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Central nervous system pericytes in health and disease

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Pericytes are uniquely positioned within the neurovascular unit to serve as vital integrators, coordinators and effectors of many neurovascular functions, including angiogenesis, blood-brain barrier (BBB) formation and maintenance, vascular stability and angioarchitecture, regulation of capillary blood flow and clearance of toxic cellular byproducts necessary for proper CNS homeostasis and neuronal function. New studies have revealed that pericyte deficiency in the CNS leads to BBB breakdown and brain hypoperfusion resulting in secondary neurodegenerative changes. Here we review recent progress in understanding the biology of CNS pericytes and their role in health and disease.

In mammals, especially humans, proper cerebrovascular function is essential for the homeostasis and survival of the CNS. To efficiently meet the demands of highly metabolic nervous tissue, an intricate and highly evolved network of branching conduits comprising the cerebrovascular tree has developed. The cerebrovascular tree originates from large, interconnected arteries forming the circle of Willis at the base of the brain. These arteries sequentially divide, giving rise to pial arteries, penetrating intracerebral arteries and, finally, arterioles and a vast capillary network once inside the parenchyma. To understand the importance of cerebrovascular function, one need look no further than its structure. In the adult human, it is estimated that the total perfused cerebral vascular length is approximately 600–700 km (ref. 1). In small mammals, such as the mouse, the distance from each neuronal cell body to a neighboring capillaries is ~15 μm (ref. 2).

The cerebrovasculature is not simply a passive conduit, but rather a highly dynamic multicellular structure capable of integrating and responding to both systemic and neural cues. At the core of its proper functionality is the intimately connected and highly coordinated neurovascular unit (NVU) comprising endothelial cells, pericytes at the capillary level, vascular smooth muscle cells (VSMCs) at the arterial level, astrocytes, microglia and neurons (**Fig. 1**). Proper communication and functional interdependence of these diverse, but equally important, cell types is essential for effective CNS homeostasis. Furthermore, miscommunication and malfunction of members of the NVU are important in many neurologic diseases[3–6]. The present review will focus on the rapidly evolving roles of CNS pericytes in health and disease.

Pericytes and the neurovascular unit

Pericytes are uniquely positioned within the NVU, serving as vital integrators, coordinators and effectors of neurovascular functions including regulation of blood brain barrier (BBB) permeability[7–9], regulation of cerebral blood flow (CBF)[9,10] and clearance of toxic cellular byproducts[5,9–11]. Despite their discovery almost 150 years ago[11], most of the insights into pericyte biology have come from relatively recent studies.

Anatomically, pericytes are located directly on the capillary wall and share a common basement membrane with endothelial cells (**Fig. 1**). Both pericytes and endothelial cells are attached to extracellular matrix proteins of the basement membrane by different intregins[11,12]. Pericytes project elongated, stellate-shaped finger-like processes that ensheath the capillary wall. In areas lacking a basement membrane, interdigitations of pericyte and endothelial cell membranes make direct peg-and-socket contacts containing cell-to-cell junction proteins. These include N-cadherin, the adherens junction protein[13–15] and the connexin-43 (CX43) hemichannels that form gap junctions allowing transfer of nutrients, metabolites, secondary messengers and ions between the two cell types[16] (**Fig. 1**, inset). Connexins CX43 and CX30 contribute to astrocyte-endothelial and astrocyte-neuronal gap junctions[17]. At some points of contact, adhesion plaques composed predominately of fibronectin mediate the connection of the basement membrane to the plasma membrane and the underlying actin cytoskeletal networks of pericytes and endothelium[11]. CNS endothelial cells are connected with each other by different types of tight and adherens junctions, forming the BBB[5]. The maintenance of the adherens, gap and tight junctions between different cell types within the NVU is essential for CNS vascular homeostasis[5,9,14–16]. The CNS endothelium has significantly higher pericyte coverage[7–9] than peripheral tissues[18], suggesting that pericytes may have heightened functional importance in the CNS.

Origin of CNS pericytes

Effective establishment of microvascular networks in the developing brain, spinal cord and retina are required for normal CNS growth, maturation and function. Initial CNS vascularization occurs through invading, sprouting angiogenesis (branching of existing vessels) that originates from a juxtaposed, mesoderm-derived perineural vascular plexus located outside the CNS[19]. As endothelial cells penetrate the embryonic neural tube and migrate through the neuropil toward the ventricles, they secrete molecular cues that recruit pericytes to

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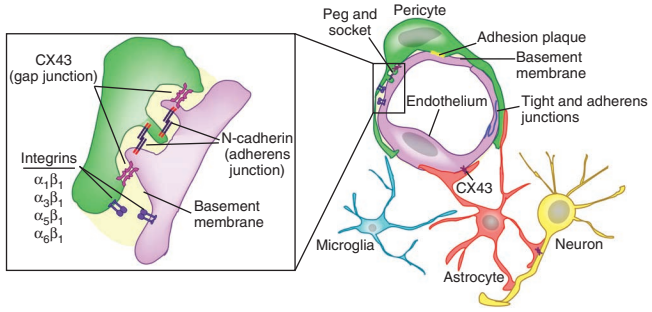

Figure 1 Structural and molecular pericyte connections within the neurovascular unit. Right: pericytes (green) and endothelial cells (purple) are connected to a shared basement membrane (yellow) by several types of integrin molecule. In areas lacking the basement membrane, interdigitations of pericyte and endothelial cell membranes, called peg and socket contacts, form direct connections and contain several different transmembrane junctional proteins (inset). N-cadherin is the key adherens junction protein between pericytes and endothelium. Pairs of connexin 43 (CX43) hemichannels expressed respectively in pericytes and endothelium form gap junctions that allow transfer of molecules between pericytes and endothelial cells. Adhesion plaques similar to desmosomes contain fibronectin deposits in the intercellular spaces between pericytes and endothelial cells. CX43 is also abundant at astrocyte–endothelial cell and astrocyte-neuron interfaces. Different types of tight junction proteins, tight junction adaptor proteins and adhesion junctions regulate direct endothelial cell–endothelial cell contacts forming the anatomical blood-brain barrier.

the nascent capillary tube. CNS pericytes originate from both mesoderm-derived mesenchymal stem cells and neuroectoderm-derived neural crest cells, depending on the location within the developing cerebrovascular tree (**Fig. 2**). Critical insights into the distinct developmental origins of CNS pericytes have come from a series of avian chimerization studies in which quail neuroectoderm or mesoderm were transplanted into developing chick embryos. Transplanted neuroectoderm was found to give rise to pericytes in the forebrain, whereas transplanted mesoderm gives rise to pericytes in the midbrain, brainstem, spinal cord and peripheral organs[20–22].

During embryonic development and the early postnatal period, the CNS relies on the proliferative expansion of preexisting pericyte pools, a process termed longitudinal recruitment[23–25] (**Fig. 2**). At the site of angiogenesis, endothelial cells stimulate the proliferation, migration and attachment of pericytes along the adjacent endothelial tube[23–28]. Whether this process continues during vascular remodeling in the adult and aging CNS has yet to be experimentally proven. More recently, circulating mesoderm-derived bone marrow progenitor cells have been shown to contribute to adult CNS pericyte pools after ischemia[29–31]. The relative contributions of circulating pericyte progenitor cells and local, preexisting CNS pericyte pools to renewal of pericytes in the adult CNS under normal conditions and after CNS acute and chronic injury remain to be determined. Future studies are also needed to determine the distinct molecular transcriptional switches mediating pericyte differentiation from their blood-borne peripheral precursors.

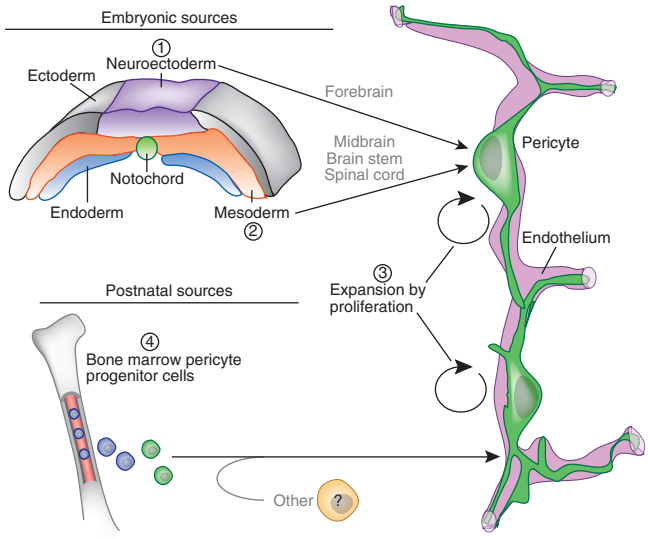

Figure 2 Origin of pericytes in the CNS. The embryonic sources of pericytes include (1) neuroectoderm-derived neural crest cells, which give rise to pericytes of the forebrain, (2) mesoderm-derived mesenchymal stem cells, which give rise to pericytes in the midbrain, brain stem and spinal cord, and (3) expansion by proliferation from the newly established pericyte pools. Postnatal sources of pericytes include (3) expansion by proliferation from the existing pericyte pools and (4) mesoderm-derived circulating mesenchymal stem cells (bone marrow pericyte progenitor cells) and presently undetermined 'other' sources.

Signaling: platelet-derived growth factor B

The cross-talk and functional coupling between pericytes and endothelial cells is the result of several signal transduction cascades, including but not limited to platelet-derived growth factor B (PDGF-B), transforming growth factor-β (TGF-β), Notch, sphingosine-1 phosphate and angiopoietin signaling[32]. The present review focuses on the roles of PDGF-B, TGF-β and the more recently described Notch signaling.

PDGF-B and PDGF receptor-β (PDGFRβ) are essential for recruitment of CNS pericytes (**Fig. 3**)[32–34]. Homozygous deletion of the *Pdgfb* or *Pdgfrb* gene results in a complete lack of CNS pericytes and is embryonic lethal[23,35,36]. Genetic manipulations maintaining some residual PDGF-B and/or PDGFRβ activity lead to viable mouse models of pericyte deficiency[7–9,34,37]. The endothelium secretes PDGF-B as a disulfide-linked homodimer (PDGF-BB)[7,38,39]. After secretion, a positively charged C terminal sequence within each PDGF-B monomer termed the retention motif electrostatically interacts with negatively charged heparin sulfate proteoglycans contained within the extracellular matrix, leading to localized PDGF-BB retention[40]. A steep periendothelial PDGF-BB concentration gradient is necessary for proper pericyte recruitment, including proliferation, migration and attachment (**Fig. 3a**,**b**)[7,24,37,41]. The attachment and migration seem to be retention-dependent events, whereas pericyte proliferation can be induced by freely diffusible PDGF-BB[24,41]. However, the molecular mechanisms mediating differential biological responses to retained and diffusible PDGF-BB are unknown.

PDGFRβ is a tyrosine kinase receptor, expressed in vascular mural cells in the developing mouse neural tube[23,33], adult mouse CNS[9,42] and human brain (E.A.W., R.D.B. and B.V.Z., unpublished data). Binding of PDGF-BB to PDGFRβ leads to receptor dimerization, autophosphorylation and activation of several downstream signal transduction cascades (**Fig. 3a**)[34,40]. The number of pericytes contained within the embryonic neural tube correlates with PDGFRβ receptor abundance and the number of PDGFRβ signal transduction pathways available for activation[34]. Recent studies have suggested that sustained PDGF-BB–PDGFRβ signaling in the adult CNS is required for pericyte cell survival (**Fig. 3c**)[9,43].

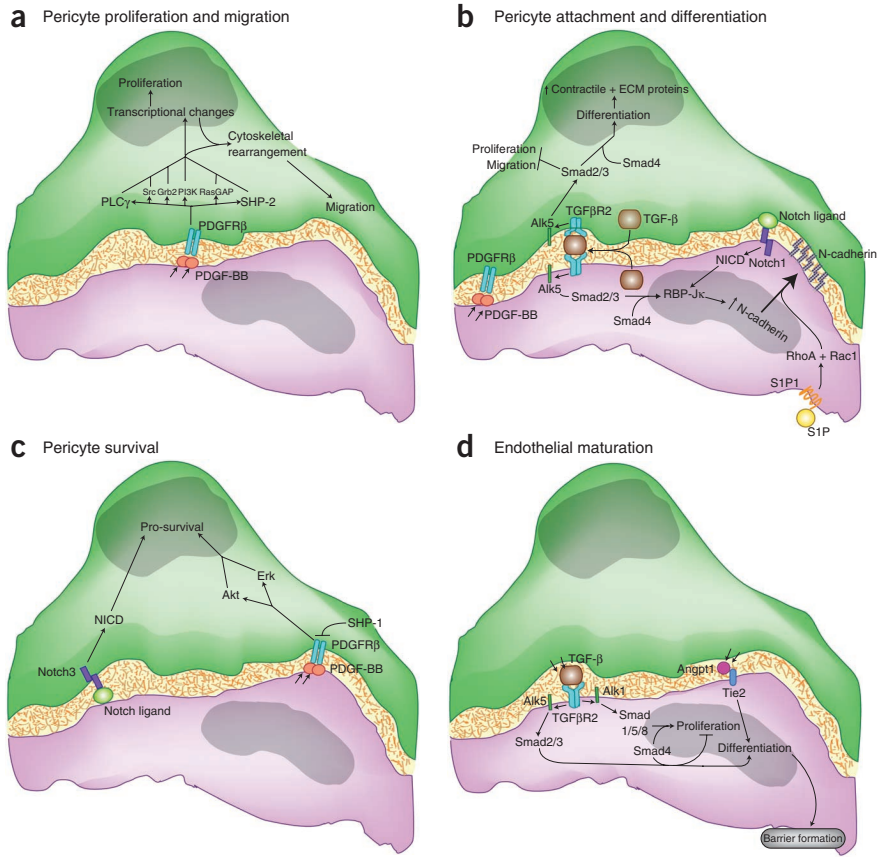

Figure 3 Pericyte-endothelial signaling. (**a**) Pericyte proliferation and migration. Endothelial cell (EC)-secreted PDGF-BB is retained within the extracellular matrix (ECM). PDGF-BB binds to PDGFRβ on the pericyte (PC) plasma membrane, leading to PDGFRβ dimerization, autophosphorylation and activation of several downstream signal transduction cascades (for example, Src, the Grb2 adaptor protein, phosphatidylinositol-3-OH kinase (PI3K), Ras GTPase activating protein (RasGAP), phospholipase C (PLC)-γ, SHP-2 tyrosine phosphatase), resulting in pericyte proliferation and cytoskeletal rearrangements facilitating migration. (**b**) Pericyte attachment and differentiation. In both pericytes and endothelium, TGF-β binding to TGFβR2 leads to activation of the ALK5-SMAD2/3 pathway and nuclear translocation of the Smad2/3/4 complex with unique consequences in the two cell types. In pericytes, it inhibits proliferation and leads to expression of contractile and ECM proteins. In endothelium, it also inhibits proliferation and cooperates with Notch signaling to increase expression of N-cadherin. Specifically, when Notch1 on the endothelial cell binds to an unspecified Notch ligand on the pericyte, activation leads to nuclear translocation of the Notch intracellular domain (NICD). NICD and the Smad2/3/4 complex interact with the transcription factor RBP-Jκ, promoting the upregulation of N-cadherin. Sphingosine-1 phosphate (S1P)-mediated activation of endothelial S1P1 facilitates N-cadherin trafficking to the endothelial cell membrane by the action of the GTPases RhoA and Rac1. Elevated endothelial N-cadherin leads to increased homophilic interactions with N-cadherin on pericytes, resulting in endothelial cell–pericyte adhesion. PDGF-BB/PDGFRβ signaling may also contribute to endothelial cell-pericyte attachment. However, the mechanism by which this occurs and whether N-cadherin is involved remain to be determined. (**c**) Pericyte survival. Activated PDGFRβ leads to activation of Akt and Erk serine/threonine kinases and downstream survival pathways. Some studies implicate Notch3 signaling in pericyte survival. (**d**) Endothelial maturation. Pericyte-derived TGF-β binds to TGFβR2 in endothelium and activates ALK5-Smad2/3/4 and ALK1-Smad1/5/8 pathways, exerting opposing effects on endothelial proliferation. Smad2/3/4 and angiopoietin-1 (Angpt1)/Tie2 signaling contribute to BBB formation.

Signaling: transforming growth factor-β

TGF-β is a multifunctional cytokine that exerts different effects on vascular development, including induction of pericyte differentiation and adhesion (**Fig. 3b**)[14,32,44–46]. TGF-β is secreted by endothelial cells, pericytes, neurons and glia as a protein-bound, latent form that is activated by thrombospondin or integrins[32,45]. After activation, TGF-β binds to the type 2 TGF-β receptor (TGFβR2), which leads to recruitment and phosphorylation of a type 1 TGF-β receptor, activin-like kinase 1 or 5 (ALK1 or ALK5), activating Smad signal transduction proteins, which results in transcriptional changes (**Fig. 3b**,**d**)[45]. Genetic ablation of key steps in the TGF-β signaling cascade, including genes for TGF-β (*Tgfb1)*, the receptors *Tgfbr2*, *Acvrl1* (encoding ALK1), *Tgfbr1* (encoding ALK5) and endoglin (*Eng*), and the downstream *Smad4* and *Smad5*, is embryonic lethal as a result of erroneous vascular development[32]. Furthermore, reduction of TGF-β in the human fetal germinal matrix, a structure rich in neuronal and glial precursor cells located in the thalamostriate groove just beneath the ventricular ependyma, is associated with region-specific reductions in pericytes causing vascular fragility[47,48]. The current concept states that intact bidirectional TGF-β signaling in pericytes and endothelium is required for proper vascular development[32,49].

Endothelially secreted TGF-β binds to TGFβR2 in pericytes, resulting in activation of the ALK5-Smad2/3 pathway and nuclear translocation of Smad2-Smad3-Smad4 protein complex, which inhibits pericyte proliferation while inducing pericyte contractile protein expression and extracellular matrix production[44,50] (**Fig. 3b**). TGF-β signal transduction in endothelium facilitates proper pericyte attachment, in coordination with Notch signal transduction, by means of the upregulation of the adhesion molecule N-cadherin[13–15] (**Fig. 3b**). Disruption of this pathway through endothelial cell–specific *Smad4* deletion restricted to the cerebrovasculature results in pericyte detachment, reduced pericyte coverage of the capillary tube and intraventricular hemorrhage during the perinatal period[14,15]. N-cadherin is also regulated through another important mediator of vascular maturation, sphingosine-1-phosphate signaling, thus probably representing a key point of convergence of molecular cascades regulating pericyte–endothelial cell attachment[32,51] (**Fig. 3b**).

In endothelium, TGF-β binding to TGFβR2 exerts differential effects by means of the endoglin and ALK1-Smad1/5/8 pathway, favoring proliferation and migration, and the ALK5-SMAD2/3 pathway, favoring quiescence, maturation and vascular stability[45,52] (**Fig. 3d**). After successful recruitment and attachment, pericytes stabilize the nascent capillary tube through secretion of TGF-β and activation of the TGFβR2-ALK5-Smad2/3 pathway[50,53], resulting in inhibition of endothelial proliferation[14] and promotion of endothelial maturation, including BBB formation[46,53] and basement membrane formation and stabilization[12,50,54] (**Fig. 3d**). Pericytes further promote endothelial stabilization, differentiation and barrier formation through secretion of angiopoietin-1 (ANGPT1) and activation of

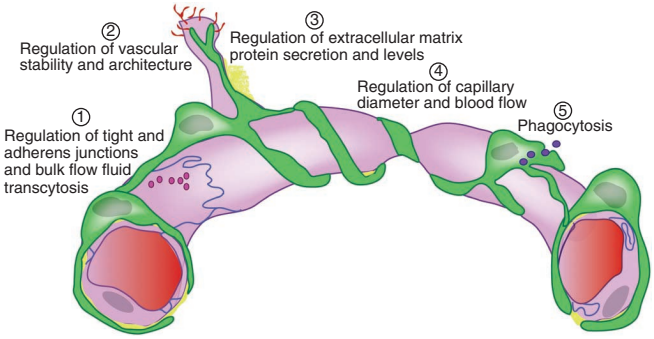

Figure 4 Pericytes are multi-functional members of the neurovascular unit. Pericytes (1) control BBB integrity by regulating the orientation and abundance of endothelial tight and adherens junction proteins, as well as the rate of bulk flow fluid transcytosis (transendothelial transport of fluid-filled vesicles); (2) regulate the stability and architecture of newly formed cerebral microvessels; (3) contribute to secretion and regulate the levels of extracellular matrix proteins forming the basement membrane; (4) regulate capillary diameter and blood flow; and (5) provide clearance and phagocytotic functions in brain.

its endothelial receptor Tie2 (refs. 55,56) (**Fig. 3d**). Despite signaling from pericytes to endothelial cells, ANGPT1 is not believed to promote pericyte recruitment[57].

Signaling: Notch

Notch signaling has well defined roles in embryonic vascular development, angiogenesis and arterial-venous specification[58]. In mammals, there are four distinct heterodimeric receptors, Notch1–Notch4, and five ligands, delta-like (DLL) 1, 3 and 4 and jagged (JAG) 1 and 2, all of which are transmembrane proteins. Cell-to-cell contact is required for efficient signal transduction. After ligand–receptor binding, sequential proteolytic cleavage releases the Notch intracellular domain (NICD), which translocates to the nucleus and binds the transcription factor recombination signal binding protein Jκ (RBP-Jκ), leading to downstream transcriptional changes.

Recent studies have suggested that Notch signaling could be important for pericyte attachment to endothelial cells and survival[14,15,59–63]. For example, endothelial cell–specific ablation of RBP-Jκ was shown to impair pericyte adhesion, resulting in reduced pericyte coverage and perinatal hemorrhage[14,15] (**Fig. 3b**). Moreover, endothelial cell–mural cell contact upregulates Notch3 protein in CNS pericytes and VSMCs[59]. Some studies have suggested that Notch3 could be important in survival of both pericytes and VSMCs[60,63] (**Fig. 3c**). Notch has also been implicated in the regulation of PDGFRβ in mural cells[64]. Therefore, it remains to be determined whether Notch3 signaling is protective in mural cells, including pericytes, directly or indirectly through PDGFRβ signaling. It has been also suggested that Notch ligand DLL4 may function in pericyte differentiation[62]. However, more work is needed to establish the exact role of Notch signaling in pericytes.

Function: BBB formation and maintenance

Studies over the past two decades have begun to reveal the pivotal role of pericytes in the regulation of several neurovascular functions necessary for proper CNS homeostasis, including BBB formation and maintenance, vascular stability and angioarchitecture, and capillary diameter and blood flow (**Fig. 4**).

Successful formation and maintenance of the BBB ensures the optimal chemical composition of the neuronal microenvironment that is needed for normal synaptic transmission and function of neuronal circuits. The BBB is a highly regulated, continuous endothelial cell membrane characterized by the presence of tight and adherens junctions and low levels of bulk flow transcytosis, which eliminate significant paracellular flow between cells and uncontrolled transendothelial fluid transport across cells, respectively[5]. Pericytes, although long speculated to fill a critical position in the BBB, have only recently been demonstrated to help form and maintain the BBB *in vivo* in both the developing and adult brain[7–9,14,65].

Recent work using mice with deficient Pdgfrβ signaling and resulting deficits in embryonic pericyte recruitment have demonstrated that the BBB forms early in embryogenesis, during a time period that coincides with initial pericyte recruitment and precedes astrocyte generation[8]. Furthermore, careful molecular characterization has shown not only that pericytes are necessary for the formation of the BBB but also that pericyte coverage of the endothelial capillary wall regulates important functional aspects governing BBB integrity, including the formation of tight junctions and transendothelial vesicle trafficking[8] (**Fig. 4**). Notably, pericyte-deficient mice do not have a generalized developmental reduction in endothelial expression of BBB-specific genes such as *Slc2a1*, encoding glucose transporter-1 (refs. 7,14), but instead fail to downregulate genes associated with increased vascular permeability, such as angiopoietin-2 (*Angpt2*) and plasmalemma vesicle-associated protein (*Plvap*)[8]. In addition, during BBB development, pericytes suppress expression of molecules that increase vascular permeability and CNS immune cell infiltration, including intercellular adhesion molecule 1 (*Icam1*), activated leukocyte adhesion molecule (*Alcam*) and lectin, galactose binding, soluble 3 (*Lgals3*)[8]. Together, these data suggests that pericytes may be important for suppressing a 'leaky' and proinflammatory phenotype associated with undifferentiated cerebrovascular endothelial cells during embryogenesis.

The role of pericytes in regulating BBB integrity, however, is not limited to the prenatal period. Recent studies using pericyte-deficient mouse lines with genetically disrupted Pdgfrβ signaling have shown that pericytes are integral in maintaining the BBB during adulthood[7,9] and brain aging[9] (**Fig. 4**). During early to mid-adulthood, loss of brain pericytes has been shown to increase transendothelial fluid flow[7,9] and paracellular transport as a result of reduced tight junction protein expression[9], both causing BBB disruption. For example, the levels of key tight junction transmembrane proteins such as occludin and claudin 5; the adaptor protein zonula occludens 1, which links tight junction proteins with the underlying cytoskeleton; and the adherens junction protein vascular endothelial cadherin are all progressively reduced with aging in pericyte-deficient mice[9]. Furthermore, it was shown that reductions in tight junction and adherens junction proteins and resulting increases in paracellular leakage to several exogenous vascular tracers and endogenous blood-derived macromolecules progress in correlation with age-dependent decreases in CNS pericyte coverage. The BBB breakdown caused by pericyte deficiency was also associated with a progressive age-dependent microvascular degeneration accompanied by a loss of cerebral capillaries[9]. However, unlike in the embryonic CNS, brain pericyte deficiency did not result in upregulation of genes for proinflammatory molecules, including *Icam1*, interleukin-1β (*IL1b*), interleukin-6 (*IL6*), tumor necrosis factor-α (*Tnfa*) or chemokine C-C motif ligand 2 (*Ccl2*), nor did it result in increased CNS immune cell response in early to mid-adulthood, but only in aged pericyte-deficient mice[9].

Notably, a loss of brain pericytes and resulting BBB breakdown have been shown to impair CNS function through leakage and deposition of several potentially vasculotoxic and neurotoxic blood-derived macromolecules, including fibrin[66], thrombin[67,68], plasmin[69] and hemoglobin-derived hemosiderin, which causes accumulation of

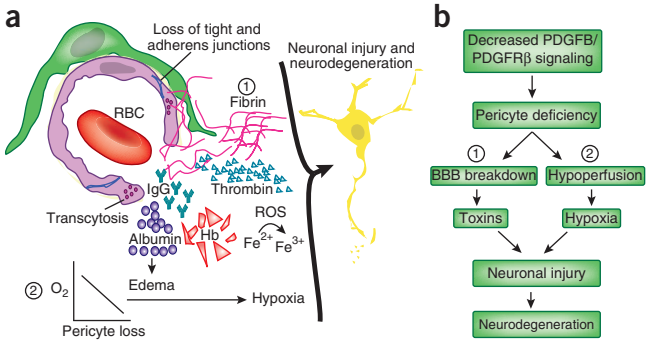

Figure 5 Pericyte loss can trigger primary vascular dysfunction leading to neurodegeneration. (**a**) (1) Blood-brain barrier (BBB) breakdown due to disrupted BBB tight and adherens junctions and increased bulk flow fluid transcytosis leads to brain influx of serum proteins (for example, albumin, immunoglobulin G (IgG)), causing edema, and of blood-derived vasculotoxic and neurotoxic macromolecules (for example, fibrin, thrombin, hemoglobin (Hb)-derived iron), causing neuronal injury and neurodegenerative changes. RBC, red blood cell; ROS, reactive oxygen species. (2) Reductions in capillary blood flow due to microvascular degeneration and pericapillary edema aggravate chronic hypoperfusion and hypoxia, depriving metabolically active neurons of oxygen and other essential nutrients, which leads to neuronal dysfunction. (**b**) Flow chart diagram depicting how deficient PDGFB/PDGFRβ signaling leads to pericyte loss resulting in (1) BBB breakdown and (2) hypoperfusion and hypoxia, as shown in **a**. Both arms 1 and 2 contribute to secondary neuronal degenerative changes.

iron and reactive oxygen species[9,70,71]. Vasculotoxic proteinaceous accumulations may amplify microvascular degeneration and the development of edema obstructing capillary flow, which results in local tissue hypoperfusion and hypoxia. Ultimately, vascular damage leading to accumulation of neurotoxins on the one hand and chronic hypoxia on the other may interact at the neuronal interface, initiating neuronal functional and structural changes resulting in secondary, vascular-mediated neurodegeneration[9] (**Fig. 5**).

Function: vascular stability and angioarchitecture

Vessels lacking pericytes are frequently described as showing focal and/or diffuse dilations, being exceedingly tortuous, and having frequent rupture-prone microaneurysms[7,14,33,39,72], suggesting that pericytes are critical for the regulation of vascular stability and angioarchitecture (**Fig. 4**). In the human CNS, region-specific reductions in pericyte coverage have been found in hemorrhage-prone, progenitor-rich regions of the developing telencephalon, such as the germinal matrix[47,73]. In the developing mouse CNS, a near complete loss or detachment of pericytes causes endothelial hyperproliferation but normal vascular density and branching[14,72]. In contrast, a wide spectrum of pericyte-deficient states induced by endothelial cell–specific deletion of *Pdgfb* may lead to either vascular regression or vascular hyperproliferation, depending on the magnitude of pericyte loss, suggesting that pericytes may have diverse, dose-dependent effects on vascular architecture[39]. Once in adulthood and during brain aging, pericyte-deficient states of variable magnitude induced through *Pdgfb or Pdgfrb* deficiencies have consistently lead to increased vascular regression and reductions in capillary density[7,9].

Although classically thought to be confined to a stabilizing role during embryonic vascular development[72], pericytes have been suggested to be more dynamic in nature, having several angiogenic actions after initial embryonic CNS vascular establishment. For example, pericytes express several matrix metalloproteinases (MMP2, MMP3 and MMP9)[74,75] and urokinase plasminogen activator receptor[76] capable of enhancing extracellular matrix degradation early in angiogenesis, thereby removing mechanical restraints to endothelial cell migration and facilitating the release of more matrix-sequestered angiogenic factors (**Fig. 4**). In addition, pericytes directly contribute to the synthesis of essential extracellular matrix proteins[11,12,50] including laminin, nidogen and fibronectin, and they secrete tissue inhibitor of metalloproteinase 3 (TIMP3), a potent inhibitor of several MMPs (for example, MMP1, MMP10, MMP14) and ADAM15 (a disintegrin and metallopeptidase domain 15)[55], which during the vessel stabilization phase inhibits degradation of basement membrane proteins. Pericytes have also been suggested to stimulate endothelial survival, proliferation and sprout formation through expression of angiogenic factors, such as vascular endothelial growth factor-A (VEGF-A)[27,77]. Whether endothelial tube formation precedes and guides pericyte migration or, alternatively, pericyte migration precedes and guides tube formation remains debatable[26,27,74,78].

Pericytes may therefore have multiple and sometimes opposing roles in angiogenesis, promoting endothelial cell survival and migration early but ultimately reducing proliferation and inducing quiescence during later stages[14,27,72,77]. During embryonic development, when a growth factor–enriched environment is present, primary pericyte loss results in endothelial cell proliferation without increases in branching or vessel density due to failed migration and inability to induce endothelial quiescence. Conversely, in established vascular beds in the adult CNS with comparatively low levels of extra-pericytic vascular growth factors, pericyte deficiency deprives endothelial cells of much-needed vasculotrophic support, leading to vascular regression. However, to effectively investigate whether pericytes have differential effects on CNS angioarchitecture during development and in the adult CNS, an inducible model of pericyte deficiency must be created and characterized.

Function: capillary diameter and blood flow

The role of pericytes in the regulation of capillary diameter and blood flow in response to synaptic transmission and the release of vasoactive mediators has been speculated for a long time, but has only been experimentally supported recently by a few studies[9,10]. Pericytes have been shown to express not only contractile properties[79], but also receptors for vasoactive molecules, including catecholamines, endothelin-1, vasopressin, and angiotensin II (refs. 11,80). In organotypic slices, pericytes dilate and constrict upon stimulation with a variety of different neurotransmitters[10]. Furthermore, changes in pericyte tonicity have been shown to depend on intracellular calcium fluxes[10,80,81] as in other contractile cells including cerebral VSMCs[82]. Pericyte contraction has also been shown to play an essential role in obstructing capillary flow during cerebral ischemia[83].

Recent findings have further supported that pericytes may contribute to regulation of functional hyperemia, as shown by attenuated *in vivo* CBF responses to brain activation in pericyte-deficient mice[9]. However, another study confirmed that pericytes contract *in vivo* but failed to show their role in functional hyperemia[84] thus raising questions regarding the functional significance of pericytes' contractile phenotype *in vivo*. Future studies are needed to more clearly delineate the role of pericytes in the context of the well-established role of VSMCs of small pial and intracerebral arteries in mediating CBF responses to neuronal activity[80,85].

Other functions

Pericytes have been shown to have direct macrophage-like phagocytotic properties contributing to the ability of the microcirculation to clear toxic cellular byproducts[9,11] (**Fig. 4**). Moreover, a recent study has

demonstrated that neural crest cell–derived pericytes control T-cell transmigration across the endothelium from the thymus gland into circulation[86]. Circulating T cells may infiltrate the brain during neurodegenerative diseases such as multiple sclerosis. Adhesion molecules on CNS pericytes including vascular adhesion molecule-1 have been suggested to regulate T-cell entry into the brain[87]. More studies are needed to establish the role of pericytes in immune responses of the CNS.

It has been also suggested that pericytes in culture can differentiate into neuron-like and glia-like lineages under different *in vitro* conditions[88]. However, at present there is no evidence that such differentiation takes place in brain *in vivo*.

Disease: diabetic retinopathy

Abnormal pericyte function, deficiency or both has been noted in many CNS diseases. These include diabetic retinopathy, neonatal intraventricular hemorrhage and some neurodegenerative disorders.

Diabetic retinopathy is a frequent debilitating complication of type I and type II diabetes mellitus characterized by an early loss of retinal pericytes that may be divided into two phases: (i) nonproliferative and (ii) proliferative retinopathy[89]. During the nonproliferative phase, vessels show frequent microaneurysms, are prone to intraretinal hemorrhages, and are in various stages of vasoregression as evidenced by increased numbers of acellular, occluded capillaries surrounded by a thickened basement membrane. In contrast, proliferative diabetic retinopathy represents excessive proliferation of abnormal, rupture-prone vessels that may give rise to subsequent hemorrhage and retinal detachment. Although the stimulus for transition between nonproliferative and proliferative phases has yet to be definitively identified, many believe that it results from a hypoxia-driven excess of proangiogenic factors in response to progressive microvascular regression[89]. However, retinal hypoxia has yet to be conclusively experimentally proven in human subjects[90].

A primary pericyte deficiency evoked through either genetic reduction of *Pdgfb* or exogenous application or overexpression of angiopoietin 2, a potent pericyte detachment stimulus, is able to recapitulate the characteristic vascular regression of nonproliferative diabetic retinopathy[91,92]. Endothelial cell–specific deletion of *Pdgfb* resulting in a wide spectrum of pericyte deficient states confirmed that pericyte deficiency leads to pathologic hallmarks roughly equivalent to nonproliferative diabetic retinopathy. However, when pericyte coverage reductions exceeded 50%, regions of vascular hyperproliferation were noted[39] raising a question as to whether a threshold of pericyte loss may represent a transition point between the two pathogenic phases.

Much attention has been placed on identifying the mechanism(s) of retinal pericyte dropout and means of therapeutic intervention. Notably, it does not seem that all pericyte subpopulations are equally affected; pericytes located on straight capillary tubes may be especially vulnerable[89]. Hyperglycemia-mediated dephosphorylation of PDGFRβ has been shown to result in PDGFRβ resistance to endothelially secreted PDGF-B, ultimately leading to pericyte apoptosis[43] (**Fig. 3c**). Whether the PDGFRβ pathway or other prosurvival pathways, such as Notch3, may be therapeutically exploited to mitigate diabetes-induced microangiopathy remains to be determined.

Disease: neonatal intraventricular hemorrhage

Neonatal intraventricular hemorrhage is a substantial cause of morbidity and mortality in premature infants[73]. The relative susceptibility of the germinal matrix to hemorrhage is believed to arise partially from a region-specific paucity of pericytes and resulting vascular instability in this highly angiogenic niche[47]. Vascular fragility in combination with altered hemodynamics then combine to promote vessel rupture and hemorrhage[73]. Some studies have suggested that neonatal intraventricular hemorrhage may result from a region-specific deficiency in TGF-β signaling[14,15,47]. As discussed above, mouse models with endothelial cell–specific deletion of *Smad4* or the Notch-related *Rbpj* (encoding RBP-Jκ) recapitulate the perinatal hemorrhagic phenotype[14]. Furthermore, antenatal glucocorticoid administration exerts protective effects by increasing TGF-β, enhancing pericyte coverage and suppressing VEGF-driven angiogenesis[48]. Future studies are required to determine whether the TGF-β signaling pathway may be targeted therapeutically to increase vascular stability and reduce the risk of hemorrhage.

Disease: neurodegeneration

Neurovascular dysfunction is associated with neurodegenerative conditions[5]. Recently, work using pericyte-deficient mice has demonstrated that an initial loss of brain pericytes triggers two parallel pathways of neurodegeneration involving breakdown of the BBB with toxic extravasations of circulating plasma proteins, and a chronic hypoperfusion and hypoxia. These two pathways act in synergy to generate a vascular-mediated secondary neurodegenerative phenotype[9] (**Fig. 5**). The vascular damage in pericyte-deficient mouse models precedes neuronal damage and neuroinflammation, suggesting that primary vascular lesions can lead to neurodegenerative changes[65]. Two other recent reports have also shown that pericytes are essential for BBB integrity during development[8] and adulthood[7]. Together these three studies confirm the role of brain pericytes in maintaining cerebral vascular homeostasis necessary to protect the CNS from injury.

The role of pericytes in specific neurodegenerative disorders such as Alzheimer's disease is less well understood. Some studies have suggested that Alzheimer's disease entails pericyte abnormalities[5,93]. The exact role of pericytes in neurovascular dysfunction in this disease, however, including focally reduced microvessel density and hypoperfusion[94], is unclear. Amyloid-β peptide (Aβ), a key neurovascular toxin present in Alzheimer's disease, accumulates on and around capillaries and pericytes[95,96]. *In vitro* studies have demonstrated that various Aβ species can cause pericyte cell death at high concentrations[97]. It is also known that transgenic models of Alzheimer's disease overexpressing Aβ have reduced cerebral vascular density[1], impaired vascular reactivity[98,99] and BBB breakdown[66], before appreciable neuronal loss or amyloid deposition. It remains unclear, however, how Aβ influences *in vivo* pericyte-mediated capillary contractility and BBB protection. As pericytes have been established to be key regulators of BBB integrity and likely contribute to neurovascular coupling, it is tempting to speculate that if Aβ and/or other Alzheimer's disease comorbidities trigger a loss of pericytes, corresponding reductions in CBF and BBB breakdown may amplify the rate of Aβ deposition and neuronal loss, establishing a self-propagating vicious cycle. Transcriptional changes in mural cells in Alzheimer's disease, namely upregulation of myocardin and serum response factor, have been shown to lead to cerebral artery and arteriole hypercontraction, reducing CBF[82] and Aβ clearance[100]. Future studies are required to determine whether similar molecular changes occur in pericytes.

Future directions and perspectives

Remarkable recent progress in the field of CNS pericyte biology has lead to a greater appreciation of their functional importance in the CNS in health and disease. We have begun to uncover how pericytes maintain their physical association with other cell types (namely, endothelium, astrocytes and microglia), as well as how they

communicate with their neighboring cells and regulate each others' function, ultimately influencing neuronal function. Exciting and novel research based on the neurovascular model of neurological diseases has started to change the way we now think about neurodegeneration. It will continue to provide insights ultimately leading to development of new therapeutic approaches. At present, relatively little is yet known about the role of pericytes in major human brain diseases including acute injury (that is, stroke, traumatic brain injury) and neurodegenerative proteinaceous disorders such as Alzheimer's disease, prion disease, amyotrophic lateral sclerosis and Parkinson's disease. Future studies should better define key molecules mediating neurovascular links between pericytes and other cell types within the NVU, and the manner in which cellular cross-talk is disrupted in brain diseases. There is no question that addressing these issues will create new opportunities not only to better understand the molecular basis of pericyte cell-cell signaling within the NVU in health and disease, but also to develop new types of neurovascular medicine.

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The authors declare no competing financial interests.

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