in fact, join the Folkman group at Children's Hospital, Boston in the late 1990s. Incredibly enough, while Dr. Folkman's seminal hypotheses and experimentation in tumor angiogenesis have paved the way towards effective cancer treatments for innumerable patients, these scientific accomplishments are perhaps dwarfed by his legendary humanity and generosity towards colleagues and patients alike. May future studies in the vascular biology field continue Dr. Folkman's rich scientific heritage in a manner that would have made him proud.

Acknowledgments

Support for this review was provided by NIH grants 1 R01 NS052830-01 and 1 R01 HL074267-01 (C. J. K.). MRM is a trainee of the Medical Scientist Training Program at Stanford University. The authors thank generous support from the Janower and Peabody Endowed Chairs of the Brain Tumor Society and the Center for Children's Brain Tumors at Stanford University.

Support provided by the National Institutes of Health, the Brain Tumor Society, and the Center for Children's Brain Tumors at Stanford University.

References

1. Risau, W. Development of the Vascular System of Organs and Tissues. Schaper, W.; Schaper, J., editors. Kluwer Academic Publishers; Boston: 1993. p. 17-28.
2. Risau W. Mechanisms of angiogenesis. *Nature*. 1997; 386:671–674. [PubMed: 9109485]

3. Louissaint A Jr, Rao S, Leventhal C, Goldman SA. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron*. 2002; 34:945–960. [PubMed: 12086642]
4. Raab S, et al. Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. *Thromb Haemost*. 2004; 91:595–605. [PubMed: 14983237]
5. Shen Q, et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science*. 2004; 304:1338–1340. [PubMed: 15060285]
6. Pardridge WM. Molecular biology of the blood-brain barrier. *Methods Mol Med*. 2003; 89:385–399. [PubMed: 12958435]
7. Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. *J Neurosci*. 2006; 26:13007–13016. [PubMed: 17167090]
8. Fabel K, et al. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *Eur J Neurosci*. 2003; 18:2803–2812. [PubMed: 14656329]
9. Dore-Duffy P. Isolation and characterization of cerebral microvascular pericytes. *Methods Mol Med*. 2003; 89:375–382. [PubMed: 12958434]
10. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis*. 2004; 16:1–13. [PubMed: 15207256]
11. Hogan KA, Ambler CA, Chapman DL, Bautch VL. The neural tube patterns vessels developmentally using the VEGF signaling pathway. *Development*. 2004; 131:1503–1513. [PubMed: 14998923]
12. Breier G, Risau W. The role of vascular endothelial growth factor in blood vessel formation. *Trends Cell Biol*. 1996; 6:454–456. [PubMed: 15157501]
13. Breier G, Albrecht U, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. *Development*. 1992; 114:521–532. [PubMed: 1592003]
14. Herken R, Gotz W, Wattjes KH. Initial development of capillaries in the neuroepithelium of the mouse. *J Anat*. 1989; 164:85–92. [PubMed: 2606797]
15. Saint-Geniez M, D'Amore PA. Development and pathology of the hyaloid, choroidal and retinal vasculature. *Int J Dev Biol*. 2004; 48:1045–1058. [PubMed: 15558494]
16. Fruttiger M. Development of the retinal vasculature. *Angiogenesis*. 2007; 10:77–88. [PubMed: 17322966]
17. Gerhardt H, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol*. 2003; 161:1163–1177. [PubMed: 12810700]
18. Gariano RF, Gardner TW. Retinal angiogenesis in development and disease. *Nature*. 2005; 438:960–966. [PubMed: 16355161]
19. Haigh JJ, et al. Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. *Dev Biol*. 2003; 262:225–241. [PubMed: 14550787]
20. Ridley AJ, et al. Cell migration: integrating signals from front to back. *Science*. 2003; 302:1704–1709. [PubMed: 14657486]
21. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003; 9:669–676. [PubMed: 12778165]
22. Matsumoto T, Mugishima H. Signal transduction via vascular endothelial growth factor (VEGF) receptors and their roles in atherogenesis. *J Atheroscler Thromb*. 2006; 13:130–135. [PubMed: 16835467]
23. Dumont DJ, et al. Vascularization of the mouse embryo: a study of flk-1, tek, tie, and vascular endothelial growth factor expression during development. *Dev Dyn*. 1995; 203:80–92. [PubMed: 7647376]
24. Millauer B, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell*. 1993; 72:835–846. [PubMed: 7681362]
25. Ogunshola OO, et al. Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain. *Brain Res Dev Brain Res*. 2000; 119:139–153.

26. Ruhrberg C, et al. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes Dev.* 2002; 16:2684–2698. [PubMed: 12381667]
27. Kawasaki T, et al. A requirement for neuropilin-1 in embryonic vessel formation. *Development.* 1999; 126:4895–4902. [PubMed: 10518505]
28. Gu C, et al. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. *Dev Cell.* 2003; 5:45–57. [PubMed: 12852851]
29. Gerhardt H, et al. Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. *Dev Dyn.* 2004; 231:503–509. [PubMed: 15376331]
30. Hellstrom M, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature.* 2007; 445:776–780. [PubMed: 17259973]
31. Fleming RJ. Structural conservation of Notch receptors and ligands. *Semin Cell Dev Biol.* 1998; 9:599–607. [PubMed: 9918871]
32. Lubman OY, Korolev SV, Kopan R. Anchoring notch genetics and biochemistry; structural analysis of the ankyrin domain sheds light on existing data. *Mol Cell.* 2004; 13:619–626. [PubMed: 15023333]
33. Iso T, Hamamori Y, Kedes L. Notch signaling in vascular development. *Arterioscler Thromb Vasc Biol.* 2003; 23:543–553. [PubMed: 12615665]
34. Karsan A. The role of notch in modeling and maintaining the vasculature. *Can J Physiol Pharmacol.* 2005; 83:14–23. [PubMed: 15759046]
35. Gordon WR, et al. Structural basis for autoinhibition of Notch. *Nat Struct Mol Biol.* 2007; 14:295–300. [PubMed: 17401372]
36. Chitnis A. Why is delta endocytosis required for effective activation of notch? *Dev Dyn.* 2006; 235:886–894. [PubMed: 16425217]
37. Sainson RC, et al. Cell-autonomous notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis. *Faseb J.* 2005; 19:1027–1029. [PubMed: 15774577]
38. Gale NW, et al. Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development. *Proc Natl Acad Sci U S A.* 2004; 101:15949–15954. [PubMed: 15520367]
39. Ferrara N, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature.* 1996; 380:439–442. [PubMed: 8602242]
40. Carmeliet P, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature.* 1996; 380:435–439. [PubMed: 8602241]
41. Lobov IB, et al. Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. *Proc Natl Acad Sci U S A.* 2007; 104:3219–3224. [PubMed: 17296940]
42. Noguera-Troise I, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Novartis Found Symp.* 2007; 283:106–120. discussion 121–105. [PubMed: 18300417]
43. Ridgway J, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature.* 2006; 444:1083–1087. [PubMed: 17183323]
44. Taylor KL, Henderson AM, Hughes CC. Notch activation during endothelial cell network formation in vitro targets the basic HLH transcription factor HESR-1 and downregulates VEGFR-2/KDR expression. *Microvasc Res.* 2002; 64:372–383. [PubMed: 12453432]
45. Williams CK, Li JL, Murga M, Harris AL, Tosato G. Up-regulation of the Notch ligand Delta-like 4 inhibits VEGF-induced endothelial cell function. *Blood.* 2006; 107:931–939. [PubMed: 16219802]
46. Kidd T, et al. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell.* 1998; 92:205–215. [PubMed: 9458045]
47. Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. *Nature.* 2005; 436:193–200. [PubMed: 16015319]
48. Andrews WD, Barber M, Parnavelas JG. Slit-Robo interactions during cortical development. *J Anat.* 2007; 211:188–198. [PubMed: 17553100]

49. Park KW, et al. Robo4 is a vascular-specific receptor that inhibits endothelial migration. *Dev Biol.* 2003; 261:251–267. [PubMed: 12941633]
50. Jones CA, et al. Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. *Nat Med.* 2008; 14:448–453. [PubMed: 18345009]
51. Proctor JM, Zang K, Wang D, Wang R, Reichardt LF. Vascular development of the brain requires beta8 integrin expression in the neuroepithelium. *J Neurosci.* 2005; 25:9940–9948. [PubMed: 16251442]
52. McCarty JH, et al. Selective ablation of alphav integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. *Development.* 2005; 132:165–176. [PubMed: 15576410]
53. Zhu J, et al. beta8 integrins are required for vascular morphogenesis in mouse embryos. *Development.* 2002; 129:2891–2903. [PubMed: 12050137]
54. Bader BL, Rayburn H, Crowley D, Hynes RO. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell.* 1998; 95:507–519. [PubMed: 9827803]
55. McCarty JH, et al. Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins. *Mol Cell Biol.* 2002; 22:7667–7677. [PubMed: 12370313]
56. Hellstrom M, et al. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol.* 2001; 153:543–553. [PubMed: 11331305]
57. Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science.* 1997; 277:242–245. [PubMed: 9211853]
58. Hoch RV, Soriano P. Roles of PDGF in animal development. *Development.* 2003; 130:4769–4784. [PubMed: 12952899]
59. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development.* 1999; 126:3047–3055. [PubMed: 10375497]
60. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res.* 2005; 97:512–523. [PubMed: 16166562]
61. Minami T, Rosenberg RD, Aird WC. Transforming growth factor-beta 1—mediated inhibition of the flk-1/KDR gene is mediated by a 5′-untranslated region palindromic GATA site. *J Biol Chem.* 2001; 276:5395–5402. [PubMed: 11098056]
62. Niimi H, Pardali K, Vanlandewijck M, Heldin CH, Moustakas A. Notch signaling is necessary for epithelial growth arrest by TGF-beta. *J Cell Biol.* 2007; 176:695–707. [PubMed: 17325209]
63. Blokzijl A, et al. Cross-talk between the Notch and TGF-beta signaling pathways mediated by interaction of the Notch intracellular domain with Smad3. *J Cell Biol.* 2003; 163:723–728. [PubMed: 14638857]
64. Holderfield MT, Hughes CC. Crosstalk between vascular endothelial growth factor, notch, and transforming growth factor-beta in vascular morphogenesis. *Circ Res.* 2008; 102:637–652. [PubMed: 18369162]
65. Roelen BA, van Rooijen MA, Mummery CL. Expression of ALK-1, a type 1 serine/threonine kinase receptor, coincides with sites of vasculogenesis and angiogenesis in early mouse development. *Dev Dyn.* 1997; 209:418–430. [PubMed: 9264265]
66. Lebrin F, et al. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *Embo J.* 2004; 23:4018–4028. [PubMed: 15385967]
67. Goumans MJ, Lebrin F, Valdimarsdottir G. Controlling the angiogenic switch: a balance between two distinct TGF-b receptor signaling pathways. *Trends Cardiovasc Med.* 2003; 13:301–307. [PubMed: 14522471]
68. Oh SP, et al. Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. *Proc Natl Acad Sci USA.* 2000; 97:2626–2631. [PubMed: 10716993]
69. Urness LD, Sorensen LK, Li DY. Arteriovenous malformations in mice lacking activin receptor-like kinase-1. *Nat Genet.* 2000; 26:328–331. [PubMed: 11062473]

70. Larsson J, et al. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *Embo J*. 2001; 20:1663–1673. [PubMed: 11285230]
71. Oshima M, Oshima H, Taketo MM. TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol*. 1996; 179:297–302. [PubMed: 8873772]
72. Li DY, et al. Defective angiogenesis in mice lacking endoglin. *Science*. 1999; 284:1534–1537. [PubMed: 10348742]
73. Johnson DW, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*. 1996; 13:189–195. [PubMed: 8640225]
74. McAllister KA, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet*. 1994; 8:345–351. [PubMed: 7894484]
75. Bobik A. Transforming growth factor-betas and vascular disorders. *Arterioscler Thromb Vasc Biol*. 2006; 26:1712–1720. [PubMed: 16675726]
76. Harrison RE, et al. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet*. 2003; 40:865–871. [PubMed: 14684682]
77. Sorensen LK, Brooke BS, Li DY, Urness LD. Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific TGFbeta coreceptor. *Dev Biol*. 2003; 261:235–250. [PubMed: 12941632]
78. Bourdeau A, Faughnan ME, Letarte M. Endoglin-deficient mice, a unique model to study hereditary hemorrhagic telangiectasia. *Trends Cardiovasc Med*. 2000; 10:279–285. [PubMed: 11343967]
79. Carvalho RL, et al. Defective paracrine signalling by TGF-beta in yolk sac vasculature of endoglin mutant mice: a paradigm for hereditary haemorrhagic telangiectasia. *Development*. 2004; 131:6237–6247. [PubMed: 15548578]
80. Lebrin F, Deckers M, Bertolino P, Ten Dijke P. TGF-beta receptor function in the endothelium. *Cardiovasc Res*. 2005; 65:599–608. [PubMed: 15664386]
81. Torsney E, et al. Mouse model for hereditary hemorrhagic telangiectasia has a generalized vascular abnormality. *Circulation*. 2003; 107:1653–1657. [PubMed: 12668501]
82. Letarte M, et al. Reduced endothelial secretion and plasma levels of transforming growth factor-beta1 in patients with hereditary hemorrhagic telangiectasia type 1. *Cardiovasc Res*. 2005; 68:155–164. [PubMed: 15907823]
83. Toporsian M, et al. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. *Circ Res*. 2005; 96:684–692. [PubMed: 15718503]
84. Krebs LT, et al. Characterization of Notch3—deficient mice: normal embryonic development and absence of genetic interactions with a Notch1 mutation. *Genesis*. 2003; 37:139–143. [PubMed: 14595837]
85. Domenga V, et al. Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. *Genes Dev*. 2004; 18:2730–2735. [PubMed: 15545631]
86. Joutel A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996; 383:707–710. [PubMed: 8878478]
87. Ruchoux MM, et al. Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Acta Neuropathol*. 1995; 89:500–512. [PubMed: 7676806]
88. Maisonpierre PC, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science*. 1997; 277:55–60. [PubMed: 9204896]
89. Sato TN, et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature*. 1995; 376:70–74. [PubMed: 7596437]
90. Dumont DJ, et al. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev*. 1994; 8:1897–1909. [PubMed: 7958865]
91. Suri C, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996; 87:1171–1180. [PubMed: 8980224]

92. Hackett SF, Wiegand S, Yancopoulos G, Campochiaro PA. Angiopoietin-2 plays an important role in retinal angiogenesis. *J Cell Physiol.* 2002; 192:182–187. [PubMed: 12115724]
93. Gale NW, et al. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. *Dev Cell.* 2002; 3:411–423. [PubMed: 12361603]
94. Lyden D, et al. Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. *Nature.* 1999; 401:670–677. [PubMed: 10537105]
95. Wang QK. Update on the molecular genetics of vascular anomalies. *Lymphat Res Biol.* 2005; 3:226–233. [PubMed: 16379592]
96. Black GC, et al. Coats' disease of the retina (unilateral retinal telangiectasis) caused by somatic mutation in the NDP gene: a role for norrin in retinal angiogenesis. *Hum Mol Genet.* 1999; 8:2031–2035. [PubMed: 10484772]
97. Liu Y, et al. Edg-1, the G protein-coupled receptor for sphingosine-1—phosphate, is essential for vascular maturation. *J Clin Invest.* 2000; 106:951–961. [PubMed: 11032855]
98. Abbott N. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat.* 2002; 200:527. [PubMed: 17103724]
99. Ferrara N, LeCouter J, Lin R, Peale F. EG-VEGF and Bv8: a novel family of tissue-restricted angiogenic factors. *Biochim Biophys Acta.* 2004; 1654:69–78. [PubMed: 14984768]
100. Kuehbach A, Urbich C, Dimmeler S. Targeting microRNA expression to regulate angiogenesis. *Trends Pharmacol Sci.* 2008; 29:12–15. [PubMed: 18068232]
101. Chen Y, Gorski DH. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. *Blood.* 2008; 111:1217–1226. [PubMed: 17957028]
102. Stenman JM, et al. Canonical signaling regulates organ-specific assembly and differentiation of CNS vasculature. *Science.* 2008; 322:1247–1250. [PubMed: 19023080]
103. Liebner S, et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *J Cell Biol.* 2008; 183:409–417. [PubMed: 18955553]