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Cell adhesion and signaling networks in brain neurovascular units

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Abstract

Purpose of review—Central nervous system (CNS) neurovascular units are multicellular complexes consisting of neurons and astrocytes, vascular endothelial cells and pericytes, as well as an assortment of growth factors and extracellular matrix (ECM) proteins. Here, I will discuss the current knowledge of signaling networks essential for the development and physiology of CNS neurovascular units, particularly in the brain.

Recent findings—Molecular genetic studies have identified various signaling proteins that regulate the formation and function of CNS neurovascular units. These include members of the integrin family of ECM adhesion receptors, ECM proteins such as Wnts and latent transforming growth factor β s, and various transcriptional regulators, including β -catenin and the inhibitors of DNA binding (Ids).

Summary—Neurovascular units are the cellular and molecular interfaces between the circulatory system and the CNS. Recent molecular genetic analyses in mice and other model organisms have revealed the first mechanisms underlying bidirectional communication between neural and vascular components. In particular, ECM-mediated adhesion and signaling pathways have been identified as essential for neurovascular development and physiology. Understanding how these various gene products normally control neurovascular unit formation and function will lend new insights into the causes and possible treatments of debilitating neurovascular-related diseases such as birth defects, stroke, and age-related dementia.

Keywords

angiogenesis; band 4.1 proteins; $\beta 8$ integrin; neurogenesis; transforming growth factor β ; vascular niche

Introduction

The mammalian circulatory system is composed of an elaborate array of arteries, veins, and capillaries that transport cells and blood through mostly every organ and tissue of the body. The traditional view of the circulatory system as a mundane, static network of blood vessels has been challenged in recent years by the discovery that both vascular cells and vascular-derived molecules dynamically modulate cell growth, survival, and differentiation in a diverse array of embryonic and postnatal organs [1–3,4•]. Here, I will focus exclusively on the roles of blood vessels in regulating neural cell functions in the vertebrate central nervous system

(CNS). A specific emphasis will be placed on mechanisms of bidirectional neural–vascular cell adhesion and communication in the embryonic brain.

The human CNS, consisting of hundreds of billions of neurons and glia and their myriad connections, is interlaced with a complex web of blood vessels [5,6]. In addition to providing vital oxygen and nutrients to meet the high metabolic demands of the CNS, blood vessels dynamically communicate with neurons and glia, and these events are essential for normal CNS formation and function [7,8]. In turn, neural cells and neural-derived cues influence many vascular functions, including modulation of blood vessel dilation and constriction [9,10], as well as homeostatic regulation of the blood–brain barrier (BBB) [11,12]. At the cellular and molecular levels, communication between the circulatory system and the CNS occurs within integrated, multicellular structures, termed neurovascular units [13]. The major objective of this review is to summarize the current knowledge of signaling pathways necessary for the development and physiology of neurovascular units as well as to relate some of these findings to CNS neurovascular disorders.

Neural stem and progenitor cells regulate neurovascular development during embryogenesis

Neovascularization of the mammalian CNS occurs entirely via angiogenesis [14]. Bidirectional communication between neural cells and endothelial cells initiates at early stages of CNS development [15–17], when angiogenic blood vessels invade the CNS parenchyma in response to a gradient of neural-derived vascular endothelial growth factor (VEGF) [18,19]. Upon entering the CNS parenchyma, blood vessels migrate along a preformed latticework of neuroepithelia and radial glia [20,21], which are neural stem and progenitor cells that give rise to differentiated neurons and astrocytes in the CNS [22]. Cell–cell communication between blood vessels and radial glia occurs primarily via intervening vascular basement membranes that contain a milieu of growth factors and extracellular matrix (ECM) proteins [4,23] (Fig. 1). Recent molecular genetic analyses in mice have uncovered critical insights about neural and vascular communication within neurovascular units, particularly related to cell–ECM adhesion and signaling. Some examples of these experimental findings are summarized below.

Wnts are lipid-modified, ECM-associated proteins that modulate intracellular signaling cascades via their Frizzled and low-density lipoprotein receptor-related protein (LRP) cell surface receptors [24]. Canonical Wnt signaling involves stabilization and activation of the transcription factor β -catenin, which in the absence of Wnt receptor engagement is targeted for degradation by the proteasome. Stenman *et al.* [25••] have shown that ablation of both the *Wnt7a* and *Wnt7b* genes in mouse CNS neural stem and progenitor cells leads to lethal neurovascular phenotypes, including abnormal BBB maturation and CNS-specific hemorrhage. Liebner *et al.* [26••] have also shown that Wnt stimulation induces BBB-like properties in cultured endothelial cells, and these events are dependent on canonical β -catenin signaling. Genetic ablation of β -catenin in vascular endothelial cells also induces CNS neurovascular disorders; however, these phenotypes appear more severe than those in the *Wnt7a* and *Wnt7b* double knockouts [25••], suggesting that Wnt-independent β -catenin signaling pathways are also important in endothelial cells [27]. Collectively, these findings reveal that canonical Wnt signaling pathways, in part, mediate neurovascular development during mouse embryogenesis.

Other molecular genetic studies in mice have identified transcriptional regulators as essential for proper neurovascular unit development during embryogenesis. For example, combined deletion of the Id1 and Id3 transcription factors causes CNS-specific neurovascular abnormalities and embryonic lethality [28]. Id1/Id3 mutants display premature differentiation of CNS radial glial cells, leading to increased numbers of neurons. This raises the interesting

possibility that radial glial cells, which also serve as scaffolds for angiogenic blood vessels in the CNS, may be progressively depleted in Id1/Id3 mutant mice, thus contributing to abnormal blood vessel morphogenesis and hemorrhage. However, Id1 and Id3 likely provide additional functions in CNS neurovascular development, as induction of abnormal radial glial differentiation via other pathways, such as Notch1 activation, is not reported to induce obvious neurovascular disorders [29].

Zebrafish genetics have also proven to be a powerful tool for uncovering mechanisms of neurovascular unit development [30]. Liu *et al.* [31••] have characterized signaling defects in the zebrafish bubblehead mutant, which develop embryonic CNS-specific neurovascular phenotypes. These phenotypes are due to mutations in the β -Pix gene, leading to defective interactions between β -Pix protein, a GTP exchange factor, and the p21-activated kinase 2a (Pak2a). As a result, Pak2a is mislocalized within the cell and does not effectively activate the Rac and Cdc42 small GTPases at the plasma membrane. It remains to be determined whether the signaling defects in bubblehead mutants occur in vascular cells or perivascular neural cells. Furthermore, it will be interesting to identify upstream effectors of the β -Pix and Pak2a signaling pathway.

The α v β 8 integrin – transforming growth factor β signaling axis in neurovascular development

Integrins are heterodimeric cell surface proteins consisting of α and β subunits that serve as receptors for many ECM proteins [32]. Many integrins play critical roles in adult CNS neurovascular units [33]; however, various molecular genetic data reveal that α v β 8 integrin and its ECM ligands, the latent transforming growth factor (TGF) β s, are absolutely essential for embryonic neurovascular development [34]. β 8 integrin is reported to pair only with the α v subunit [35,36], and mouse embryos harboring ablated α v or β 8 integrin genes in all cells develop CNS-specific neurovascular disorders that include abnormal blood vessel morphogenesis and hemorrhage [37,38]. Cre-mediated ablation of α v or β 8 integrin genes in CNS neural stem and progenitor cells also leads to similar CNS-specific neurovascular phenotypes [39,40]. In contrast, deletion of the α v or β 8 integrin genes in vascular endothelial cells does not cause obvious CNS vascular disorders [39,40]; however, these mice develop autoimmunity and cancer due to dendritic cell dysfunction [41•,42•]. The neurovascular phenotypes in α v and β 8 integrin mutant embryos are mostly resolved in the postnatal period, although adult mutant mice develop progressive neurological abnormalities likely because of the prior neurovascular damage. Interestingly, repair of these severe neurovascular disorders occurs within a postnatal period when CNS blood vessels transition from an angiogenic to a quiescent status [14], suggesting that α v β 8 integrin provides essential roles in modulating active phases of CNS angiogenesis similar to those that occur during embryonic development.

α v β 8 integrin is reported to bind to multiple ECM ligands [43–45]; however, the neurovascular disorders in α v and β 8 integrin mutant mice are due primarily to deficient integrin-mediated TGF β activation [46]. TGF β s are produced by cells as inactive, ECM-associated complexes [47]. Dissociation of the bioactive portions, TGF β s, from latency-associated peptides (LAPs) leads to release from the ECM, cell surface receptor engagement, and activation of intracellular signaling cascades via the canonical SMADs as well as other noncanonical effectors [48]. LAPs for TGF β 1 and TGF β 3 contain arginine–glycine–aspartic acid (RGD) peptide sequences that serve as integrin-binding motifs. Although all five α v-containing integrins can adhere to LAP–TGF β s via these RGD sequences, only α v β 6 and α v β 8 integrin have been reported to activate TGF β s [49,50]. The functional significance of integrin-mediated TGF β activation has been demonstrated using gene ‘knock-in’ technology, in which the RGD sequence within the endogenous *TGF β 1* gene was mutated to RGE, resulting in inhibition of integrin adhesion. These mutant mice develop lethal phenotypes that are identical to those observed in *TGF β 1*–/–

– mice, revealing that integrins play central roles in TGF β activation during embryogenesis [51••]. Furthermore, mice deficient for both active TGF β 1 and genetically null for TGF β 3 develop CNS-specific neurovascular phenotypes that are nearly identical to those observed in α v and β 8 integrin knockout mice [52••]. TGF β s modulate intracellular signal transduction pathways primarily via cell transmembrane receptor serine/threonine kinases [53]; however, neuropilin-1 (NRP1), a receptor for semaphorins and VEGF [54], was recently reported to bind to latent and bioactive forms of TGF β 1 [55••]. This is intriguing, because genetic ablation of the *NRP1* gene in mice leads to CNS neurovascular phenotypes that are similar to phenotypes in α v β 8 integrin and TGF β 1/TGF β 3 mutants [56,57]. It will be fascinating to determine whether integrin-activated TGF β s regulate neurovascular development via only the canonical TGF β receptors or whether NRP1 also plays a role in these processes.

Neurovascular phenotypes that develop in mutant mice lacking components of the α v β 8 integrin and TGF β signaling axis are quite similar to those reported for the various mouse and zebrafish mutants described above, suggesting possible functional links between these pathways (Fig. 1). For example, intracellular cross-talk may take place between TGF β and Wnt-mediated signaling pathways within cerebral endothelial cells, as has been shown in other cell types [58]. The Band 4.1s are a family of cytoskeletal adaptor proteins that directly interact with the β 8 integrin cytoplasmic tail [59,60].

Although the functional significance of the β 8 integrin–Band 4.1 associations have not been determined, it is enticing to speculate that Band 4.1s, which contain FERM domains that mediate protein–protein interactions [61], may serve to couple β 8 integrin with other signaling effectors. This is particularly intriguing for the β Pix, Pak2a, and Rac signaling pathway identified in the zebrafish bubblehead mutant [31••], as β 8 integrin has been reported to activate Rac signaling [62]. An exciting future challenge will be to decipher how these different signaling pathways are functionally connected during neurovascular unit development and physiology in the CNS.

Neurovascular units are niches for neural stem and progenitor cells in the adult brain

Neurogenesis in the adult mammalian brain occurs in two specific regions: the subgranular layer (SGL) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle. Neural stem cells within these regions display many radial glial-like properties [63] and intimately associate with specialized neurovascular structures, termed vascular niches [64,65]. Neural stem cell vascular niches were first discovered in the SGL by Palmer *et al.* [66] during efforts to quantify adult stem cell self-renewal and differentiation. These studies revealed DNA synthesis in both SGL neural stem cells as well as capillary endothelial cells; furthermore, these authors found that most neural stem cells within the SGL were preferentially clustered within 10 μ m of angiogenic capillaries. These data suggested an important functional relationship between neural stem cells and cerebral blood vessels, with vascular-derived factors likely influencing stem cell growth and fate determination. Indeed, Lie *et al.* [67] subsequently showed that Wnts, possibly secreted by vascular cells in the SGL niche, induce canonical signaling cascades within neural stem cells that are essential for proper self-renewal and differentiation.

More recent studies have shown that neural stem cells also localize to vascular niches within the SVZ. Tavazoie *et al.* [68••] have reported the intriguing finding that SVZ neural stem cells preferentially associate with capillary regions displaying enhanced BBB permeability, suggesting cells or factors from the circulation are important for modulating neural stem cell behaviors. It will be fascinating to determine what these factors are and the mechanisms underlying how their access to neural stem cells is regulated at the temporal and spatial levels.

Ultrastructural analyses by Mercier *et al.* [69] first showed that blood vessels within the SVZ extend specialized basal laminar structures, termed fractones, that are enriched in heparin sulfate proteoglycans, laminins, and probably other ECM proteins. These authors also reported that fractones directly contact neural stem cells and postulated that these interactions regulate cell behaviors via ECM adhesion as well as presentation of growth factors such as basic fibroblast growth factor [70••]. Shen *et al.* [71••] have shown that SVZ neural stem cells utilize $\alpha 6 \beta 1$ integrin to bind to laminins in vascular basement membranes and fractones. Inhibition of $\alpha 6 \beta 1$ integrin-mediated adhesion and signaling *in vivo* promotes neural stem cell proliferation, indicating that stem cell interactions with laminins in vascular niches suppress neural stem cell expansion. $\alpha 6 \beta 1$ is also utilized by embryonic neural stem cells to adhere to laminins, suggesting shared regulatory mechanisms between embryonic and adult stem cell niches [72•,73].

My laboratory has recently identified $\beta 8$ integrin as a critical regulator of neurovascular homeostasis and neurogenesis in the adult mouse brain [74]. $\beta 8$ integrin is expressed in cultured SVZ neural stem cells, and ablation of $\beta 8$ gene expression *in vivo* results in reduced SVZ cell proliferation and survival, likely owing to uncoupling of neural stem cells from ECM components in the vascular niche. As described above, $\alpha v \beta 8$ integrin regulates blood vessel morphogenesis and neurovascular development in the embryonic brain, and these processes are dependent on integrin-mediated TGF β activation. It will be interesting to determine how $\beta 8$ integrin-mediated TGF β activation is involved in regulating neural stem cell functions in vascular niches of the adult brain.

Conclusion

Neurovascular units are the cellular and molecular interfaces between the circulatory system and CNS. In recent years, various gene knockout strategies in mice – most of which were not initially aimed at studying neurovascular biology – have yielded the first insights about pathways important for neurovascular development and homeostasis. Large-scale mutagenesis screens in mice [75,76] will be necessary for identifying gene networks involved in neurovascular formation and function. Genomic screens to identify neurovascular mutants in the zebrafish should also provide important mechanistic insights. Gaining a comprehensive understanding of the molecular pathways underlying neurovascular unit development will provide novel insights into the causes and possible treatments of debilitating human neurovascular-related diseases such as birth defects, stroke, cancer, and age-related neurodegeneration.

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References and recommended reading

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- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 231).

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Figure 1. A summary of adhesion and signaling pathways in neurovascular units

(a) The multicellular composition of a central nervous system (CNS) neurovascular unit composed of vascular endothelial cells and pericytes, neurons, and glia. Extracellular matrix (ECM)-rich basement membranes separate the various neural and vascular cell types. (b) A more detailed view of the boxed area in (a) showing a vascular endothelial cell and perivascular glial cell separated by a vascular basement membrane. The $\alpha v\beta 8$ integrin–transforming growth factor (TGF) β adhesion and signaling axis is essential for proper linkage between perivascular glial cells and vascular endothelial cells within neurovascular units. Paracrine TGF β signaling events in endothelial cells likely involve the SMADs. In perivascular cells, $\alpha v\beta 8$ integrin interacts with the Band 4.1s, which may link this integrin with multiple intracellular signaling effectors, including β -Pix. Wnt7a and Wnt7b are ECM-associated proteins expressed by neural cells of the CNS parenchyma that regulate the development of the neurovascular unit. Wnt-mediated signaling pathways require β -catenin in vascular endothelial cells. BBB, blood–brain barrier.