Pericytes of the neurovascular unit: key functions and signaling pathways

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Pericytes are vascular mural cells embedded in the basement membrane of blood microvessels. They extend their processes along capillaries, pre-capillary arterioles and post-capillary venules. CNS pericytes are uniquely positioned in the neurovascular unit between endothelial cells, astrocytes and neurons. They integrate, coordinate and process signals from their neighboring cells to generate diverse functional responses that are critical for CNS functions in health and disease, including regulation of the blood–brain barrier permeability, angiogenesis, clearance of toxic metabolites, capillary hemodynamic responses, neuroinflammation and stem cell activity. Here we examine the key signaling pathways between pericytes and their neighboring endothelial cells, astrocytes and neurons that control neurovascular functions. We also review the role of pericytes in CNS disorders including rare monogenic diseases and complex neurological disorders such as Alzheimer's disease and brain tumors. Finally, we discuss directions for future studies.

The neurovascular unit (NVU) is comprised of vascular cells (pericytes, vascular smooth muscle cells (VSMCs), endothelial cells), glial cells (astrocytes, microglia, oligodendrocytes) and neurons[1–3]. Pericytes are centrally positioned in the NVU between endothelial cells, astrocytes and neurons (**Fig. 1a**). They receive signals from their neighboring cells and generate functional responses that are essential for proper CNS functioning[2,4–6] (**Fig. 1b**).

Endothelial cells form the blood–brain barrier (BBB), which sanctions entry of macromolecules, cells and pathogens from blood into the CNS. Brain endothelium also regulates CNS transport of energy metabolites, nutrients and ions, and clearance of neurotoxic metabolites[1,7]. The BBB's integrity is maintained chiefly by pericytes[8–10]. Endothelial tight junctions and lack of fenestrae contribute to a physical barrier[5,7–11] that prevents transport of peptides and proteins into the brain[12,13] unless they have specific carriers and/or receptors in brain endothelium[14,15]. The BBB's integrity is vital for normal CNS functions as illustrated by rare genetic human diseases where specific gene defects in pericytes, endothelial cells or astrocytes lead to NVU disruption and neurological disorders[7]. Pericyte degeneration and BBB breakdown are found in complex neurological disorders such as Alzheimer's disease[1,3,16]. Additionally, pericytes contribute to CNS tumor angiogenesis and growth[5]. Here we review the functions and signal transduction pathways in CNS pericytes in health and disease.

Pericytes: characterization, function and dysfunction

Characterization. Pericytes are embedded in the basement membrane of small blood vessels including capillaries, pre-capillary arterioles and post-capillary venules[2]. Though the cells themselves were originally described by Charles Rouget, the term "pericytes" was coined by Zimmermann in 1923; he proposed several subtypes of pericytes based on their morphology, location within the vascular network, and function[17,18]. Pericytes express several contractile and cytoskeletal proteins (for example, α-smooth muscle actin, vimentin, desmin, myosin, and nestin)[5,6,19–21] and cell surface antigens (for example, the transmembrane chondroitin sulfate proteoglycan NG2, platelet-derived growth factor receptor-β (PDGFRβ), aminopeptidases A and N (CD13), regulator of G-protein signaling-5 (RGS5) and cell surface glycoprotein MUC18 (CD146)) (refs. 5,18,21,22), some of which are also found on VSMCs[2,21,22].

Recent studies of the cortical angioarchitecture in mice expressing fluorescent proteins under control of the *NG2* (*Cspg4*) and *Pdgfrb* promoters have identified several pericyte subpopulations, including VSMC–pericyte hybrids on pre-capillary arterioles, thin-strand helical pericytes on the capillary itself and mesh pericytes with stellate morphology on post-capillary arterioles and venules[19,20]. Future single-cell RNA-seq and proteomic studies, as used successfully to characterize subpopulations of cortical progenitor cells[23] and drug-resistant tumor cells[24], may also contribute to better understanding of different pericyte subtypes.

Function. Pericytes regulate BBB permeability, angiogenesis, clearance, cerebral blood flow (CBF), neuroinflammation and stem cell activity (**Fig. 1b**).

BBB permeability. Pericytes control the expression of endothelial BBB tight and adherens junction proteins, the alignment of tight junction proteins and bulk-flow transcytosis of fluid-filled vesicles across the BBB[8–10]. The molecular pathways between endothelial cells and pericytes that can be manipulated to open the BBB 'on demand' for delivery of neuropharmaceuticals and/or to reverse BBB breakdown in neurological disorders[7] remain largely unknown.

Angiogenesis. Pericytes regulate angiogenesis, microvascular stability and angioarchitecture during CNS development and vascular remodeling[2,5,25].

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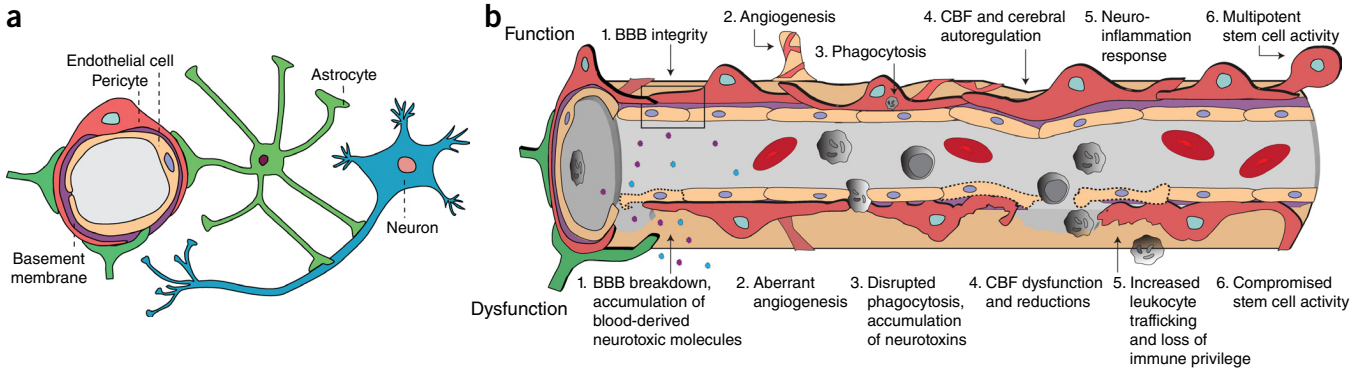

Figure 1 The multifunctional role of CNS pericytes at the NVU. (**a**) A simplified NVU diagram showing the interactive cellular network at the level of brain capillaries that comprises vascular cells, glial cells, and neurons. Intricate cell–cell communication and signal transduction mechanisms of NVU cell types are highly controlled to regulate numerous functions in the CNS. (**b**) Under physiological conditions (top row), pericytes regulate (1) BBB integrity, i.e., tight or adherens junctions and transcytosis across the BBB; (2) angiogenesis, i.e., microvascular remodeling, stability and architecture; (3) phagocytosis, i.e., clearance of toxic metabolites from the CNS; (4) CBF and capillary diameter; (5) neuroinflammation, i.e., leukocyte trafficking into the brain; and (6) multipotent stem cell activity. Pericyte dysfunction (bottom row) is characterized by (1) BBB breakdown causing leakage of neurotoxic blood-derived molecules into the brain (for example, fibrinogen, thrombin, plasminogen, erythrocyte-derived free iron and anti-brain antibodies); (2) aberrant angiogenesis; (3) impaired phagocytosis causing CNS accumulation of neurotoxins; (4) CBF dysfunction and ischemic capillary obstruction; (5) increased leukocyte trafficking promoting neuroinflammation; and (6) impaired stem cell-like ability to differentiate into neuronal and hematopoietic cells. Pericyte dysfunction is present in numerous neurological conditions and can contribute to disease pathogenesis.

Clearance. Pericytes can also act as perivascular tissue macrophages to clear tissue debris and foreign proteins injected systemically and/or locally in the CNS[2,9,10,26,27] and participate in clearance of Alzheimer's amyloid-β toxin, as shown in a murine Alzheimer's disease model[26].

CBF. VSMCs control dilation and constriction of arterioles and small brain arteries[28,29]. However, recent studies in cortical and cerebellar brain slices and retinal explants, as well as *in vivo* studies of cortical, retinal, olfactory bulb and ear microcirculation, have demonstrated that capillaries contribute to hemodynamic responses[19,30–34]. It has been shown that pericytes regulate capillary tone and diameter[6,18,35], as discussed in greater detail in the "Arachidonic acid pathway" section of this review. Some recent studies failed to find regulation of capillary blood flow by pericytes[36], but the controversy has been attributed to a drift in pericyte definition, particularly renaming the mid-capillary pericytes into VSMCs[19].

Neuroinflammation. Studies using transgenic pericyte-deficient mice have shown that pericytes control endothelial cell–mediated leukocyte adhesion and transmigration into the CNS[37], and studies in wild-type mice demonstrate enhanced leukocyte trafficking in microvascular regions lacking pericyte coverage[38,39]. *In vitro* studies have also suggested that pericytes influence neuroinflammation[40,41]. Taken together, these findings suggest that immune activation of brain pericytes may contribute to communicating inflammatory signals in the NVU.

Stem cell activity. In vitro studies have suggested that cultured pericytes have multipotent stem cell potential[17,42]. Moreover, primary murine pericytes isolated from brain following ischemic stroke exhibit multipotential stem cell activity and differentiate into neural and vascular lineage cells[42].

Dysfunction. Pericyte degeneration leads to BBB breakdown causing brain accumulation of blood-derived neurotoxic molecules[10,43–45]. Pericyte ischemic injury results in contractile rigor and obstruction of capillary blood flow[30,46]. Pericyte-specific genetic defects lead to primary familial brain calcification, or Fahr's disease[47,48]. Pericytes degenerate and likely play a role in cerebrovascular dysfunction in complex neurological diseases such as Alzheimer's disease[26,49,50], amyotrophic lateral sclerosis (ALS)[51] and type 2 diabetes mellitus–related microangiopathy and retinopathy[52–54]. Pericyte dysfunction has been also associated with HIV-related dementia[55], epilepsy[56], cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)[57] and brain cancer[58,59].

Pericyte–endothelial cell signal transduction

PDGF-BB–PDGFRβ pathway characterization. The platelet-derived growth factor (PDGF) family contains four ligands (A through D), which bind to two receptors (α and β)[59]. PDGF receptor tyrosine kinases (PDGFRs) form three active-conformation dimers: αα, ββ and αβ. The PDGF ligands differentially bind PDGFRs; specifically, where * denotes high affinity ligand-receptor interactions, PDGFR-αα's ligands are PDGF-AA*, PDGF-AB, PDGF-BB, and PDGF-CC*; PDGFR-αβ's ligands are PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD; and PDGFR-ββ's ligands are PDGF-BB* and PDGF-DD[59]. Here we will focus on PDGF-BB secreted by endothelial cells and PDGFRβ, a receptor on pericytes[5,60,61].

Precise spatial and temporal regulation of PDGF-BB signaling is achieved via its retention motif, a region of positively charged amino acids at the C terminus. This motif binds to negatively charged heparin sulfate proteoglycans of the extracellular matrix, resulting in retention of PDGF-BB that generates a concentration gradient[5], as shown by *in vitro* studies. PDGF-BB binds to PDGFRβ, causing non-covalent dimerization and autophosphorylation of the receptor on up to 13 cytoplasmic tyrosine residues, which activates PDGFRβ[62] (**Fig. 2**). Once activated, distinct phosphorylated tyrosine residues of PDGFRβ are bound by specific Src homology 2 (SH2) domain–containing proteins, including phospholipase Cγ, Src family kinase, growth factor receptor-bound protein 2 (Grb2), phosphatidylinositol-3-OH kinase (PI3K), GTPase activating protein (GAP), SH2 tyrosine phosphatase (SHP2) and Stat5. These induce downstream signaling, which promotes pericyte survival, proliferation, migration and recruitment to the vessel wall[2,62].

RasGAP binding to phospho-PDGFRβ causes simultaneous binding of the Grb2 and son of sevenless homolog 1 (SOS1) complex to Ras that offsets the activation of Ras and reduces the downstream activity of the extracellular signal-regulated kinase-1 (ERK1) and ERK2, as

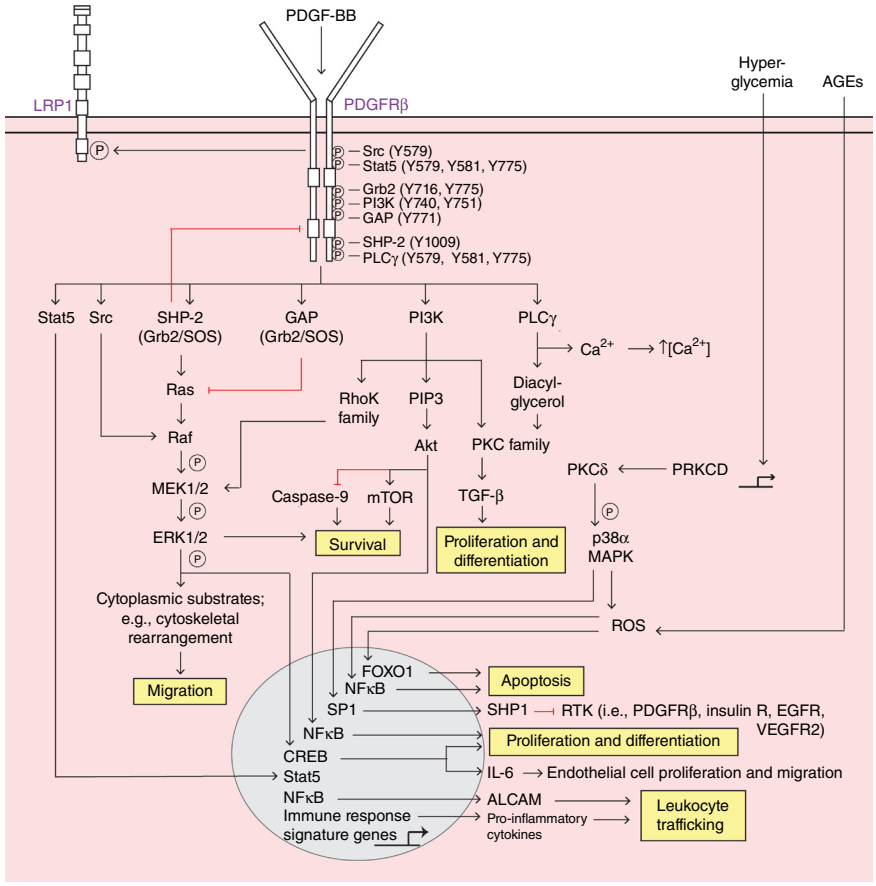

Figure 2 PDGF-BB–PDGFRβ signaling in pericytes. PDGF-BB secreted by endothelial cells binds to PDGFRβ on pericytes, causing receptor dimerization, autophosphorylation and activation. Several SH2 domain–containing proteins bind to distinct phosphorylated (P) tyrosine residues, including Stat5, Src, SHP2, growth factor receptor-bound protein 2 (Grb2), GAP, PI3K and phospholipase Cγ (PLCγ). SH2 domain–containing proteins bound to PDGFRβ differentially activate downstream signaling pathways to regulate pericyte survival, migration, apoptosis, proliferation and differentiation, as well as leukocyte trafficking. Survival is promoted via PI3K–Akt activation of mammalian target of rapamycin (mTOR) and inhibition of caspase-9 and the SHP2–Grb2/SOS complex-mediated MAPK pathway involving sequential phosphorylation of MEK and ERK. Cell migration is promoted by the MAPK pathway's induction of cytoskeletal rearrangement. Specifically, Src-activated Raf, SHP2-activated Ras, and PI3K–Rho kinase (RhoK) phosphorylation of MEK synergistically activates the MAPK pathway whereas GAP inhibition of Ras decreases MAPK signaling. Extracellular advanced glycation end products (AGEs) induce intracellular ROS and FOXO1-mediated apoptosis. Additionally, hyperglycemia promotes *PRKCD* transcriptional expression of protein kinase C-δ (PKCδ), which activates p38α MAPK. p38α MAPK activates the specificity protein-1 (SP1) element of the *PTPN6* promoter to express SH2 domain-containing phosphatase-1 (SHP-1) which inhibits several receptor tyrosine kinases (RTK) (for example, PDGFRβ, insulin receptor, epidermal growth factor receptor (EGFR) and VEGFR2) and induces downstream production of ROS, causing mitochondrial cytochrome *c* release, resulting in apoptosis. Proliferation and differentiation are promoted by the PI3K pathway, specifically via PKC–TGF-β signaling, phosphatidylinositol-3,4,5-triphosphate (PIP3)–Akt transcriptional activation of NFκB and MAPK activation of cAMP response element-binding protein (CREB) to express interleukin-6 (IL-6). Peripheral leukocyte trafficking into the CNS is regulated in part by PDGFRβ–mediated proinflammatory responses, including transcriptional expression of immune response signaling genes (for example, cytokines and chemokines) and also Akt-induced activation of NFκB and transcriptional expression of activated leukocyte cell adhesion molecule (ALCAM). Red lines, inhibitory mechanisms; black lines, activator mechanisms; gray circle, cell nucleus.

shown *in vitro*[63]. Activation of Notch ligands and/or Notch receptors and activation of a Notch signal integrator transcription factor, CSL, can modulate PDGFRβ signaling independently of PDGF-BB, as shown using *in vitro* cultures and Notch3-deficient mice[64] (**Fig. 2**).

PDGF-BB–PDGFRβ pathway function. Developmental studies using *Pdgfb* and *Pdgfrb* transgenic mouse models[60,62] show that PDGF-BB–PDGFRβ signaling is important for endothelial–mesenchymal communication and CNS blood vessel development and stabilization[5]. Both *Pdgfb* null and *Pdgfrb* null mutations are embryonically lethal in mice and lead to development of CNS microvascular instability, endothelial hyperplasia, microaneurysms and blood vessel ruptures with microhemorrhages, whereas deletion of the single allele does not result in an apparent vascular phenotype in the developing CNS.

In contrast, during postnatal development, adulthood and aging, brain pericytes may fulfill a different regulatory role, as suggested by recent work in mouse models with partially disrupted PDGF-BB–PDGFRβ signaling as a result of either mutation in the PDGF-BB retention motif ($Pdgfb^{ret/ret}$) or deficient PDGFRβ signaling, both of which result in age-dependent BBB breakdown and accumulation of blood-derived neurotoxic proteins in the neuropil and brain interstitial fluid[8–10]. Deficient PDGFRβ signaling also leads to microvascular reductions, which in parallel with BBB breakdown may contribute to secondary neurodegeneration[10,43].

In animal models of diabetic retinopathy, hyperglycemia leads to diminished PDGFRβ signaling resulting in pericyte apoptosis[52], whereas studies of tumor angiogenesis have shown that pericyte loss may lead to endothelial apoptosis[65]. PDGFRβ signal transduction in pericytes also mediates proinflammatory responses at the BBB by transcriptional regulation of several chemokines that promote endothelial expression of monocyte chemoattractant protein-1, nitric oxide (NO), interleukins IL-1, IL-6, IL-12, and tumor necrosis factor-α, resulting in transvascular trafficking of macrophages and leukocytes into the brain, as shown in pericyte-deficient $Pdgfrb^{+/-}$ mice[37]. Disrupted PDGF-BB–PDGFRβ signaling upregulates vascular endothelial growth factor (VEGF)-A, which accelerates vascular abnormalities, as shown *in vivo* in *Pdgfb* and *Pdgfrb* mutant mice[66]. Dysfunction in the PDGF-BB–PDGFRβ signaling pathway contributes to various CNS pathophysiologies, as discussed below.

PDGF-BB–PDGFRβ signaling in Fahr's disease. Fahr's disease is characterized by migraines, mood swings, motor symptoms (for example, Parkinsonism) and dementia, and its etiology includes loss-of-function mutations in *PDGFB* and *PDGFRB* genes[47,48] implicating

involvement of pericytes. Mutations in the retention motif of PDGF-BB in *Pdgfb*$^{ret/ret}$ pericyte-deficient transgenic mice[9] and *PDGFB* mutations in humans lead to calcifications in capillaries and small microvessels, mainly in the basal ganglia, which correlate with the degree of pericyte deficiency and BBB breakdown as shown in the murine model of this disease[47].

Loss-of-function mutations in *SLC20A2* gene, encoding the sodium-dependent phosphate transporter PiT-2, are also associated with Fahr's disease[67,68] and likely involve changes in phosphate transport at the BBB that promote regional brain accumulation of inorganic phosphate, which subsequently causes calcium phosphate deposition[67].

PDGF-BB–PDGFRβ signaling in Alzheimer's disease. Pericytes degenerate in Alzheimer's disease, as shown by post-mortem brain tissue studies in humans[50,69–71] and animal models of Alzheimer's disease[26,72,73]. Moreover, plasma PDGF-BB levels are increased in people with Alzheimer's disease[74], and soluble PDGFRβ levels, reflecting pericyte injury[75], are increased in cerebrospinal fluid (CSF) of people with mild dementia, transgenic Alzheimer's disease mouse models, and pericyte-deficient mice[73], suggesting dysfunction in PDGF-BB–PDGFRβ pathway as compared to control subjects or littermate control mice.

In transgenic mice, deficient PDGFRβ signaling leads to pericyte loss, causing BBB disruption and microvascular reductions followed by neurodegenerative changes independently of amyloid-β (ref. 10). However, studies in mice overexpressing amyloid-β precursor protein (APP) crossed with pericyte-deficient *Pdgfrb*$^{+/-}$ mice (*APP*$^{Sw/0}$;*Pdgfrb*$^{+/-}$ mice) indicate that defective PDGF-BB–PDGFRβ signaling leads to faulty amyloid-β clearance from brain interstitial fluid by diminishing low-density lipoprotein receptor-related protein 1 (LRP1)-mediated amyloid-β clearance in pericytes[26]. Compared to control *APP*$^{Sw/0}$ mice, which develop a moderate pericyte loss[26,72], *APP*$^{Sw/0}$;*Pdgfrb*$^{+/-}$ mice have an earlier onset of cerebral amyloid angiopathy and amyloid-β load and increased amyloid-β_{40} and amyloid-β_{42} levels in the brain[26]. Accelerated pericyte degeneration in *APP*$^{Sw/0}$;*Pdgfrb*$^{+/-}$ mice also leads to tau pathology and neuronal loss, which is not normally seen in *APP*$^{Sw/0}$ mice[26]. These data suggest that a double vascular and amyloid-β hit is needed for the development of full-spectrum Alzheimer's disease–like pathology in mice. Whether the same double hit contributes to the pathogenesis of late-onset Alzheimer's disease in humans, which is characterized by pericyte degeneration[50,69–72], is unclear.

Mutations in *SORL1*, *SORCS1*, *SORCS2* and *SORCS3* genes, encoding proteins containing vacuolar protein sorting-10 (Vps10) domains—namely, sorL1 (also known as sorLA) sorCS1, sorCS2 and sorCS3—are risk factors for sporadic Alzheimer's disease[76,77] and diabetes[78]. Under normal conditions, sorL1 and sorCS1–3 interact with the retromer complex to facilitate intracellular trafficking, recycling, sequestration and metabolism of different proteins, including APP (ref. 79). Single nucleotide polymorphisms in the genes encoding these proteins have been suggested to promote either aberrant APP clearance and/or processing[76]. PDGF-BB binds to sorL1, sorCS1 and sorCS3 (refs. 80,81), which may influence its interaction with and/or downstream signaling from PDGFRβ that in turn might lead to pericyte dysfunction and/or degeneration, as seen in late-onset Alzheimer's disease[50,69–71,73]. In addition to PDGF-BB, sorL1 binds other LRP1 ligands similarly to LRP1 (ref. 81), which may influence LRP1-mediated BBB clearance[82]. More studies are needed to evaluate the effects of interactions of PDGF-BB with sorL1 and sorCS1–3 on downstream PDGFRβ signaling in pericytes and whether these Vps10 proteins can provide a molecular link between Alzheimer's disease and diabetes pathogenesis.

Presenilin-1 (*PSEN1*) and *PSEN2* mutations, the most frequent cause of autosomal-dominant Alzheimer's disease (ADAD)[83,84], both result in reduced PDGFRβ mRNA and protein levels, reduced PDGF-BB binding sites, and reduced PDGFRβ activation and autophosphorylation that consequentially suppress the downstream mitogen-activated protein kinase (MEK)–ERK and PI3K–Akt signaling pathways, as shown in *PSEN1* and *PSEN2* knockout cells[85]. These changes may lead to the pericyte degeneration and BBB disruption reported in post-mortem brains affected by ADAD[84] and *PSEN1* transgenic mutants[86]. Elucidating the exact mechanism by which *PSEN1* and *PSEN2* mutations impair PDGF-BB–PDGFRβ signaling would inform how pericyte and microvascular dysfunction contributes to ADAD pathophysiology.

Several dominantly inherited rare vasculotropic *APP* mutations in amyloid-β residues 21 to 23 (for example, Dutch, Flemish, Iowa and Arctic) primarily affect the cerebrovascular system, leading to BBB breakdown, cerebral amyloid angiopathy and hemorrhages with recurrent strokes, as recently reviewed[87]. Although *APP* mutations lead to degeneration of mural cells, whether deficient PDGF-BB–PDGFRβ signaling might contribute to loss of pericytes, rupture of blood vessels and/or BBB disruption is unknown. A possible mechanism illustrating how pericyte dysfunction and degeneration resulting from a deficient PDGF-BB–PDGFRβ pathway can contribute to dementia and Alzheimer's disease pathology in late-onset and early inherited familial cases is shown in **Figure 3**.

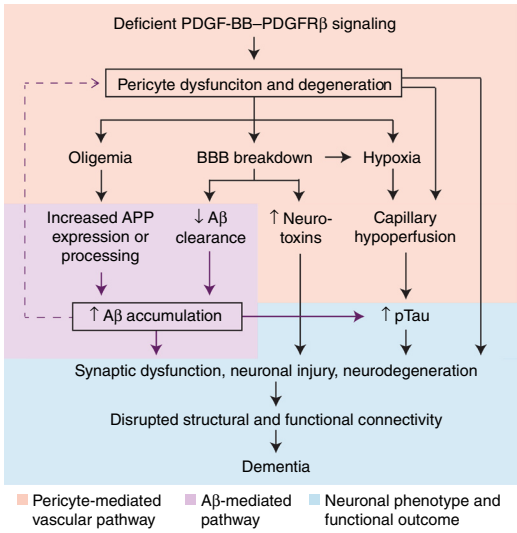

Figure 3 Deficient PDGF-BB–PDGFRβ signaling in pericytes may promote neuronal dysfunction and degeneration in Alzheimer's dementia. In the amyloid-β (Aβ)-independent pathway (pink box), deficient PDGF-BB–PDGFRβ signaling leads to pericyte dysfunction and/or degeneration, resulting in microvascular and CBF reductions and oligemia (brain hypoperfusion), and to BBB breakdown with accumulation of blood-derived toxic products in the brain. BBB breakdown leads to capillary edema contributing to capillary hypoperfusion and hypoxia. In the Aβ-dependent pathway (purple box), oligemia leads to increased Aβ production, whereas BBB breakdown and deficient PDGFRβ signaling can both lead to faulty Aβ clearance, which in turn promotes Aβ accumulation in the brain. Synergistic action of the Aβ-independent and Aβ-dependent pathways leads to accelerated tau hyperphosphorylation (pTau), formation of neurofibrillary tangles, synaptic dysfunction and loss and neuronal degeneration, which together promote behavioral deficits and dementia (blue box).

PDGF-BB–PDGFRβ signaling in ALS. Microvascular pathology is present in people with sporadic and familial ALS carrying superoxide dismutase 1 (*SOD1*) mutations[1,51]. Pericyte degeneration in people with ALS coincides with BBB and blood–spinal cord barrier (BSCB) breakdown in motor-neuron-dense regions of the spinal cord and motor cortex[51]. Consistently, ALS model transgenic *SOD1* mutant mice develop BSCB disruption, pericyte deficiency and erythrocyte extravasation before motor neuron dysfunction[43,51,88]. Moreover, preventing BSCB breakdown delays the onset of motor neuron disorder in *SOD1* mutants[44]. Whether patients with the most frequent genetic cause of ALS, expanded hexanucleotide repeat of GGGGCC in a noncoding region of the *C9Orf72* gene[89], also develop pericyte degeneration and BBB breakdown and/or disrupted PDGF-BB–PDGFRβ signaling is unknown at present.

PDGF-BB–PDGFRβ signaling in diabetes. In type 2 diabetes mellitus and diabetic retinopathy, hyperglycemia influences the downstream PDGFRβ signal transduction cascade to induce pericyte apoptosis, as shown *in vivo* in rats and *in vitro* in retinal cultures[52–54] (**Fig. 2**). Hyperglycemia-induced apoptosis *in vivo* can be prevented by inhibition of the protein kinase C-δ–p38α–mitogen-activated protein kinase-14 (MAPK14)–SHP1 pathway[52] and advanced glycation end product (AGE)-induced apoptosis by PDGFRβ downstream activation of Akt and nuclear factor-κB (NFκB)[54].

PDGF-BB–PDGFRβ signaling in HIV-induced neurocognitive deficits. HIV-1–positive individuals can develop HIV-associated neurocognitive disorders and HIV-associated dementia. Increased BBB permeability and pericyte loss, present in HIV-associated neurocognitive disorders and dementia, is thought to promote neurological impairments via increased transport of the HIV-1 viral neurotoxic protein Tat101 into the brain[55]. Interestingly, Tat101 increases PDGF-BB expression and PDGF-BB–PDGFRβ signaling, specifically via MAPK and NFκB activation, resulting in elevated pericyte migration and deficiency[55].

PDGF-BB–PDGFRβ signaling in brain cancer. The exact role of PDGFRβ signaling in pericytes in regulating growth and maturation of blood vessels in brain tumors remains still largely unexplored. Angiogenesis studies in the transgenic mouse model of pancreatic islet carcinogenesis (RIP1-Tag2) found that the broad-spectrum receptor tyrosine kinase inhibitor SU6668, which preferentially targets PDGFRβ, leads to regression of blood vessels by detaching pericytes from tumor vessels, which restricts tumor growth[90]. Similarly, SU6668 diminishes pericytes in xenograft tumors and restricts tumor growth[91]. Tumor-derived PDGFRβ$^+$ perivascular progenitor cells can differentiate into mature pericytes, eliciting vascular stabilization, maturation and survival[65]. Moreover, specific inhibition of PDGFRβ signaling eliminates PDGFRβ$^+$ perivascular progenitor cells and mature pericytes around tumor vessels, leading to endothelial cell apoptosis in transgenic RIP1-Tag2 mice with pancreatic islet tumors[65].

A recent study has shown that PDGFRβ regulates cell proliferation and invasion of medulloblastomas via JAG2 (ref. 58), suggesting that PDGFRβ could be a potential therapeutic target for medulloblastomas. It remains unclear, however, whether medulloblastoma cells have the potential to differentiate into pericytes, as shown for some other types of brain neoplastic cells that can generate pericytes to control blood vessel function during tumor growth[92,93].

TGF-β–TGFβR2 pathway characterization. Transforming growth factor-β (TGF-β) is expressed in a latent form by pericytes, endothelial cells, neurons and glia. Studies using *in vitro* primary cocultures of endothelial cells and pericytes indicate that TGF-β activation at the BBB requires pericyte–endothelial cell interaction[94]: specifically, formation of the gap junction connexin-43 hemichannels[95]. Activated TGF-β binds TGF-β receptor 2 (TGFβR2) on pericytes and endothelial cells[2,5]. TGF-β–TGFβR2 signals downstream via activin receptor–like kinase 5 (Alk5) in both pericytes and endothelial cells or Alk1 only in endothelial cells, as shown in primary bovine retinal cultures[96] (**Fig. 4**). In pericytes, Alk5–mothers against decapentaplegic homolog 2 (Smad2)/Smad3/Smad4 signaling inhibits proliferation and promotes differentiation[2,5] (**Fig. 4**). For example, mouse embryos with reduced TGFβR2–Alk5–Smad2/3 signaling exhibit enhanced pericyte proliferation[97], and depleting TGF-β attenuates mural cell differentiation in murine embryonic mesenchymal cells and endothelial cell cocultures[98]. In endothelial cells, Alk5 and Alk1 induce opposing effects via distinct downstream signal transduction events[2,5,96]. Specifically, *in vitro* studies in murine embryonic endothelial cells reveal that TGF-β–TGFβR2 signals downstream via Alk5–Smad2/3/4 to induce PDGF-BB expression and activate the recombination signal sequence–binding protein-Jκ (RBP-Jκ) transcription factor to express N-cadherin and promote attachment, Alk1–Smad1/5/8 to promote proliferation, and the PI3K–Akt pathway to promote survival and transcriptionally induce proliferation[2,99,100] (**Fig. 4**).

TGF-β–TGFβR2 pathway function. TGF-β–TGFβR2 signaling promotes cell differentiation, maturation, proliferation, migration and attachment of endothelial cells and pericytes, as shown by *in vitro* studies of endothelial cells and pericytes of mouse embryos or bovine retinas[96–98] and confirmed *in vivo* in murine models[99]. Studies using transgenic mice with disrupted TGF-β–TGFβR2 downstream signaling—as in, for example, *Smad1*, *Smad2* and *Smad4* knockout mice[100,101]—indicate that TGF-β–TGFβR2 downstream pathways regulate BBB formation, particularly the endothelial-to-mesenchymal transition during normal development and vascular stabilization, and that aberrant TGF-β–TGFβR2 signaling in this pathway leads to development of brain hemorrhages[99].

Recent studies have shown that the forkhead transcription factor Foxf2 is specifically expressed in brain pericytes and that Foxf2 knockout embryos develop intracerebral hemorrhage, perivascular edema, thinning of the vascular basal lamina, an increase in luminal endothelial caveolae and BBB breakdown[97].

TGF-β–TGFβR2 signaling in neonatal intraventricular hemorrhage. Intraventricular hemorrhage (IVH) is a major cause of mortality in premature infants, where it results from vascular instability due to pericyte deficiency[100]. TGF-β signaling induces N-cadherin expression to promote BBB integrity, whereas regional disruption in TGF-β signaling leads to the development of IVH, as shown in transgenic mice with endothelial cell–specific *Smad4* knockout[100]. Mice are protected from IVH via perinatal glucocorticoid administration that increases TGF-β signaling, resulting in increased pericyte coverage[102].

TGF-β–TGFβR2 signaling in cerebral cavernous malformation. Cerebral cavernous malformation (CCM) is characterized by raspberry-like lesions of microvessels that exhibit pericyte deficiency and by enlarged endothelial cells with pinocytotic vesicles and poorly developed tight and adherens junctions, making the vessels prone to hemorrhaging[99,103]. This phenotype is present in sporadic and familial CCM, including loss-of-function mutations in *CCM1*, *CCM2* and *CCM3* (ref. 104). An analogous vascular phenotype develops

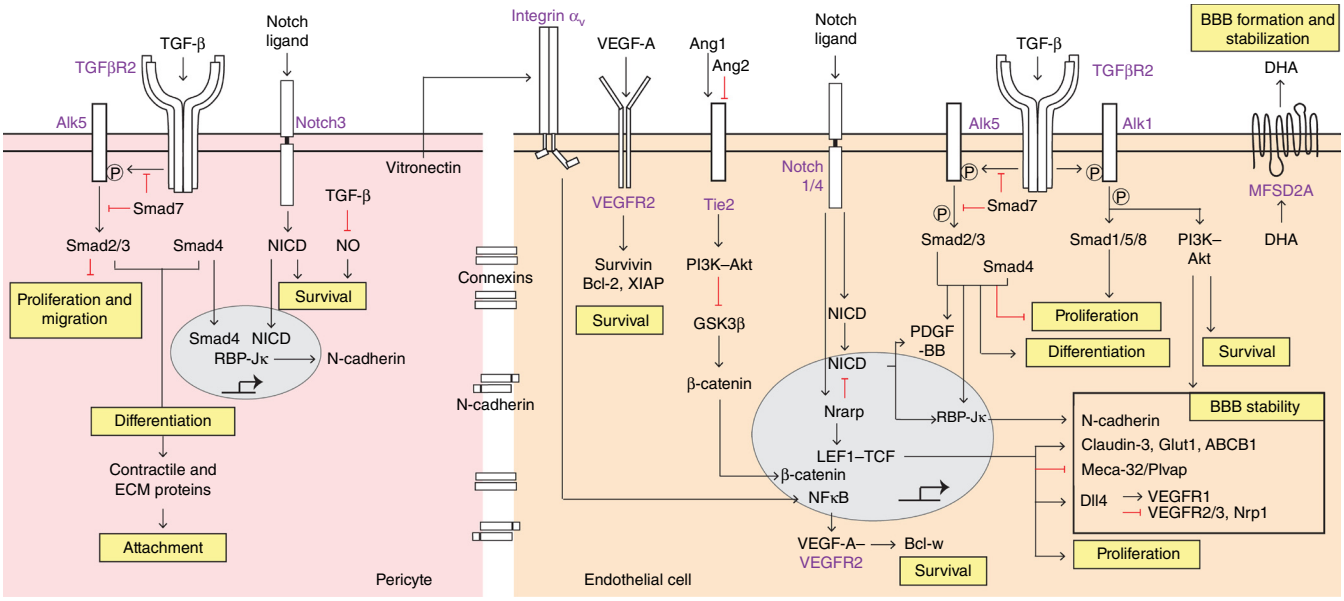

Figure 4 TGF-β–TGFβR2, Notch, VEGF-A–VEGFR2, Ang–Tie2 and MFSD2A signaling pathways. Pericytes (left, pink): TGF-β secreted by endothelial cells and pericytes binds to TGFβR2, which phosphorylates Alk5 (inhibited by Smad7), activating the downstream Smad signaling cascade. Activated Smad2/3 inhibits pericyte proliferation and migration. Recruitment of Smad4 to the Smad2/3 complex transcriptionally promotes pericyte differentiation, expression of contractile and extracellular matrix proteins, and pericyte attachment. TGF-β also inhibits nitric oxide generation, promoting survival. Activated Notch3 receptor cleaves NICD, which promotes survival. The Notch–NICD pathway works cooperatively with the TGF-β–TGFβR2–Smad-4 pathway to stimulate *Rbpj*-mediated expression of N-cadherin, increasing BBB stability. Endothelial cells (right, orange): the VEGF-A–VEGFR2 autocrine–paracrine pathway promotes survival via increased expression of anti-apoptotic Bcl-2, survivin and XIAP. Vitronectin secreted by pericytes acts on integrin α_V on endothelial cells, resulting in NFκB-mediated transcriptional expression of VEGF-A and intracrine-mediated VEGF-A–VEGFR2-dependent Bcl-w expression promoting survival. Pericyte-derived Ang1 acts on endothelial Tie2 receptor and endothelial cell–secreted Ang2 blocks Ang1 binding to Tie2, acting as a Tie2 antagonist. Ang1–Tie2 activates the PI3K–Akt pathway resulting in inhibition of glycogen synthase kinase 3β (GSK3β), an inhibitor of β-catenin; this leads to β-catenin nuclear translocation, activating TCF–LEF transcription factors that control expression of several proteins promoting BBB stability (for example, upregulation of claudin-5, Glut1, and ATP-binding cassette subfamily B member 1 (ABCB1), downregulation of plasmalemma vesicle associated protein (Plvap)–Meca23, and DLL4-mediated expression of VEGFR1 and inhibition of VEGFR2, VEGFR3 and neuropilin-1 (Nrp1); see box). Signaling through Notch1 or Notch4 receptors contributes to β-catenin-mediated BBB stability via Notch-regulated ankyrin repeat protein (Nrarp), which increases β-catenin nuclear signaling by inhibiting LEF1 degradation and decreases Notch signaling via NICD destabilization. Additionally, the Notch–NICD pathway stimulates PDGF-BB expression and RBP-Jκ–mediated expression of N-cadherin, contributing to BBB stability. As in pericytes, the TGF-β–TGFβR2 pathway in endothelium similarly activates (i) the Alk5–Smad2/3/4 complex to transcriptionally promote differentiation, inhibit proliferation and induce RBP-Jκ–mediated expression of N-cadherin, (ii) the Alk1–Smad1/5/8 complex to promote proliferation and (iii) the Alk1–PI3K–Akt pathway to promote survival and BBB stability. MFSD2A facilitates luminal-to-abluminal transport of docosahexaenoic acid (DHA), an essential omega-3 fatty acid, and controls formation of the BBB; its expression depends on the presence of pericytes. Red lines, inhibitory mechanisms; black lines, activator mechanisms; gray circle, cell nucleus.

in mice with endothelial cell–specific inducible ablation of *Ccm1* (*iCcm1*$^{\Delta EC/EC}$) (refs. 99,103) and *Ccm2* or *Ccm3* mutations (ref. 99). Disrupted TGF-β signaling at the BBB is reported in studies using *iCcm1*$^{\Delta EC/EC}$ mice, brain tissue from people with CCM, and cultured human brain vascular cells[99,103] and contributes to CCM by inducing structural instability of capillaries. Human CCM3 also binds to paxillin, a scaffolding protein; and CCM3 and paxillin colocalize in mouse cerebral pericytes[105].

TGF-β–TGFβR2 signaling in brain cancer. Several studies indicate that TGF-β signaling[93,106,107] and pericytes[93,108] affect cancer growth and tumor-associated blood vessel function. For example, high TGF-β–Smad activity was found in aggressive, highly proliferative gliomas, and this activity confers poor prognosis in patients with glioma[106]. Transcriptomic analysis of primary cultured human gliomas and human glioma biopsies indicates that the TGF-β–Smad pathway promotes proliferation through the induction of PDGF-B in gliomas with an unmethylated *PDGFB* gene[106].

More recent studies reveal that self-renewing tumorigenic glioma stem cells give rise to pericytes to support vessel formation and tumor growth[92,93]. Glioma stem cells are recruited to endothelial cells by SDF-1, an α-chemokine that binds to G-protein-coupled CXCR4, and are induced to become pericytes predominantly by TGF-β (ref. 93). TGF-β signaling has also been shown to regulate vascular phenotypes in gliomas and is sufficient to invoke many of the changes found in a gene signature associated with pathologically altered vessels in human glioblastoma grade IV (ref. 107).

TGF-β–TGFβR2 signaling in ischemic stroke. Multiple studies indicate that TGF-β–TGFβR2 signaling mediates angiogenic responses to brain injury due to ischemia or hypoxia[109]. In this context, TGF-β signaling stabilizes newly formed microvessels[110].

Notch pathway characterization. Pericytes express Notch3 and endothelial cells express Notch1 and Notch4 receptors (**Fig. 4**). Notch ligands such as delta-like ligand 4 (Dll4) and Jagged activate the receptor to induce cleavage of the Notch intracellular domain (NICD). NICD translocates to the nucleus and interacts with a Notch signal integrator transcription factor, the DNA-binding protein CSL complex (comprising C-promoter binding factor 1 (CBF-1)–RBP-Jκ, suppressor of hairless, and Lag-1), to induce transcription of downstream target genes, as shown in *Notch3* knockout mice[111].

In endothelial cells, the Notch pathway is modulated by canonical Wnt signaling to influence vascular sprouting and remodeling[112]. The Wnt–β-catenin pathway in brain endothelium regulates differentiation of the brain vasculature, angiogenesis and BBB integrity, as shown by *in vitro* studies using primary mouse and rat brain microvascular endothelial cells and endothelial cells with conditional activation of β-catenin and by *in vivo* studies using *Pdgfb-iCreERT2;Ctnnb1*$^{lox/lox}$ mice, *Pdgfb-iCreER*$^{+/-}$*;Nrarp*$^{-/-}$*;Ctnnb1*$^{lox(ex3)/lox(ex3)}$ mice, TOP-Gal Wnt reporter mice and zebrafish[112,113].

Notch pathway: function. The Notch pathway regulates angiogenic sprouting and microvascular remodeling[111]. During sprouting angiogenesis, specialized endothelial tip cells lead the outgrowth of blood vessel sprouts toward gradients of VEGF-A (refs. 114,115). Studies using *Dll4*$^{+/-}$ mice and mice with inducible endothelium-specific inactivation of *Notch1* (*Cdh5-CreER*^{T2}*;R26R-Notch1*$^{loxP/loxP}$ mice), as well as Notch inhibition by γ-secretase inhibitors or activation with soluble Jagged-1 peptide, have demonstrated that Dll4–Notch1 signaling between endothelial cells in the angiogenic sprout restricts tip-cell formation in response to VEGF (ref. 116). This model offers not only an explanation for the dose dependency and haploinsufficiency of the *Dll4* gene[117,118], but also suggests that γ-secretase inhibitors and/or modulators of *Dll4* or Notch signaling developed originally for Alzheimer's disease could also be used as pharmacological regulators of angiogenesis.

Notch signaling in neurological disorders. Several studies using transgenic models, human tissue and/or cultured brain vascular cells report that CCM is associated with disruptions in TGF-β and Notch signaling at the BBB[99,103]. Much like disrupted TGF-β signaling, dysfunction in Notch signaling is associated with IVH, as observed in mouse knockouts of *Rbpj* (encoding the Notch-related RBP-Jκ transcription factor)[100]. In cultured glioblastoma stem cells, activating Notch1 signaling induces a vascularization switch and causes the glioblastoma stem cells to differentiate into pericyte-like cells (expressing PDGFRβ, NG2 and α-smooth muscle actin) with upregulated expression of angiogenic factors including cytokines, matrix metalloproteinase-9 (MMP-9) and adhesion molecules[92].

VEGF-A–VEGFR2 pathway characterization and function. Pericytes (paracrine signaling) and endothelial cells (autocrine signaling) secrete VEGF-A, which activates the VEGF receptor-2 (VEGFR2) pathway to increase expression of antiapoptotic Bcl-2, survivin and X-linked inhibitor of apoptosis protein (XIAP), as shown by *in vitro* studies of human brain pericyte and endothelial cell cocultures[119] (**Fig. 4**). For intracrine signaling, pericytes secrete vitronectin (in a PDGF-BB–PDGFRβ-dependent process) that activates integrin α_V–NFκB signaling in endothelial cells to upregulate VEGF-A, which signals intracellularly via VEGFR2 to promote Bcl-w expression and cell survival, as shown by *in vitro* studies of human brain pericyte and endothelial cell cocultures[119] (**Fig. 4**). VEGF-A–VEGFR2 signal transduction promotes cell survival, angiogenesis and vascular permeability[2,119].

VEGF-A–VEGFR2 signaling in ischemic brain injury and hypoxia. *In vivo* studies in cats have shown that brain pericytes migrate away from capillaries rapidly following a hypoxic insult[120], and the pericyte–endothelial cell ratio in the brain is reduced 1 week after hypobaric hypoxia in rodents[121]. During hypoxia, VEGF levels are upregulated in pericytes within 24 h and upregulated in astrocytes after 4 d (ref. 121). In *in vitro* endothelial and pericyte cocultures, inhibiting VEGF in chronic mild hypoxia (1% O_2) promotes angiopoietin-1 (Ang1)-mediated endothelial cell tight junction stabilization, whereas severe hypoxia (0.1% O_2) promotes apoptosis of endothelial cells[122]. Hypoxia activates hypoxia-inducible factor 1α, which translocates to the nucleus and binds hypoxia-response elements to induce transcription of target genes to promote angiogenesis (for example, by upregulating VEGF-A and MMPs), anaerobic metabolism, apoptosis, and cell survival and proliferation[123].

VEGF-A–VEGFR2 signaling in brain cancer. Inhibition of VEGF-induced angiogenesis suppresses growth of human glioblastoma cells *in vivo* in mice[124]. Clinical trials with bevacizumab, a monoclonal antibody against VEGF-A, have shown it slows tumor growth in people with glioblastoma, but growth inevitably recurs[125]. Treatment with an anti-VEGF antibody was least effective in human subjects with glioblastoma exhibiting heightened CBF and angiogenesis[125]. VEGF–VEGFR2 signaling can also function as a negative regulator of receptor tyrosine kinases including PDGFRβ in pericytes[126] and mesenchymal–epithelial transition (MET) receptor in glioblastoma cells[127]. Pericytes *in vitro* are also reported to express VEGFR2; VEGF ablation in tumor cells disrupts PDGFRβ–VEGFR2 heterocomplex formation and downstream PDGFRβ signaling in pericytes and increases tumor vessel maturation[126]. MET is more abundantly expressed in glioblastoma tissue of patients who exhibit more resistance to bevacizumab[127], suggesting that VEGFR2's association with receptor tyrosine kinases in pericytes may be important in promoting cancer cell maturation and/or tumor growth.

Ang–Tie2 pathway characterization and function. Ang1, secreted by pericytes, binds to the endothelial cell–specific Tie2 receptor tyrosine kinase and activates the downstream PI3K–Akt pathway in endothelial cells[5]. Angiopoietin-2 (Ang2), expressed by endothelial cells, was originally shown to inhibit Ang1-mediated phosphorylation of Tie2, as well as cellular responses during embryonic development[5]. Ang2 also has proangiogenic activities in adult tissues and cultured endothelial cells, independent of Ang1. For example, in the absence of Ang1, Ang2 binds endothelial Tie2 and activates the PI3K–Akt pathway in cultured endothelial cells, acting as a Tie2 agonist, but when Ang1 is present, Ang2 dose-dependently inhibits Ang1-induced Tie2 phosphorylation and endothelial cell survival, acting as a Tie2 antagonist[128]. Ang2 also binds to endothelial integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$) to induce phosphorylation of the integrin adaptor protein focal adhesion kinase (FAK) and activation of Rac1, which promotes endothelial cell migration and sprouting, as demonstrated in cultured Tie2-silenced endothelial cells and *in vivo* in human xenotransplanted endothelial cells in mice[129].

Ang1 knockout mice exhibit pronounced angiogenic deficits in brain resulting in embryonic lethality, similarly to *Tie2* knockout mice[130]. Studies using *in vivo* assays demonstrate attenuated vascular responses to histamine, bradykinin and VEGF in *Ang2*$^{-/-}$ mice[131]. Recombinant Ang1 attenuates retinal vascular disruptions caused by pericyte loss[132], whereas overexpressing Ang2 in the retina results in pericyte loss and aberrant retinal angiogenesis[133]. Furthermore, cytokine-induced intracellular calcium influx is impaired in *Ang2*-null endothelioma cells, consistent with reduced phospholipase activation *in vivo*[131]. Thus, the Ang–Tie2 system has a critical role in regulating angiogenesis and vascular permeability, but Ang1 and Ang2 exert diverse effects on endothelial cell functions depending on experimental conditions and models.

Ang–Tie2 signaling in neurological disorders. In the db/db transgenic mouse model of diabetes, ischemic stroke leads to enhanced BBB breakdown associated with increased expression of Ang2 and

with decreased expression of Ang1, Tie2 and tight junction endothelial proteins[134]. Ang2 is upregulated in tumors and thought to denote the onset of angiogenic sprouting[135].

MFSD2A pathway characterization and function. The major facilitator superfamily domain-containing 2a (MFSD2A) is a sodium-dependent BBB transporter of long-chain fatty acids that is expressed in the brain exclusively in the endothelium, as indicated by studies in mice and humans[136,137]. Studies in transgenic *Mfsd2a* null mice have shown that MFSD2A facilitates transport of docosahexaenoic acid (DHA), an essential omega-3 fatty acid, into the brain[136]. Its expression appears to depend on the presence of pericytes[137], but the exact molecular pathway remains to be elucidated. MFSD2A exhibits dual functions at the BBB by regulating the formation and maintenance of BBB integrity[137] and delivery of essential fatty acids to the brain, as shown *in vitro* in endothelial cell cultures and *in vivo* in murine models[136,137].

MFSD2A signaling in microcephaly. *Mfsd2a*-deficient mice develop a reduced brain size, termed microcephaly[136]. Microcephaly syndrome in humans was recently shown to be caused by inactivating mutations in *MFSD2A*, and the syndrome's severity correlates with the degree of functional inactivation of the MFSD2A protein[138,139].

Ephrin–Eph pathway characterization and function. The Eph receptor tyrosine kinases and their membrane-tethered ephrin ligands provide critical guidance cues at points of cell-to-cell contact[140]. EphB receptors and their ephrin-B ligands are critical in the regulation of developmental angiogenesis and pericyte–endothelial cell interactions during vascular assembly, as shown *in vivo* by genetic loss-of-function studies[140]. Ephrin-B ligands and EphB receptors mark angiogenic vessels *in vivo* in the developing murine retina[141]. Studies in transgenic *Pdgfrb-Cre;Efnb2*$^{lox/lox}$ mice reveal that ephrin-B2–EphB4 signaling controls pericyte directional migration and adhesion to maturing vessels[142]. Studies using genetically engineered mice in which the *lacZ* coding region substitutes and reports for the *Efnb2* coding region have shown that ephrin-B2 is expressed in brain pericytes and endothelial cells[143]. Moreover, EphB4 controls blood vascular morphogenesis during postnatal angiogenesis[144]. Thus, ehprin-B2 and its receptor EphB4 can participate in vascular remodeling and in different aspects of NVU formation. The ephrin-B–EphB pathway is activated bidirectionally[143]: ephrin-B binding to EphB receptors causes phosphorylation of both the ephrin-B transmembrane ligand and its EphB receptor[141].

Other pericyte–endothelial cell communication pathways. Signaling between integrin $\alpha_4\beta_1$ expressed by proliferating but not quiescent endothelial cells and vascular cell adhesion molecule-1 (VCAM-1) expressed by proliferating but not quiescent pericytes is critical for cell–cell adhesion events required for survival of endothelial and mural cells during vascularization, as shown both by *in vitro* and *in vivo* studies[145]. Blocking N-cadherin function leads to defective pericyte adhesion, increased pericyte recruitment and disturbed vascular morphogenesis, as shown in chicken embryos[146]. Studies using *in vitro* pluripotent embryonic stem cells demonstrate that N-cadherin is required for the maturation of endothelial sprouts by interacting with pericytes[147]. Endosialin is not expressed in the normal human adult brain[148,149] or the adult mouse brain[150], but is abundantly expressed in brain pericytes in the developing murine CNS[150] and tumor vessel–associated pericytes[149], suggesting that its expression in sites of active tissue remodeling and neovascularization might have implications for angiogenesis, tumor growth and metastasis.

Pericyte–astrocyte signal transduction

CypA–NFκB–MMP-9 pathway characterization and function. Astrocyte-secreted apolipoprotein E (APOE) interacts with cell-surface LRP1 on pericytes, which regulates activation of the proinflammatory BBB-degrading cyclophilin A (CypA)–NFκB–MMP-9 pathway in an isoform-specific manner (i.e., APOE4 but not APOE2 or APOE3 acts in this way)[11] (**Fig. 5**). The increased MMP-9 activity triggered by APOE4, but not APOE2 and APOE3, in the vessel wall leads to degradation of endothelial tight junction and basement membrane proteins causing BBB breakdown[11] which in turn leads to brain accumulation of blood-derived neurotoxic molecules and erythrocytes and to secondary neurodegenerative changes.

CypA–NFκB–MMP-9 signaling in Alzheimer's disease. The cerebrovascular contributions to dementia and Alzheimer's disease are particularly salient in individuals carrying the *APOE4* gene, the major genetic risk factor for late-onset sporadic Alzheimer's disease[83]. Increases in CypA and MMP-9 CSF levels were recently reported to correlate with BBB breakdown in human *APOE4* carriers, but not in age-matched *APOE2* or *APOE3* carriers with intact BBBs[49]. Additionally, post-mortem analysis in *APOE4*-positive people with Alzheimer's disease compared to non-carriers reveals increased CypA and MMP-9 protein levels in hippocampal and cortical pericytes, as well as pericyte degeneration[50,151]. Animal studies have demonstrated that BBB breakdown in *APOE4* transgenic mice, but not in *APOE3* or *APOE2* transgenic mice[11,152,153], results in neuronal injury and neurodegeneration[11].

Arachidonic acid pathway. Astrocytes have been proposed to regulate pericyte tone via some of the same signaling pathways as reported for astrocyte-mediated regulation of VSMCs tone[6,30] (**Fig. 5**). The arachidonic acid pathway was shown to have a critical role in the regulation of pericyte tone and capillary diameter in studies using rat brain slices and retinal explants[35]. *In vivo* studies in the murine cortex have shown that arachidonic acid is metabolized in astrocytes to prostaglandin E_2 (PGE_2) by cyclooxtgenase-1, which has been shown to regulate hemodynamic responses[154]. Studies using rat cerebellar slices and retinal explants have shown that PGE_2 activates EP4 receptors in pericytes, leading to pericyte relaxation after addition of glutamate to slices[30]. Arachidonic acid secreted by astrocytes is metabolized to 20-hydroxyeicosatetraenoic acid (20-HETE) in pericytes by membrane-bound cytochrome P450 4A, as shown in rat cerebral arterial microsomes and brain slices[30,155]. In turn, 20-HETE leads to contraction of pericytes in cerebral slices and retinal explants[30,35]. Although the idea that the arachidonic acid pathway in astrocytes controls pericyte tone and capillary diameter is supported by studies in brain slices, *in vivo* evidence is still lacking.

$Ca^{2+}{}_i$–calmodulin–myosin light chain pathway. Intracellular Ca^{2+} ($Ca^{2+}{}_i$) increases in response to voltage-gated Ca^{2+} channels, as shown *in vitro* in primary rat brain pericytes[156] and isolated rat retinal microvascular pericytes[157], and in response to reactive oxygen species (ROS), as shown *in vitro* in human microvascular pericytes[158], *ex vivo* in rat cerebellar slices[30] and *in vivo* in murine pericytes following ischemic stroke[46]. An increase in extracellular K^+ activates voltage-gated Ca^{2+} channels, resulting in $Ca^{2+}{}_i$ increases and depolarization and contraction of primary rat brain pericytes[156]. Increased $Ca^{2+}{}_i$ in pericytes is shown to promote contraction, possibly via downstream signaling through calmodulin and myosin light chain kinase to phosphorylate myosin light chain and induce contraction, by analogy to events described in VSMCs of isolated rat cerebral arteries[159].

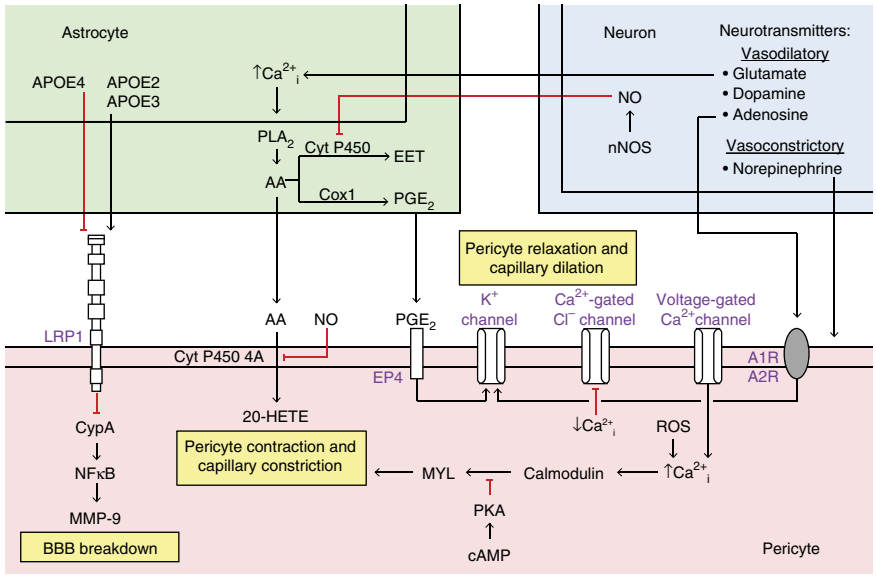

Figure 5 Pericyte–astrocyte and pericyte–neuron signaling pathways. Astrocytes (top left, green) secrete APOE2 and APOE3 that bind to lipoprotein LRP1 receptor on pericytes (bottom, pink) to inhibit the downstream CypA–NFκB–MMP-9 pathway. In contrast, APOE4 binds weakly to LRP1, which activates the proinflammatory CypA–NFκB–MMP-9 cascade leading to BBB breakdown. Astrocyte $Ca^{2+}{}_i$ increases in response to neuronal factors such as glutamate, which promotes phospholipase A_2 (PLA_2)-mediated arachidonic acid (AA) generation. In astrocytes, AA is metabolized into PGE_2 via cyclooxygenase-1 (Cox1), as well as into epoxyeicosatrienoic acids (EET) via cytochrome (Cyt) P450. Astrocytic AA is metabolized into 20-HETE in mural cells via membrane-bound Cyt P450 4A, which promotes pericyte contraction. PGE_2 from astrocytes binds to the pericyte EP4 receptor, which alters K^+ conductance and promotes pericyte relaxation. In neurons (top right, blue), neuronal nitric oxide synthase (nNOS) generates NO, which inhibits Cyt P450 in astrocytes and Cyt P450 4A in pericytes to prevent AA to EET and AA to 20-HETE metabolism, respectively. In pericytes, increased cAMP signals via protein kinase A (PKA) to inhibit myosin light chain (MYL) phosphorylation and prevent pericyte contraction. Pericyte $Ca^{2+}{}_i$ increases in response to voltage-gated Ca^{2+} channels and ROS. Increased $Ca^{2+}{}_i$ in pericytes promotes contraction, possibly via downstream signaling through calmodulin (CaM) and myosin light chain kinase (MLCK) which phosphorylates MYL to induce contraction, as shown in VSMCs. Conversely, decreasing $Ca^{2+}{}_i$ in pericytes inhibits Ca^{2+}-gated chloride channels which promotes relaxation. Furthermore, neurotransmitters promote pericyte relaxation (for example, glutamate, dopamine, adenosine) or contraction (for example, norepinephrine). Specifically, adenosine signals through adenosine A1 and A2 receptors (A1R, A2R) on pericytes to alter K^+ conductance and promote pericyte relaxation. Red lines, inhibitory mechanisms; black lines, activating mechanisms.

Pericytes were recently shown to express *Myl9*, which encodes myosin light chain regulatory polypeptide 9, in single-cell RNA-seq analysis of the murine cortex[160]. Treating isolated rat retinal microvascular pericytes with a cyclic guanosine monophosphate analog inhibits voltage-gated Ca^{2+} channels, decreases $Ca^{2+}{}_i$ and inhibits Ca^{2+}-gated chloride channels, which decreases whole-cell Ca^{2+} and Cl^- currents and promotes pericyte relaxation[157].

Pericyte–neuron interactions

Neuronal innervation of pericytes covering brain capillaries is not as well understood as neuronal innervation of VSMCs surrounding arterioles and small arteries[161] although capillaries are more numerous and more densely spaced than arterioles, as shown, for example, in the mouse cortex[162]. Moreover, the average distance between a neuron and a capillary is 8–23 µm in the mouse hippocampus, while the average distance between a neuron and an arteriole is 70–160 µm (ref. 163), suggesting that pericytes and capillaries are well positioned to receive chemical transmitters from activated neurons and are likely to respond earlier to changes in neuronal activity than VSMCs.

Neurotrophic factors. Cultured human brain pericytes express levels of neurotrophic factors that are low but comparable to the amounts produced by cultured astrocytes[164]. The functional importance of these findings remains unknown. A recent study using a murine hypothalamic GT1-7 cell line has shown that only pericyte-derived media, but not astrocyte or VSMC media, increase the insulin-stimulated phosphorylation of Akt in GT1-7 cells and insulin-dependent tyrosine phosphorylation of insulin receptor β (ref. 165), suggesting that pericytes rather than astrocytes and VSMCs can increase insulin sensitivity in hypothalamic neurons by releasing soluble factors. Given the strategic location of pericytes within the NVU, it is possible that pericyte-derived molecules may be distributed in the NVU and reach their neuronal targets by para-arterial CSF–interstitial fluid flow[166] or perivascular flow[167].

Neurotransmitters. Studies using bovine retinal pericytes[168] and *ex vivo* rat cerebellar slices and retinal explants[30,35] show that norepinephrine leads to pericyte contraction and reduction of capillary diameter. Neurotransmitters that lead to pericyte relaxation include GABA, as shown *ex vivo* in rat cerebellar slices[35]; adenosine, as shown *in vitro* in rat retinal pericytes[169]; glutamate, as shown *ex vivo* in rat cerebellar slices[30]; and dopamine, as shown *ex vivo* in rat retinal pericytes[170]. Studies in rat cerebellar slices and retinal explants also show that glutamate suppresses pericyte contractility through PGE_2[30,35] and that nitric oxide blocks 20-HETE-induced pericyte contraction by inhibiting arachidonic acid conversion to 20-HETE, resulting in capillary dilation[30] (**Fig. 5**). Recent *in vivo* studies have provided important evidence for neuronal control of capillary circulation by showing that capillaries dilate ahead of arterioles in the mouse cortex in response to whisker stimulation[30].

Integrating pathways: toward a systems biology approach

Recent studies have also focused on modeling the BBB, NVU and pericyte functions in blood vessels[171–173]. A computational model predicting disruption of blood vessel development incorporates endothelial, inflammatory and mural cells (i.e., pericytes)[172]. A physical three-dimensional, multi-compartment, organotypic microphysiological system representative of the NVU—an NVU on a chip—recapitulates all critical barriers in the brain, including BBB, brain–CSF barrier and blood–CSF barrier[171]. It has been suggested that *in silico* modeling might even more rapidly enhance our understanding of the NVU than *in vitro* cell-based modeling[173], but it requires a critical level of biological understanding to successfully bridge the logical connectivity of molecular pathways with computational integration systems[174].

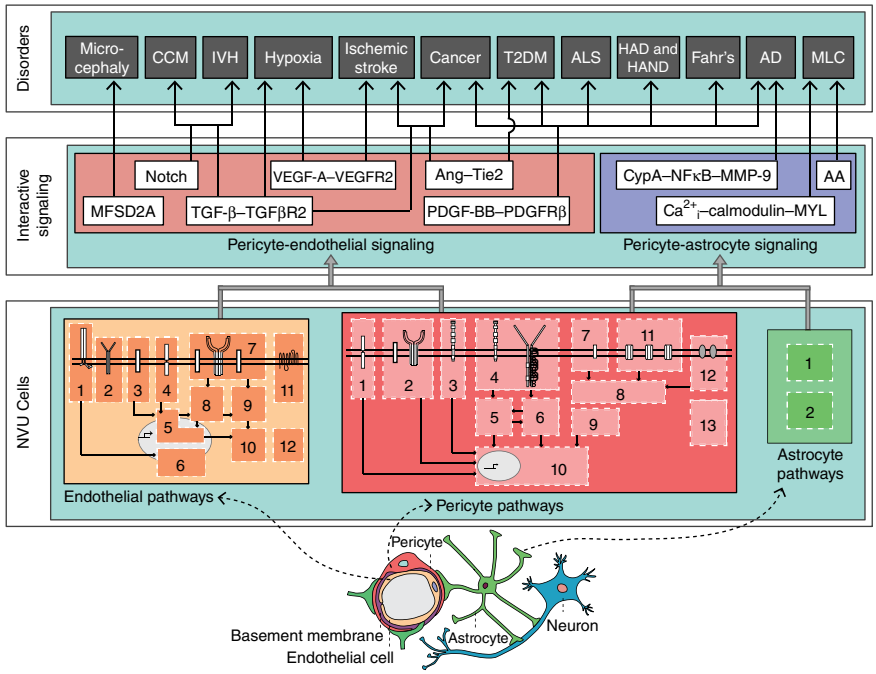

Figure 6 Integrated pathways between pericytes, endothelial cells and astrocytes in the NVU. A proposed three-layered model of the NVU. The first layer, NVU cells, is the foundational layer of cell-type-specific systems, each of which consists of integrated modular molecular pathways. We show the endothelial cell (yellow) partitioned into 12 pathways (**Supplementary Fig. 1**), the pericyte (pink) partitioned into 13 pathways (**Supplementary Fig. 2**) and the astrocyte (green) partitioned into 2 pathways (**Supplementary Fig. 3**). Each major signal transduction pathway within each NVU cell type also provides for the modular addition of new signaling pathways. The second layer, interactive signaling, instantiates the converging points of interactions of key signaling pathways between pericytes–endothelial cells and pericytes–astrocytes. The pericyte–endothelial signaling (coral box) in the second layer consists of 6 major signaling pathways: MFSD2A, Notch, TGF-β–TGFβR2, VEGF-A–VEGFR2, Ang–Tie2 and PDGF-BB–PDGFRβ. The pericyte–astrocyte signaling (purple box), also at the second layer, consists of 3 major signaling pathway: CypA–NFκB–MMP-9, arachidonic acid and $Ca^{2+}{}_i$–calmodulin–myosin light chain (MYL). The third layer, disorders, proposes major signaling pathways of CNS pericytes with neighboring NVU cell types (i.e., endothelial cells and astrocytes) that are suggested to contribute to pericyte dysfunction in neurological disorders, including microcephaly, CCM, IVH, hypoxia, ischemic stroke, cancer, type 2 diabetes mellitus (T2DM), ALS, HIV-associated dementia (HAD) and HIV-associated neurocognitive disorders (HAND), Fahr's disease, Alzheimer's disease (AD) and megalencephalic leukoencephalopathy with subcortical cysts (MLC).

Here we schematically illustrate a multilayered model of the NVU with (i) an interconnected system of pathways in endothelial cells, pericytes and astrocytes (NVU cell layer); (ii) converging points of key signaling pathways in CNS pericytes, between pericytes and endothelial cells, and between pericytes and astrocytes (interactive signaling layer); and (iii) the potential impact of such convergent pathways to neurological disorders (disorders layer) (**Fig. 6**). Since all these processes may be species, strain, disease or context dependent, we provide **Figure 6** as an all-inclusive data source and encourage the readers to explore and critically examine the specifics in the existing literature.

Future directions

Several functions of pericytes, such as capillary contractility, neuroinflammation and multipotent stem cell activity, are still to be fully characterized. It is also unclear how each pericyte subtype contributes to pericyte function, such as, for example, control of CBF versus control of BBB integrity. Developing genetic models with mural cell–specific ablation along the vascular tree combined with RNA-seq and proteomic analyses would greatly facilitate the study of CNS pericytes and other mural cell subpopulations.

Many studies have pointed toward pericytes as an attractive cellular target in rare genetic neurological disorders such as Fahr's disease, IVH, CCM and CADASIL. A growing body of evidence also indicates that targeting pericytes could be an important treatment option for controlling growth of brain tumors including glioblastoma, as well as improving vascular remodeling and stabilization of the BBB in Alzheimer's disease and possibly other neurodegenerative diseases. The potential of targeting pericytes to open the BBB on demand and/or to stabilize dysregulated CBF is another important area for future studies because of pericytes' emerging role in brain diseases affecting BBB and CBF, such as stroke and Alzheimer's disease.

The systems biology approach can help address some emerging questions in the field, such as, for example, whether some of the pathways identified in rare monogenic neurological diseases caused by genetic defects in pericytes, endothelial cells or astrocytes that lead to NVU disruption converge with complex neurological disorders, such as sporadic Alzheimer's disease, ALS and others, associated with pericyte and BBB dysfunction. Computational modeling of CNS pericytes combined with high-throughput screening of diverse libraries of compounds is also likely to advance discovery of therapeutics for neurological disorders based on correcting aberrant signaling and function of pericytes and their neighboring cell types in the NVU.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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