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# Multifaceted roles of pericytes in central nervous system homeostasis and disease

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## Abstract

Pericytes, the mural cells surrounding microcirculation, are gaining increasing attention for their roles in health and disease of the central nervous system (CNS). As an essential part of the neurovascular unit (NVU), pericytes are actively engaged in interactions with neighboring cells and work in synergy with them to maintain homeostasis of the CNS, such as maintaining the blood–brain barrier (BBB), regulating cerebral blood flow (CBF) and the glymphatic system as well as mediating immune responses. However, the dysfunction of pericytes may contribute to the progression of various pathologies. In this review, we discuss: (1) origin of pericytes and different pericyte markers; (2) interactions of pericytes with endothelial cells (ECs), astrocytes, microglia, oligodendrocytes, and neurons; (3) physiological roles of pericytes in the CNS; (4) effects of pericytes in different CNS diseases; (5) relationship of pericytes with extracellular vesicles (EVs) and microRNAs (miRs); (6) recent advances in pericytes studies and future perspective.

## Keywords

Pericyte, extracellular vesicles, microRNA, central nervous system disease, glymphatic system

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## Introduction

Pericytes surround endothelial cells (ECs) of the microcirculation which includes pre-capillary arterioles, capillaries, and post-capillary venules.[1] Here, we particularly focus on the pericytes of the capillaries within the central nervous system (CNS), where pericytes are sandwiched between ECs and astrocytes.[2] Within the blood–brain barrier (BBB), pericytes and ECs are separated by the endothelial basement membrane (BM), and together are embedded within the vascular BM that is derived from astrocytes.[3] Pericytes are most abundant in CNS where the EC to pericyte ratio can range from 1:1 to 3:1.[4] Such high regional density and strategic position of pericytes with neighboring cells may indicate distinct roles for pericytes in CNS. However, the recognized role of pericytes is limited and has long been overshadowed by ECs, due to the paucity of means to identify and distinguish these cells.[5] In recent years, advances in transgenic animal generation, in vivo microscopy techniques, and vascular single-cell transcriptomics have led to the identification of specific pericytes markers and visualization of pericytes in live animals.[6,7] However, gaps in knowledge persist. Findings in pericyte expression profiles have expanded pericyte categorization, and yet the functions of each type of pericyte in health and disease are not fully determined.[1,6] Also unclear, are pericyte contractile properties and their stem-cell potentials.[8,9] Although both benefits and drawbacks of pericytes have been broadly explored, the therapeutic translation related to pericyte function remains. In this review, we summarize the current knowledge and recent findings in the area of pericyte origin, pericyte identification, the physiological effects of pericytes as well as their multifaceted roles in CNS pathologies. We then review the interactions between pericytes and extracellular vesicles (EVs) and with microRNAs (miRs), which may provide potential therapeutic targets for the treatment of CNS diseases.[10,11] Finally, we briefly discuss recent

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advances in pericyte studies and present future perspectives.

## Pericyte origins

Pericytes vary in origin dependent on their anatomical location. Neural crest cells are the major source of pericytes in the forebrain.[12,13] In the remaining brain, pericytes are largely derived from the mesenchymal stem cells (MSCs) of the mesoderm which also generates the pericyte counterparts in peripheral organs.[14] Myeloid lineage cell is an alternative source of pericytes in the CNS,[15] and appears more specific to the pericytes in ectodermal organs like brain and skin, since no striking pericyte deficiencies are found in the mutant heart and liver and there are no detectable pericyte changes in vascular smooth muscle cells (VSMCs) in myeloid-deficient transgenic mice.[16]

## Pericyte identification

Both VSMCs and pericytes are the mural cells of the microcirculation.[3] Protruding ovoid nuclei with thin extended processes is the widely accepted characteristic morphology of pericytes, and is distinct from the ring-shaped VSMCs with circumferential processes.[1,3] This difference in morphology is very useful to distinguish pericytes from VSMCs within microcirculations, as many pericyte markers label both VSMCs and pericytes.[6] However, a transitional cell appears to exist between VSMCs and pericytes at the arteriolar level, and both have protruding cell bodies and circumferential processes.[1] Because the vessels they cover are arteriolar branches located between penetrating arterioles and capillaries, these transitional cells are referred to as "pre-capillary VSMCs."[8] Grant et al.[1] recently redefined these hybrid cells as "ensheathing pericytes". Therefore, how to identify and how to distinguish capillary pericytes from transitional cells are problematic. Here, we review pericyte markers and summarize these markers and related transgenic mouse lines in Table 1.

## Markers for both VSMCs and pericytes

### *Platelet-derived growth factor receptor beta*

This is a surface protein, is the most widely used pericyte marker,[3] and has been employed to identify pericytes during embryogenesis.[17] Although PDGFR-$\beta$ is a non-specific protein with expression in neuronal progenitor cells, glia, and fibroblast cells after injuries, the distinctive ovoid cell body and close proximity to microvessels permit specific identification of PDGFR-$\beta^+$ pericytes.[18]

### *Neural/glial antigen 2*

Neural/glial antigen 2 (NG2) is another commonly used pericyte marker,[6] although it is also expressed in oligodendrocyte progenitor cells (OPCs) and astrocytes.[19] However, during embryogenesis, NG2$^+$ pericytes (E11.5) appear prior to OPC's (E16), and its close proximity to endothelial cells (ECs) as well as the prominent ovoid cell body can easily distinguish it from OPCs.[3,20]

### *CD13, the aminopeptidase N*

CD13, the aminopeptidase N is regarded as a more specific marker for mural cells compared with PDGFR-beta and NG2.[21] However, CD13 cannot be employed to distinguish precapillary mural cells from capillary pericytes. In addition, CD13 cannot label pericytes of the lung.[22]

### *Desmin*

Desmin is an intermediate filament of muscle cells.[23] As pericytes have been visualized as smooth muscle cells at the capillary level, desmin can justifiably label pericytes in the BBB.[3,17] However, in a perilesional zone, desmin-positive pericytes do not overlap with PDGFR-$\beta$+ pericytes and resemble GFAP+ astrocytes.[24,25] Given the similarity in structure between the desmin and GFAP+ cells, the specificity of desmin is open to doubt in injured tissue regions.[26] Whether desmin represents inflamed pericytes or activated glial cells needs further confirmation.

### *Alpha-smooth muscle actin*

Alpha-smooth muscle actin ($\alpha$-SMA) has long been used as marker for both VSMCs and pericytes and until recently it was reported that SMA is not suitable for the pericytes of terminal vessel branches. Pericytes that encompass the pre-capillary arterioles are defined as "ensheathing pericytes" and express SMA, while their counterparts that occupy the downstream branches-capillaries are SMA-negative.[1] This finding may facilitate unveiling the distinct role of pericytes on different vessels.

## Novel markers for capillary pericytes

### *Vitronectin and interferon-induced transmembrane protein 1*

Vitronectin (VTN) and interferon-induced transmembrane protein 1(Ifitm-1) are two markers for capillary pericytes, which have been validated by single-cell RNA sequencing and fluorescent in situ hybridization.[6]

**Table 1.** Pericyte identification.

| Classic pericyte markers | | References |
|---|---|---|
| PDGFR-$\beta$ | (1) Surface protein that outlines the contour of the cell; | Armulik et al.[3] |
| | (2) First existing pericyte marker during embryogenesis; | Junget al.[17] |
| | (3) Labels relatively immature pericytes and VSMCs in CNS; | Goritz et al.[127] |
| | (4) Dynamic expressions in brain injuries | Kyyriainen et al.[18] |
| NG2 | (1) Integral membrane proteoglycan; | Zhu et al.[19] |
| | (2) First appears as a pericyte marker at E11.[5]; | Yotsumoto et al.[112] |
| | Expression in OPCs starts from E16 ; | Jung et al.[17] |
| | (3) Stimulates proliferation and migration of pericytes. | |
| $\alpha$-SMA | (1) Microfilaments in VSMCs and a subset of pericytes; | Jung et al.[17] |
| | (2) Exists as a pericyte marker after birth; | Grant et al.[1] |
| | (3) Positive expression in pre-capillary mural cells. | Hartmann et al.[21] |
| | May negative in capillary pericytes. | |
| | (4) In combination of morphology may help distinguish capillary pericytes from pre-capillary mural cells | |
| Desmin | (1) Intermediate microfilament; | Armulik et al.[3] |
| | (2) As pericyte marker throughout embryogenesis; | Jung et al.[17] |
| | (3) Labels mature pericytes and VSMCs in CNS; | Jung et al.[17] |
| | canonical marker of muscle cells; | Choi et al.[26] |
| | (4) May also be expressed in activated astrocytes in brain injury; | Kelly-Goss et al.[25] |
| CD13 | (1) Aminopeptidase N; | Hartmann et al.[21] |
| | (2) Exists as a pericyte marker after birth at P6; | Jung et al.[17] |
| | (3) Does not label pericytes in lung. | Vanlandewijck et al.[22] |
| RGS5 | Labels angiogenic capillary pericytes; | Mitchellet al.[28] |
| Endosialin(CD248) receptor | Labels angiogenic capillary pericytes; | Simonavicius et al.[35] |
| CD146 | Labels pericytes from E11, and may coordinate EC-pericyte interaction; | Chen et al.[30] |
| New pericyte markers | | |
| VTN/Ifitm-1 | Labels capillary pericytes; | He et al.[6] |
| NeuroTrace 500/525 | Absorbed by capillary pericytes ; | Damisah et al.[27] and Grant et al.[1] |
| Transgenicpericytesmice | | |
| $Pdgfrb^{ret/ret}$ | PDGF-B-truncating mutation | Moura et al.[151] |
| Pdgfrb-CreER mice | Tamoxifen-inducible Cre-recombinase under the control of the Pdgfrb promoter | |
| (1) Pdgfrb-P2A-CreERT2 | express Cre-ERT2 from the endogenous Pdgfrb promoter | Cuervo et al.[152] |
| (2) Pdgfrb(BAC)-$CreER^{T2}$ | Pdgfrb gene from $BAC^{f}$ library | Eilken et al.[36] |
| Pdgfrb-Cre mice + fluorescent protein reporter | Show the continuous contour of the mural cells | |
| (1) Pdgfrb-eGFP mice | | Jung et al.[17] and He et al.[6] |
| (2) Pdgfrb-tdTomato mice | | Grant et al.[1] |
| (3) Pdgfrb-YFP mice | | Berthiaume et al.[82] |
| Pdgfrb-Cre-ChR2 mice | Optogenetic stimulation cause capillary constriction | Hartmann et al.[7] and Kisleret al.[103] |
| $NG2\text{-}CreER^{TM}$ BAC transgenic mice | | Eilken et al.[36] |
| NG2-CreER mice + fluorescent protein reporter | Display the structure of individual mural cells | Jung et al.[17] and He et al.[6] |
| (1) NG2-DsRed mice | | |
| (2) NG2-tdTomato | | Grant et al.[1] and Berthiaume et al.[82] |

(continued)

**Table 1.** Continued.

| Classic pericyte markers | | References |
|---|---|---|
| NG2-Cre-ChR2 mice | Optogenetic stimulation cause arteriole constriction | Hill et al.[8] |
| Hey1-GFP (Tg(Hey1-EGFP)ID40Gsat) reporter mice | Label immature pericytes at angiogenic front | Eilken et al.[36] |
| Tbx18 | Labels all PCs, regardless of subsets of pericytes; | Guimaraes-Camboa, et al.[9] |

PDGFR-$\beta$: Platelet derived growth factor receptor beta; NG2: neural/glial antigen 2; RGS5: regulator of G-protein signaling 5; VTN/Ifitm-1:vitronectin/interferon-induced transmembrane protein 1; eGFP: estrogen-receptor; BAC: bacterial artificial chromosome; eGFP: enhanced green fluorescent protein; YFP: yellow fluorescent protein; ChR2: channelrhodopsin2.

VTN, a glycoprotein, is expressed in capillary pericytes, and has reduced abundance in pericytes that surround the arterioles and veins. Ifitm-1 shows a weak but specific signal for capillary pericytes.[6]

### *NeuroTrace 500/525*

NeuroTrace 500/525 is a Nissl dye, but does not label neurons in vivo. Surprisingly, it is exclusively absorbed by the capillary pericytes, as confirmed using both Pdgfrb-Cre:Tomato and NG2-Cre:Tomato transgenic mice.[27] NeuroTrace 500/525 generates a robust signal overlapping with endogenous PDGFR-$\beta$ and NG2-positive cells on the capillary level and is absent from immediate upstream arterioles. NeuroTrace 500/525 and $\alpha$-SMA are mutually exclusive,[1] which may provide a clear distribution of pericyte branches when using the two markers together.[1]

## Pericyte markers with angiogenesis and BBB development

### *Regulator of G-protein signaling 5*

Regulator of G-protein signaling 5 (RGS5) is particularly expressed in pericytes of angiogenic vessels. The RGS5 gene expression is more sensitive than morphological measurement. Its alteration is positively correlated with the number of activated pericytes and to a greater extent than that of NG2 and desmin during angiogenesis,[28,29] indicating RGS5 is an effective tool for pericyte quantification during angiogenesis. Likewise, endosialin is also reported to be exclusively expressed by angiogenic pericytes. CD146 is a transmembrane immunoglobulin. During BBB development, CD146 is expressed in the ECs that compose nascent vessels without pericyte coverage. However, as pericytes are recruited to the vessel sprouts, CD146 expression is diminished in ECs and enhanced in the pericytes. From E11 onward, CD146 is predominately expressed by pericytes and not by ECs of mature vessels with stable pericyte coverage. This transition indicates a coordinating role of CD146 between ECs and pericytes during BBB formation.[30]

## Pericyte effects in cell–cell interactions

We review the interactions of pericytes with ECs, astrocytes, microglia, oligodendrocytes, and neurons (Figure 1).

### *Pericytes and EC interaction (Figure 1(a)*

The interactions between pericytes and ECs are complex and have been widely studied.[31] The platelet-derived growth factor (PDGF)-BB/PDGFR-$\beta$ signaling pathway is vital for the survival, recruitment, and proliferation of pericytes during angiogenesis[32] Angiopoitin1/tyrosine protein kinase receptor (Tie2) signaling pathway is required for vascular stabilization.[33] These two pathways have been discussed extensively in previous reviews.[32] Here we review four pathways as follows:

*Vascular endothelial growth factor (VEGF)/VEGFR pathway.* VEGF-VEGFR signaling pathways play an important role in angiogenesis where it promotes the survival and proliferation of ECs. The roles of pericytes in the responses of ECs to VEGF vary among different studies. A study of tumor angiogenesis shows that pericytes promote EC survival by inducing ECs to release autocrine VEGF which subsequently activates downstream anti-apoptotic factors to stabilize vessels.[34] However, pericytes may also have destabilizing effects on vessel sprouting during both developmental and tumor angiogenesis.[35] Endosialin that is exclusively expressed by the pericytes in the angiogenic vessels can decrease the phosphorylation of VEGF/VEGFR2 downstream pathways in ECs and lead to EC apoptosis. By doing so, pericytes promote selective vessel regression, a process of pruning undesired capillaries for an organized

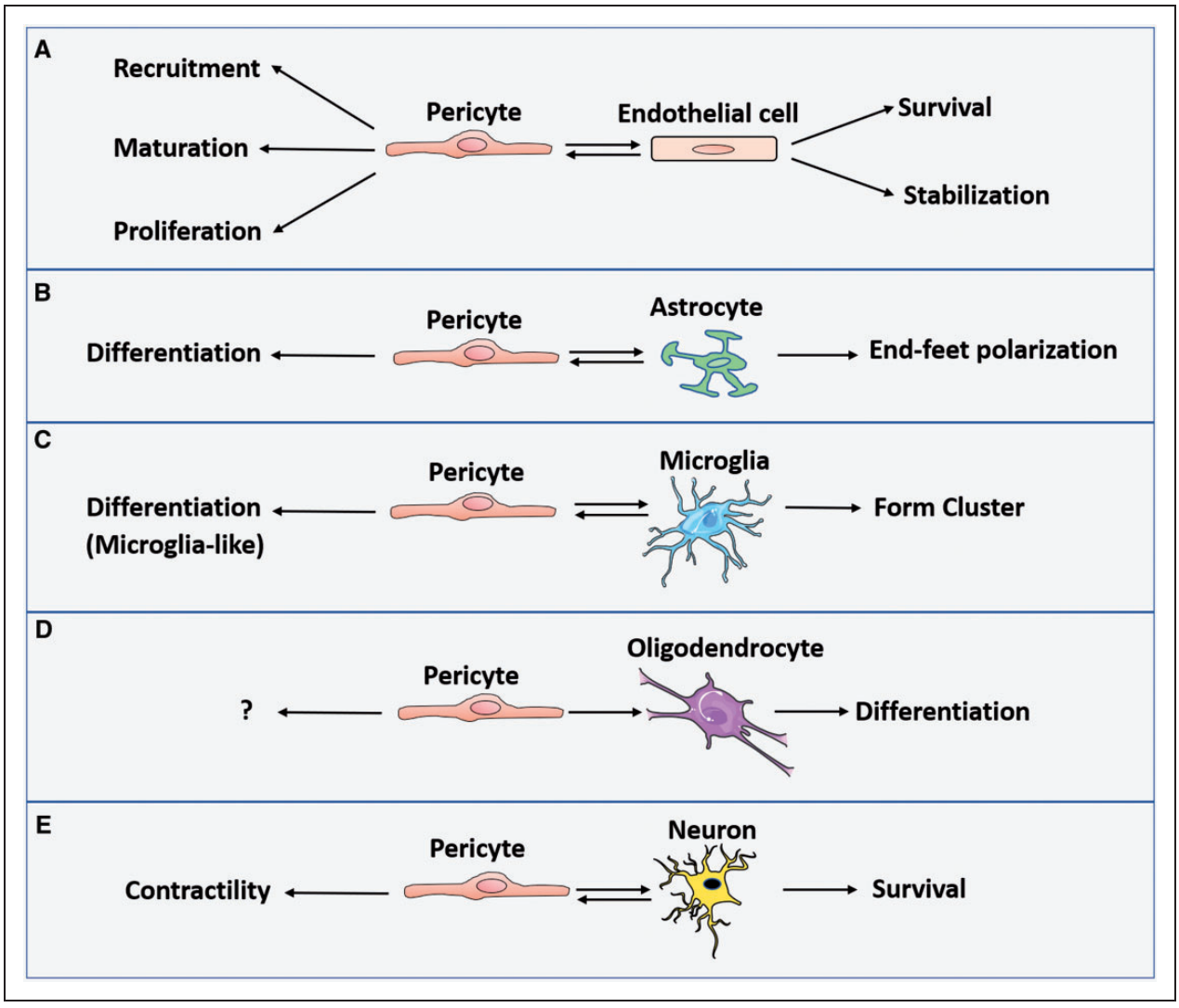


**Figure 1.** Pericyte effects in cell–cell interactions within NVU: (a) Pericyte promotes EC survival and stabilization, whereas EC enhances pericyte recruitment, promotes pericyte maturation and proliferation during embryogenesis and after birth. (b) Pericyte induces polarization of astrocytic end-feet. In turn, astrocyte help induce differentiation of pericyte. (c) Pericytes may differentiate into microglia. Pericyte and microglia can form clusters during injury. (d) Pericyte promotes OPC to differentiate into oligodendrocyte. (e) Pericyte secretes neuronal growth factor to enhance neuron survival, whereas neuron will send cues to control pericyte contractility.

vascular remodeling.[35] Pericytes are also required for the normal vessel branching during vessel sprouting. A study of retinal angiogenesis shows that deletion of pericyte-derived VEGFR-1 results in reduced vascular branch points and scattered vessel network, accompanied by an enhanced VEGF/VEGFR2 signaling pathway.[36] Soluble VEGFR1 (sVEGFR1) released by the pericytes at the front of angiogenic vessels can bind the circulating VEGF to prevent from VEGF acting on VEGFR2, and thereby inhibit EC hyperplasia that thickens the sprouting without branching. VEGF may also negatively control pericytes via disrupting pericyte recruitment and maturation by forming a PDGFR-$\beta$ and VEGFR-2 complex on pericytes.[37] However, another study of cancer-related retinopathy shows that VEGF ablates the pericytes via VEGFR1 not VEGFR2, as blockade of VEGFR2 cannot restore pericytes.[38] This discrepancy may be due to the different types of pericyte cell lines the studies tested. One used $\alpha$-SMA-positive pericyte cell line,[37] whereas the other one used NG2-positive retinal pericytes,[38] which suggests that different subsets of pericytes may respond differently to VEGF.

*Transforming growth factor beta (TGF-β).* TGF-$\beta$ is normally latent in both ECs and pericytes, and mediates angiogenesis, inflammation, and fibrosis when activated.[31] TGF-$\beta$ regulates vessel stability by inducing

pericyte differentiation.[31] Deletion of TGF-$\beta$ leads to remarkable reduction of mature pericyte coverage of vessels, which enhances vascular permeability and results in abnormal changes of retina that mimics those of diabetic retinopathy. Conversely, systemic deletion of platelet-derived growth factor receptor $\beta$ (PDGFR-$\beta$) in pericytes leads to downregulation of TGF-$\beta$, which may form a vicious cycle.[31] EC morphogenesis in developing brain partially relies on pericyte-expressed TGF-$\beta$ type1 receptor kinase and tissue inhibitor of matrix metalloproteinase 3 (TIMP3).[39] TGF-$\beta$/smad4 (Mothers against decapentaplegic homolog 4) pathway and Notch pathways in ECs cooperatively regulate the expression of N-cadherin, a key adhesion protein connecting ECs and pericytes.[40]

*Sphingosine-1-phosphate (S1P) signaling pathway.* S1P is a bioactive sphingolipid metabolite and mediates a myriad of physiological and pathological processes via binding to a family of five G protein-coupled receptors (S1PR1-S1PR5).[41] Pericyte-derived S1P increases N-cadherin and VE-cadherin expression between pericytes and ECs and reduces Angpt-2 in ECs.[31] A recent study shows that S1PR2 is upregulated in pericytes in experimental stroke.[42] S1PR2 may reduce N-cadherin expression and promote pericyte migration through NF-$\kappa$B p65 signaling, which increases BBB permeability and exacerbates neural injury.[42]

*Ephrin–erythropoietin-producing hepatoma (Eph) pathway.* EphrinB2 is vital for pericytes and ECs to assemble into a vascular structure.[43] It regulates the internalization of PDGFR-$\beta$ by pericytes.[44] Mutant mice with no EphrinB2 expression in pericytes and VSMCs are prone to develop aneurysms,[44,45] and suffer delayed neurovascular repair after stroke.[46] By contrast, diabetes induces the expression of EphrinB2 in brain pericytes, which is concomitant with immature neovascularization characterized by large diameter and tortuous appearance.[47]

*Pericyte and astrocyte interaction (Figure 1(b)).* Pericytes regulate the polarization of astrocyte end-feet.[2] Aquaporin 4 (AQP4), a principal water channel protein, is exclusively expressed by astrocytes, and mediates CNS water homeostasis, glymphatic system function, synaptic plasticity, and cognitive function.[48,49] Aqp4 is strongly expressed on the side of astrocytes that connects with pericytes.[50] Pericyte deficiency results in a redistribution of AQP4 on astrocyte end-feet where AQP4 moves away and is expressed on other side of astrocytes with no vessel contact.[2,50] This redistribution may be related with $\alpha$-syntrophin, the component of dystrophin complex that regulates AQP4 anchoring.[51] Normally, $\alpha$-syntrophin is expressed at increased density at astrocytic end-feet abutting pericytes.[50] In pericyte-deficient mice, $\alpha$-syntrophin is decreased with concomitantly reduced AQP4 expression in the perivascular site of the astrocyte end-foot.[50,52] In addition, the laminin a2 chain (Lama2), an astrocyte-derived extracellular matrix (ECM) protein, is important for the regulation of cell growth, motility, and adhesion.[53] Lama2 is greatly decreased in pericyte-deficient mice without affecting EC-derived basement membrane proteins.[2] Conversely, astrocytic laminin may also regulate pericyte differentiation via the integrin $\alpha$2 receptor.[54]

*Pericyte and microglial interaction (Figure 1(c)).* Not only do microglia acquire macrophage traits, a subset of pericytes share the same origin with macrophages.[15] Observations in CNS diseases demonstrate the potential transitions between pericytes and microglia.[55] The brain pericytes isolated from transgenic Alzheimer's mice can spontaneously differentiate into a CD11b-positive microglial-like cell types.[55] In both experimental seizures and human brain slice obtained from patients with seizure, pericytes appear with disorganized soma and ramifications and form a cluster with microglia around capillaries, yet without losing the markers NG2 and PDGFR-$\beta$.[56] By contrast, in experimental stroke, angiogenic pericytes gradually lose their characteristic morphology as well as their featured markers, gaining the microglial phenotype (CD11b-positive) seven days post stroke.[57] These transformed pericytes in stroke do not express CD68, a marker of perivascular macrophages, nor do they derive from the bone marrow, suggesting that the newly appeared microglia-like cells are local-derived.[57] Notably, not all pericytes present with microglial appearance, and the microglia-like pericytes do not overlap with the fibroblast-like pericytes that also proliferate post stroke, suggesting the divergent fates of different subsets of pericytes.[57] The microglial fate of pericytes may be attributed to the pericyte response to the compromised BBB and inflammation in AD, seizure, and stroke. These transformed pericytes may also acquire their own unique immune function, behaving differently from microglia.

*Pericyte and oligodendrocyte interaction (Figure 1(d)).* Oligodendrocytes derived from oligodendrocyte precursor cells (OPCs) myelinate the axon.[58] Pericytes and OPCs are located in close proximity in the perivascular area of cerebral white matter, and are mutually promoted.[59] Pericyte-derived Lama2 stimulates the differentiation of OPCs to oligodendrocytes during remyelination[60] and also guides neuron stem cells (NSCs) to adopt an oligodendrocyte fate.[61] All of these indicate that pericytes favor oligodendrocyte

generation, which implies a role for pericytes in demyelination diseases.

*Pericyte and neuron interaction (Figure 1(e)).* Pericytes isolated from the cortex of adult mice can promote the differentiation of NSCs from the ventricular-subventricular zone (V-SVZ).[62] Vitronectin (VTN) released by pericytes from the SVZ of adult mice can bind $\alpha v\beta 5$ integrins of astrocytes, resulting in induction of astrocytic pro-neurogenic factors.[63] Pericytes per se can also generate neurotrophic growth factors, i.e. pleiotrophin.[64] When pericytes are challenged with diphtheria toxin in vivo, adult mice exhibit loss of pericytes as well as neuronal loss. This may due to the deprivation of pericyte-derived pleiotrophin, as re-administration of pleiotrophin in pericyte-ablated mice rescues the neuronal loss, and pleiotrophin is mostly expressed in CD13-positive pericytes within NVU.[64] In addition, rapid loss of pericytes also leads to acute brain circulatory collapse including BBB breakdown, vasogenic edema, and poor blood flow, with all of these events contributing to neuronal loss.[64] Therefore, pericytes not only promote neurogenesis after birth, but also support mature neuronal survival, suggesting a potential role of pericytes in neurodegenerative diseases.

## Physiological roles of pericytes in the CNS

### *Pericytes in relationship to the BBB (Figure 2(a) and (b))*

ECs, pericytes, and astrocyte end-feet form a sandwich-like structure that provides the basic form of BBB.[3] During rodent embryogenesis, genesis of the cortical barrier (E13.5–E15.5) requires pericytes and astrocytes to cover the immature vessels where pericyte recruitment occurs prior to astrocyte generation.[65] Genetic deletion of pericytes in mouse embryos results in defective BBB development and postnatal BBB leakiness, the extent of which is proportional to the degree of pericyte deficiency and aging.[66] Pericytes maintain BBB integrity through several approaches. We will review these approaches as follows:

*Pericytes preserve the tight junctions of ECs.* Pericytes are necessary for functional tight junctions.[2] Pericyte loss leads to aberrant morphology of tight junctions that exhibit random alignment with convoluted appearance.[2] Pericyte detachment from vessels progressively decreases tight junction expression with aging.[66] N-cadherin is an integral component of heterotypic adhesion between pericytes and ECs.[67] Silencing N-cadherin in pericytes results in barrier defects, whereas overexpression of N-cadherin promotes barrier function.[67]

*Pericytes regulate transcytosis in ECs.* Vesicular trafficking, known as transcytosis, of brain ECs is much lower than that of ECs in other organs,[68] a phenomenon maintained by pericytes.[65] Pericyte-loss-related BBB leakiness does not result mainly from disruption of tight junction but from high transcytosis of ECs.[69,70] Suppression of transcytosis requires the major facilitator super family domain containing 2a (Mfsd2a) that is expressed by ECs and in proportion to pericyte coverage.[68–70] However, the ECs of blood–retina barrier of day1 postnatal mice (P1) still exhibit bulk transcytosis and high permeability, though they are covered by pericytes with functional tight junctions.[70] This inconsistency may due to the differentiation stages of pericytes which may be more crucial to transcytosis than the physical presence or the density of cells. The cytokines from mature pericytes may impact barrier integrity more than those from immature pericytes. Another interesting indication is that as transcytosis is the vesicular transport of macromolecules,[71] it may also serve as a means for molecules to shuttle between the peripheral blood and the CNS, and potentially be a target for drug delivery to the CNS.

*Pericytes and regulation of cerebral blood flow (CBF) (Figure 3(a)).* Since VSMCs control the blood flow within large vessels,[72] it is likely that pericytes also control the blood flow within the microcirculation, as pericytes are regarded as microvascular VSMCs.[8,73–80] However, the role of pericytes in the regulation of CBF has been debated among different studies. Pericyte contraction with segmental narrowing of capillaries results from prolonged impairment of the microcirculation in experimental ischemic stroke.[74] During hypoxia, pericytes dilate capillaries, an event that precedes arteriolar dilation and accounts for more than 80% of increased blood flow.[73] Hypoxia increases astrocytic [Ca2+], triggering the release of prostaglandin E2 that works on pericytes to evoke capillary dilation.[81] Hartmann et al.[7] showed that selective optogenetic stimulation of individual pericytes generates a ~20% reduction in capillary diameter as well as red blood cell velocity. Other studies further find that the microvascular constriction can spread from the proximal constricted pericytes to the distant non constricted pericytes. Selective removal of one pericyte can trigger the extension of adjacent pericyte processes that facilitates regaining the control of blood flow.[1,80,82] When obstruction occurs in pre-capillary arterioles, the ensuing low ATP will increase the intracellular [Ca2+] of distant capillary pericytes, resulting in irreversible pericyte constriction even after blood reperfusion.[74] The rigidly constricted pericytes will further lead to neurovascular uncoupling, which thereby exacerbates neural injury.[74] In contrast, some studies found

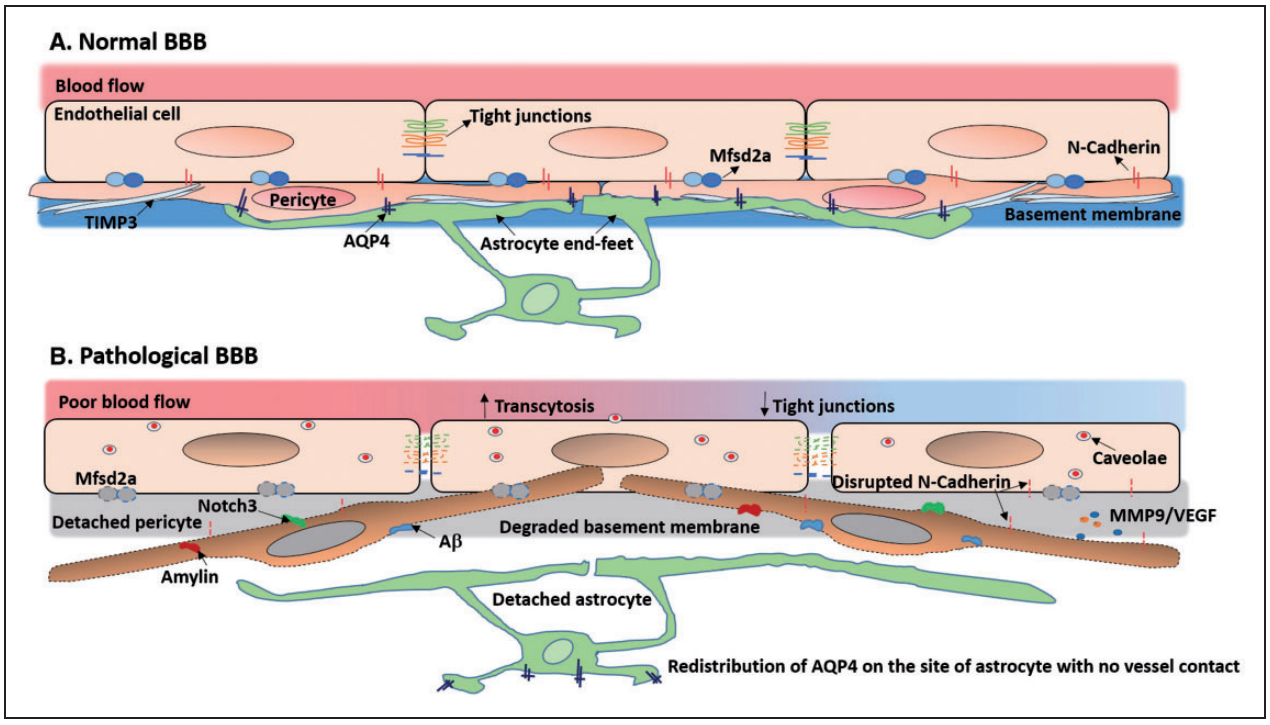


**Figure 2.** Role of pericytes in BBB function under normal and pathophysiological conditions: (a) Normally, pericyte coverage enables low transcytosis of ECs and fosters the expression of tight junction proteins and adhesion molecules between pericytes and ECs. Pericytes induce the polarization of astrocytic end-feet that AQP4 anchors at the perivascular site of astrocyte end-feet. Also, pericytes secrete ECM molecules such as TIMP3 to maintain the basement membrane. (b) In diseases, degenerated and migrated pericytes lead to high transcytosis of ECs due to loss of Mfasd2a and disrupted tight junctions. A$\beta$, amylin, and Notch3 deposition can lead to pericyte dysfunction and loss. Dysfunctional pericytes release MMP9 and VEGF and disrupt N-cadherin to further destabilize the BBB. The basement membrane is degraded as well.

that: (1) capillary pericytes exhibit contractile property, and cannot mediate functional hyperemia[8]; (2) The changes of capillary diameter are independent of pericyte coverage.[83] (3) Capillary pericytes do not contain SMA, and have no detectable contractility in response to optogenetic stimulation.[8] It is the arteriolar VSMCs that control the regional blood flow, not the downstream pericytes.[76] One possible explanation for these divergent findings is the different stimulation approaches. First, the chemical stimulating agents vary in kind and dose among studies. Pericytes may be able to constrict capillaries as long as capillaries are subjected to more potent and/or higher dose of chemical stimuli. Second, different transgenic mouse lines were used in these noted studies. The membrane-bound reporter protein in different transgenic mice may exhibit different results. For example, the fragmented fluorescent protein expression of $\alpha$-SMA in transgenic mice after tamoxifen injection may contribute to the poor detection of $\alpha$-SMA.[84,8] Additionally, transgenic mouse line carries different reporter genes that may also influence the results. For example, in NG2-Cre ChR2 mice, pericytes do not display contractile property for CBF regulation, whereas in Pdgfrb-Cre ChR2 mice, pericytes contract under two-photon stimulation, shrink capillary lumen, and block red blood cell flow, which indicate differential roles of different pericyte subtypes in regulating cerebral blood flow.[1] Next, the way that a chemical stimulant acts on regional capillary is quite different from optogenetic stimulation. Chemical stimulants influence the entire local region, whereas optogenetic stimulation directly targets the pericytes. Optogenetic stimulation has the advantage of avoiding the influence of other neighboring cells on CBF change.[85] However, this highly selective stimulation may not fully represent the natural occurrence of CBF change, as the CBF change is only part of neurovascular coupling and pericyte activity can also be affected by other CNS cells.[81] Therefore, additional investigations are warranted before concluding a definitive role for pericytes in the regulation of CBF.

*Pericytes and the glymphatic system.* The glymphatic system is a pathway within the CNS that removes brain waste and neurotoxins in cerebrospinal fluid

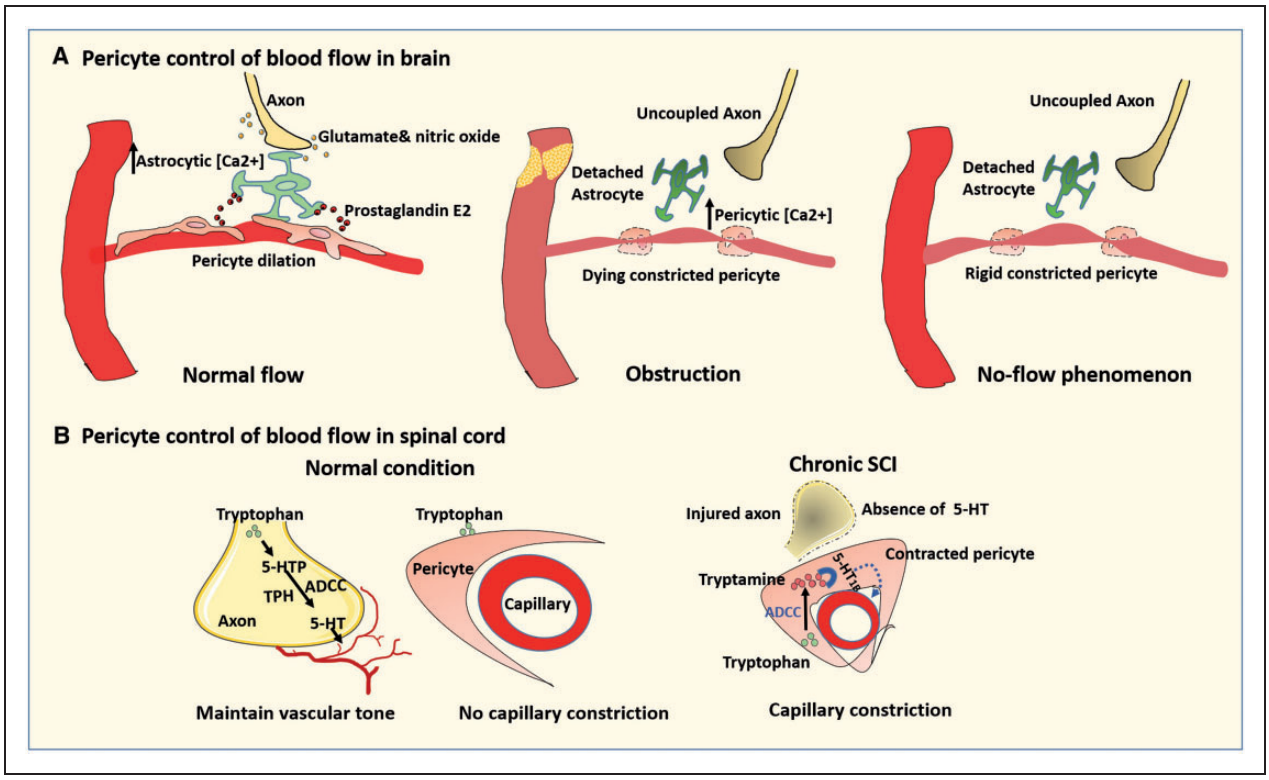


**Figure 3.** Role of pericytes in blood flow control under normal and pathophysiological conditions: (a) Normally, neurons may first send signals (glutamate and nitric oxide) to astrocytes to increase astrocytic [Ca2+], triggering the release of prostaglandin E2 to relax pericytes. When obstruction occurs in pre-capillary arterioles, the low ATP increases the intercellular [Ca2+] of distant capillary pericytes, resulting in irreversible pericyte constriction even after restoration of blood flow. (b) In normal spinal cord, the vessel constrictor 5-HT is released by axons via enzymes TPH and ADCC to maintain the basal vascular tone. Since TPH and ADCC are minimally expressed by capillary cells, there is no capillary constriction with tryptophan treatment. In chronic SCI, the compromised axon loss the ability to generate 5-HT. Blood flow is controlled by pericytes where ADCC is highly expressed. Within the pericyte, tryptamine converted from tryptophan by ADCC activates 5-HT1B that causes pericyte to contract and capillary constriction.

(CSF) and extracellular interstitial fluid.[86] In the glymphatic system, CSF enters the paravascular spaces around penetrating vessels, travels along the downstream arteries, reaches the basal lamina surrounding capillaries, continues along the paravenous routes, and finds its drainage outlet via cervical lymphatics.[48] Along this brain-throughout journey, AQP4 on end-feet is important for the mediation of CSF clearance.[48] As noted above, pericytes regulate AQP4 distribution of astrocytes end-feet.[50] Advancing age is not only related to the loss of astrocytic AQP4 polarization, but also of pericytes.[48,66] Therefore, it is highly likely that pericyte loss undermines glymphatic function by reducing AQP4 polarization, thereby decreasing the clearance of toxic wastes such as amyloid beta (A$\beta$). Pericyte loss also alters abluminal structure along the capillary bed, which may interfere with the glymphatic clearance system.[3] In this regard, preventing pericyte loss may be beneficial for renormalizing glymphatic system and waste clearance.

## Pericytes in CNS immune responses

### *Inflammation responses (Figure 4(a))*

The close proximity of pericytes to microvessels fosters a role of pericytes in immune responses.[87] During inflammation, $NG2^+$ pericytes sense the inflammatory cue early on.[87] Stimulated by proinflammatory factors, like TNF-$\alpha$, pericytes enhance expression of surface adhesion molecules to gain more interplay with leukocytes, which facilitate activation of leukocytes. Then, pericytes promote an efficient trafficking of leukocytes to inflammatory foci.[87] Hence, pericytes develop a niche-like environment to guarantee a fast and precise inflammatory reaction, which also provides an alternative target for anti-inflammatory therapies, in addition to leukocyte-endothelium reactions.

### *Pathogen infections (Figure 4(b))*

Pericytes are susceptible to certain pathogens (bacteria and viruses) and are more permissive for virus invasion

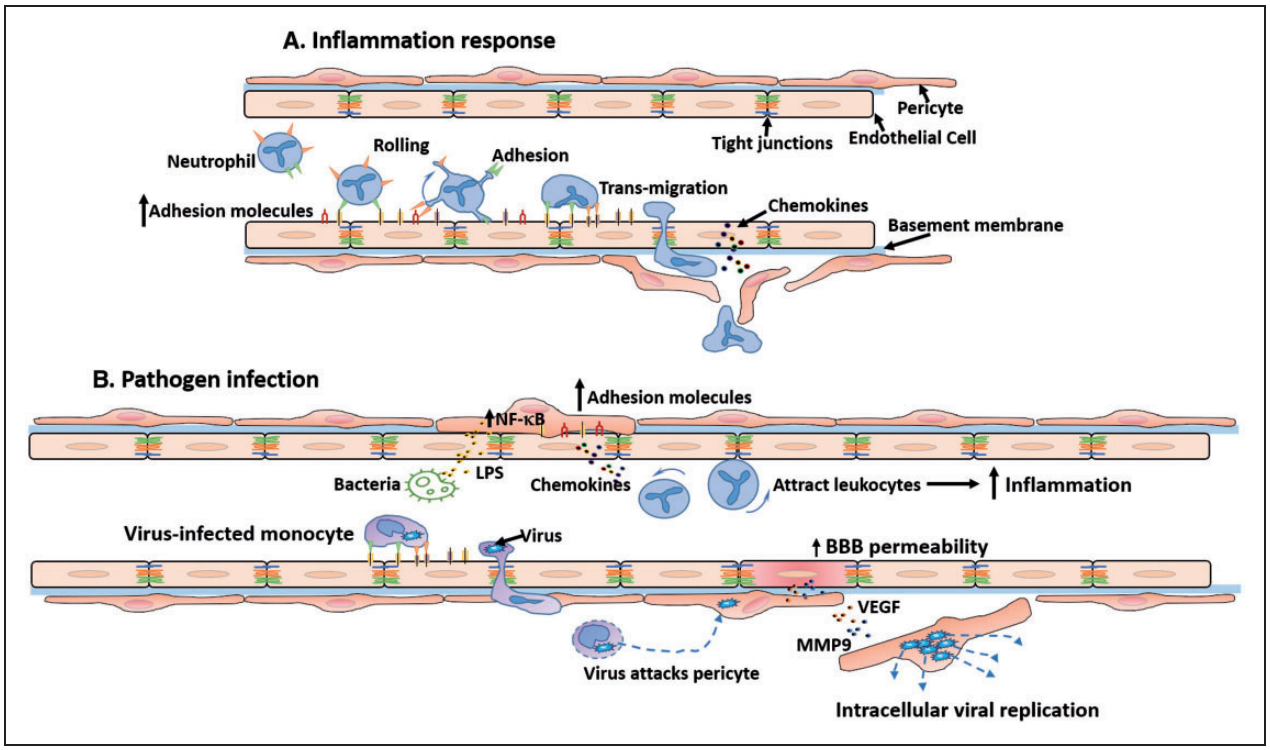


**Figure 4.** Role of pericytes in inflammatory response and pathogen infection: (a) During inflammation, pericytes sense the inflammatory cue and enhance the expression of adhesion molecules to guide and facilitate the activation of leukocytes. Then, pericytes promote an efficient trafficking of leukocytes to inflammatory foci. (b) Pericytes are permissive for virus invasion and replication, and promote virus-infected monocyte trafficking through vessels. The virus released from the monocytes may directly attack pericytes and replicate within them. The infected pericytes increase secretion of MMP9 and VEGF to increase BBB permeability. In bacterial infection, the LPS released by bacteria increase the NF-$\kappa$B and thereby induce pericyte expression of adhesion molecules and chemokines that further enhance leukocyte migration.

and replication than other CNS cells.[88,89] Pericytes express the receptors of human immunodeficiency virus (HIV) and enhance transcytosis of HIV into cells.[90] HIV-derived proteins can promote pericyte migration,[88] which may be the reason for reduced brain pericyte coverage found in both experimental HIV infection and HIV-infected patients.[88] Likewise, pericytes can be the reservoir for cytomegalovirus replication, further escalating inflammation.[91] *Escherichia coli*-attacked pericytes promote the bacterial invasion of ECs.[89] The toxins produced after infection also severely affect pericytes. Lipopolysaccharide (LPS) produced by most gram-negative bacteria affects the luminal side of ECs,[71] which then activates the NF-$\kappa$B (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway in pericytes, provoking the cells to release a myriad of proinflammatory cytokines.[92] Instead of being a defender, the infected pericytes are fragile and may actually contribute to disease progression, though pericytes may express anti-inflammatory cytokines,[93] and even transform into macrophages during infections or other pathologies.[55] Yet the exact role of these transformed pericytes is still unknown.

## Role of pericytes in CNS diseases

Normal pericytes are critical for maintaining CNS homeostasis, and dysfunction of pericytes may lead to pathologies (Figure 5). Here, we review the effects of pericytes in selected CNS diseases, as follows.

## Pericytes in ischemic stroke

Pericytes play multifaceted roles in ischemic stroke. Pericytes may exert unfavorable effects post stroke depending on timing and their location. Pericytes react rapidly to experimental ischemic stroke. In response to oxidative-nitrative stress, pericytes firmly constrict capillaries within 1 h after ischemia, causing a long-lasting no-flow phenomenon even after reperfusion.[73,74] This reaction suggests a distinct role of pericytes in pathological blood flow control, contradicting the expected mechanism that vessels tend to dilate to allow more oxygen delivery to the organ in response to hypoxia.[94] If so, timely rescue the pericytes may prevent this untoward blood redistribution after ischemic stroke, an area of research that warrants further exploration.

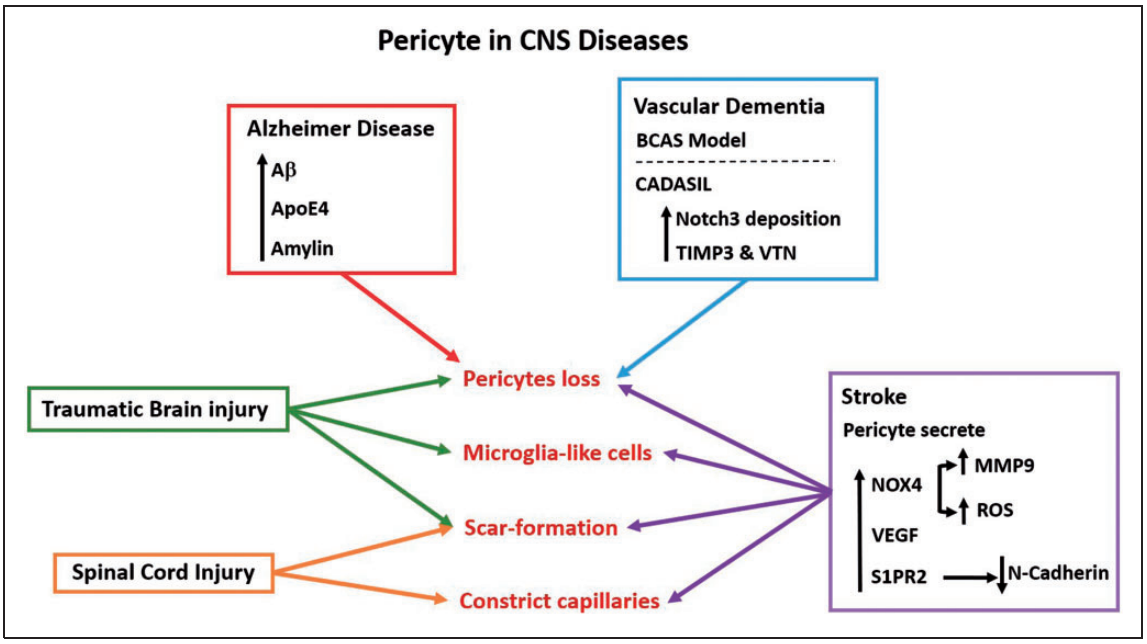


**Figure 5.** The role of pericytes in different CNS diseases: There are many factors that lead to pericyte loss. Included among the causes of pericyte loss in Alzheimer disease are genetic ApoE4 mutation and A$\beta$ deposition and amylin aggregation within pericytes. Pericytes are decreased in experimental chronic vascular dementia induced by BCAS. In genetic vascular dementia and CADASIL, mutant Notch3 deposition leads to pericyte dysfunction and loss. In traumatic brain injury, pericytes appear to initially decline. A subset of pericytes may transform into microglia-like cells, while another subset of pericyte act as activated astrocytes to form scar. In spinal cord injury, pericytes contract and limit the blood flow below the spinal lesion, and a subset of pericytes forms scar around the lesion. Stroke causes pericyte dysfunction and loss, and dysfunctional pericytes in stroke produce NOX4 which induces oxidative stress, and release MMP9 and VEGF which further destabilizes the BBB. Elevated levels of S1PR2 within pericytes lead to the loss of N-cadherin. After stroke, pericytes may also constrict capillaries and transform into microglia-like cells and scar-forming cells.

Pericytes also aggravate BBB disruption after stroke. Pericyte soma cover only 7% of cortical capillary network, yet occupy 80% of the area where BBB leakage occur post-ischemia.[73] As early as 30 min after experimental ischemic stroke, vascular leakage is preferentially located at pericyte soma and develops at a faster rate compared with non-pericyte areas. This is accompanied by increased activity of matrix metallopeptidase 9 (MMP9) around pericyte soma.[95] Twelve hours post ischemic stroke, the S1P receptor (S1PR)2 of pericytes is significantly upregulated, which disrupts N-cadherin and induces pericyte detachment, leading to severe BBB leakage that reaches a zenith at 24 h post stroke.[42,96] Between day 1 and day 7 post stroke, Nox4 (reduced nicotinamide adenine dinucleotide phosphate oxidase 4), a source of ROS, is upregulated in pericytes and drives BBB opening via activating NF$\kappa$B and MMP9.[97] However, mice with systemic knockout of pericytes suffer more severe brain edema and neurological deficits compared with control mice in focal ischemic stroke.[98] The interactions of PDGF/PDGFR-$\beta$ and TGF-$\beta$ signaling pathways may generate mature pericytes that work in the late phase of angiogenesis to stabilize nascent vessels in this scenario.[98] Additionally, a mouse study of ischemic stroke shows that long-term local administration of low-dose VEGF to brain before stroke increases pericyte coverage and thereby benefits neurological outcome after stroke.[99] This suggest that as long as the dose of VEGF kept under a threshold, a VEGF prophylactic supplement may contribute to healthy robust vasculature, which primes brain to better weather impending injuries. Intriguingly, the pericytes isolated form C57BL6/j mouse brain express neither VEGFR1 nor VEGFR2.[99] Other signaling pathways beyond VEGF/VEGFRs may exist and be involved in the interactions between pericytes and VEGF. VEGF may potentiate Angpt-Tie signaling or enhance S1P-mediated eNOS activation, both of which cause vasorelaxation.[100] Therefore, further investigations are warranted on the interaction of pericytes with multifunctional factors, like VEGF, after stroke.

In addition, pericytes may transform into multipotential cells post stroke.[57,101] Pericytes can be transformed into stromal cells that proliferate in the ischemic core and have a clear demarcation with glial scar.[102] Pericytes may also transform into microglia post stroke and the transformed cells do not overlap

with the pericyte forming the scar (for more detailed discussion please see the Pericytes and Microglia interaction section). This suggests the divergent fates of different subsets of pericytes under various conditions, and thus individual consideration of these subtypes is necessary when therapeutically manipulating the pericytes.

## Pericytes in Alzheimer's disease

### *Pericyte loss is involved in pathologies of AD (Figure 5)*

Alzheimer's disease (AD) is a common cause of cognitive decline and its progression is influenced by the neurovascular milieu.[103,104] Pericyte deficiency can cause neurovascular uncoupling,[103] and impairs the blood–axon barrier of white matter, exposing the brain tissues to the blood-derived toxins.[103] Large amounts of pericyte loss are found in brain tissue of both animal models of AD and AD patients,[105,106] and implantation of pericytes in brain can attenuate AD pathology.[107]

## Factors causing pericyte loss in AD

### *Aβ*

A$\beta$ exclusively constricts brain capillaries at pericyte sites with no changes in diameter of arterioles and venules in both AD patient brain tissue and a mouse model of AD.[108] In AD mice, the higher load of A$\beta$ deposition, the more rapid capillary constriction.[108] A$\beta$ deposition may induce NOX4-derived ROS which then triggers potent vessel constrictor endothelin-1(ET) to evoke pericyte constriction.[108]Pericyte injury is positively correlated with the ratio of A$\beta$1-42/A$\beta$1-40, two most abundant forms of A$\beta$.[109,110] Oligomeric A$\beta$1-42 drives pericyte detachment by activating MMP9 to shed NG2 on pericytes, whereas the fibril-enriched preparations of A$\beta$1-42 reduce the decline of NG2.[111] The shedding of NG2 loosens the connection between pericytes and ECs, resulting in vascular leakiness. Additionally, since pericytes regulate AQP4 distribution on astrocyte end-feet that is necessary for glymphatic clearance of A$\beta$,[2,48,112] we may speculate that impairment of glymphatic clearance of A$\beta$ via pericyte loss is another contributor to A$\beta$ deposition in AD.

### *Apolipoprotein E*

Isoforms of Apolipoprotein E (ApoE) determine the genetic risk of late-onset AD.[113] Pericyte degeneration is more severe in AD ApoE4 carriers than in AD ApoE3 carriers. The proinflammatory lipoprotein receptor-related protein-1 (LRP1)-cyclophilin A (CypA)-MMP9 pathway in pericytes is also upregulated in AD ApoE4 carriers, which causes severe BBB damage.[105]

### *Amylin*

Amylin is an amyloid polypeptide hormone derived from pancreatic $\beta$-cells.[114] Amylin-inclusions is found in AD patients and non-dementia patients, and deposits in brain microvascular pericytes.[115] The amylin afflicted pericytes exhibit blebbed or fragmented nuclei.[115] The toxic oligomeric amylin enhances caspase3/7 activity, decreases NG2 expression, and impairs autophagy.[115] The aggregated amylin acts faster and stronger than oligomeric A$\beta$1-42.[115] In addition, amylin accumulates in pancreas and brain hippocampus in patients with type 2 diabetes mellitus (DM).[116] Cytoplasmic tau and A$\beta$ protein deposits are found in both pancreas and brain in the same subjects. Amylin may serve as a potential link between the two diseases,[116] and pericytes may be the target of amylin in both these diseases, and important in the interaction between DM and AD. Another study found that plasma amylin level is reduced in AD and in patients with mild cognitive deficits compared with non-cognitively impaired patients.[117] Administration of amylin analog in AD mice decreases oxidative stress and inflammation in hippocampus and improves neurological outcome.[117] It is not clear whether there exists a causal relation between the decreased plasma level of amylin and the deposition of amylin in pericytes of the brain. Further studies are therefore warranted.

## Pericytes in vascular dementia

Vascular dementia (VD) is another common cause of dementia that is closely related with aging.[118] VD is a broad umbrella term referring to various subtypes of cognitive deficits due to vascular dysfunctions, such as microinfarcts, microbleeds, and hypoperfusion.[119] Pericyte dysfunction may render capillaries prone to ischemia and hemorrhage that contribute to cognitive decline.

### *Pericytes in chronic cerebral hypoperfusion-induced dementia*

In the animal model of chronic cerebral hypoperfusion secondary to bilateral carotid artery stenosis (BCAS), the expression of pericytes reaches a nadir in the corpus collosum on the third day post-surgery, and is accompanied by increased BBB permeability (Figure 5).[120]

This occurs prior to any evident white matter damage, which is consistent with other studies demonstrating that pericyte degeneration may lead to white matter dysfunction and be a cause of the cognitive impairment.[121] In addition, the poor brain circulation induced by BCAS may lead to pericyte contraction as seen in ischemic stroke, which will further compromise the blood flow supply to brain.[74,103] Also, the pericyte loss may interfere with the glymphatic system and thereby exacerbate neuronal toxicity.[122] Another rodent model of vascular dementia generated from administration of cholesterol crystals into the external carotid artery to form multiple microinfarcts (MMI), animals exhibit reduced cognitive function and impaired glymphatic system,[123] which may also be related to pericytes.

### *Pericytes in genetic vascular dementia-CADASIL*

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL is the most common cause of inherited monogenic stroke with cognitive impairment, characterized by the mutations of Notch3 receptor with unpaired cysteines in the epidermal growth factor-repeats.[124] Normally, Notch3 is required for pericyte development and pericyte proliferation. In CADASIL model mice, aggregated Notch3 deposits on pericytes results in age-dependent pericyte loss and associated BBB dysfunction.[124] Although Notch3 is expressed in both VSMCs and pericytes, no detectable changes are found in VSMCs, highlighting the imperative role of pericytes in the progression of CADASIL.[124] The aberrant extracellular domain of Notch3 receptor promotes the formation of Notch3/TIMP3 complex and Notch3/VTN complex, thereby impairing the homeostasis of ECM, a possible cause of vessel fibrosis.[125] Pericytes are an important source of both TIMP3 and VTN.[6,126] Dysfunctional pericytes in small-vessel diseases, like CADASIL, may cause vascular function by secreting excessive ECM, along with BBB hyperpermeability and circulatory collapse.

## Pericytes in spinal cord injury

### *Pericytes regulate blood flow in SCI (Figure 3(b))*

Pericytes impair blood flow and motor function in chronic SCI.[79] Normally, the vessel tone of spinal cord is maintained by brainstem neuron-derived monoamine serotonin (5-HT) and noradrenaline (NA) that are converted by enzymes, like tryptophan hydroxylase (TPH) and aromatic l-amino acid decarboxylase (AADC). In chronic SCI, this neuronal regulation is compromised, and pericytes take control of vessel tone and blood flow. Without 5-HT and NA, pericytes reside caudal to spinal cord transection could automatically activate 5-HT1B receptor by expressing ADCC which then constricts pericytes and curbs capillary blood flow.[79] This phenomenon may also explain the prolonged pericyte constriction in ischemic stroke after reperfusion.[73] The stressed pericytes may generate autocrine "clenching substances," like ADCC, to shift the blood from the dying vessels to the healthier ones. Yet these possible mechanisms may be organ-dependent and differ between chronic ischemia and acute no/low-flow.

### *Pericyte-derived scar in SCI*

Pericyte is another important source for scar tissues in experimental SCI, along with astrocytes.[127] From day 3 after SCI, pericytes-derived cells start to proliferate in the lesion center, which reach a peak on day 14 and then decline to a stable level for up to seven months.[127] These pericyte progeny lose their CD13 identity at day 9 and afterwards gradually present with PDGFR-$\beta$ and fibroblast marker, fibronectin. Instead of tightly covering nascent vessels like pericytes, the pericyte-derived cells detach from blood vessels, migrate through ECM and fill up the lesioned cavity with a clear demarcation with astrogliosis that surrounds the lesioned cavity. The scar densely populated by pericytes progeny may also hinder the outgrowing axons penetrating into the lesion in a murine model of SCI.[128] After moderate reduction of pericytes prior to SCI, the mice exhibit high axon regeneration and improved sensorimotor function.[128] This is accompanied by the suppression of astrogliosis and inflammation. However, severe reduction of pericytes in mice results in undesired inflammatory reactions, indicating the importance of an appropriate number of pericytes for the recovery of SCI.[128] The proliferation of pericytes may be induced by S1P/SIPR3 interaction, inhibition of which may significantly reduce glial scar formation and promote locomotor recovery.[129]

## Pericytes in traumatic brain injury

Pericytes react rapidly during the acute phase of TBI.[130] One hour after TBI, a subset of pericytes start migrating away from blood vessels in the perilesional zone, whereas the remaining immobile ones undergo degeneration (Figure 5).[130] The degenerated pericytes display enlarged endoplasmic reticulum and swollen mitochondria as well as increased transcytosis. Some exhibit hypertrophic or necrotic changes and a tendency to transform into phagocytes.[131] Pericytes decrease in perilesional zone 6 to 12 h after TBI and then re-increase from day 3 to reach a peak level on

day 5.[132] The repopulated pericytes surround the lesion with a clear demarcation with gliosis.[132] Another study observed elevated pericyte death 24 h after TBI, a phenomenon which becomes more pronounced on day 3.[133] Administration of carbon monoxide (CO) after TBI significantly improves pericyte survival and promotes the neurogenesis, which may due to the ability of CO to strengthen the cross-talk between pericytes and NSCs as observed in in vitro.[133] In addition, after TBI, the ApoE4 mice exhibit a delay of pericyte increase compared with wild type and ApoE3 mice, which is accompanied by a postponed spontaneous recovery of BBB integrity.[134,135]

## Pericytes and miRs

miRs are a family of small non-coding RNAs containing 20–30 nucleotides, and they post-transcriptionally regulate a wide spectrum of genes that are involved in cell fate determination, organ development, and disease progression, etc.[136] Hypoxia significantly increases miR-345-5p and miR − 336, but decreases miR − 140, miR − 222, miR − 24, and miR − 145 expression in cultured CNS pericytes.[137] MiR-345-5p is associated with anti-apoptosis and cell proliferation, whereas miR-140 inhibits growth and migration of cells, the change of which underlies changes in proliferation and migration of pericytes under hypoxia.[138,139] MiR-24, a pro-apoptotic factor, is decreased in pericytes in response to hypoxia, which is consistent with upregulation of anti-apoptotic miR-345-5p.[137] MiR-145 is exclusively expressed in the pericytes of brain and kidney.[137,140] It may regulate the fate and plasticity of pericytes, and thus may be alternative targets for pathologies with over-proliferating pericytes that are seen in the scar formation in brain injury and renal fibrosis. Hypoxia induces miR-126 in pericytes which may be the compensatory mechanism for pericytes to maintain vessel integrity in response to low oxygen delivery.[137] MiR-126 deficiency contributes to cardiac defects after stroke compared with stroke control mice, suggesting the potential role of miR-126 in brain–heart interactions.[141] As pericytes reside in both brain and heart, the abnormality of miR-126 may participate pericyte-related pathologies in both brain and heart.

## Pericytes and EVs

EVs are a wide spectrum of nanoparticles that include exosomes and microvesicles (MVs), both of which are able to mediate cell–cell communications.[142] Exosomes are nanosized vesicles (~30–100 nm in diameter) that intracellularly package biomolecules from the early endosome and transfer nucleic acids, proteins and lipids.[142] MVs, by contrast, have a wider range of size (100–1000 nm in diameter) and directly bud from the plasma membrane. MVs also carry and deliver nucleic acid and proteins to cells.[142]

Pericytes can be donors of EVs or recipients. As a donor, pericytes secrete vesicles with different contents according to stimuli (Figure 6(b)). Stimulated by PDGF-BB, pericyte-derived MVs carry growth factors, whereas inflamed by LPS, pericyte-derived MVs predominantly contain proinflammatory cytokines.[143] In the hypoxic milieu, pericytes can release exosomes that encompass proangiogenic factors to enhance vessel density.[11] In normoxic condition, pericytes may generate exosomes that incorporate anti-angiogenic factors to maintain vascular stability.[31]

As a recipient, pericytes may receive EVs from multiple cell sources. In diabetic wound tissue, pericytes decrease the expression of ephrin-B2 and VEGF after uptake of EC-derived MVs enriched with miR-503 (Figure 6(a)). This reduces pericyte migration and proliferation.[144] By incorporation of EC-derived MVs loaded with miR-328-3p and let-7d-3p, pericytes enhance their expression of VEGF-B associated with neuronal degeneration.[10,145] In addition to ECs, the origin of EVs delivered to pericytes are from a variety of cells. Therefore, additional investigations are required to investigate the dynamic interactions between pericytes and other cell-derived EVs.

Pericytes may negatively regulate the transportation of EVs (Figure 6(b)). First, pericyte coverage guarantees a low rate of transcytosis of ECs.[65] This may block the exosomal and MV shuttle of RNAs, proteins, and cytokines. Second, pericytes per se can secrete proteins to scavenge MVs.[146] Milk fat globule-epidermal growth factor 8 protein (MGF-E8, also known as lactadherin) is found to be produced by phagocytes and can assist the clearance of MVs coated with phosphatidylserines.[146] In brain injury, brain-derived MVs exacerbate BBB leakage and trigger systemic coagulopathy.[146] Exogenous administration of MGF-E8 significantly preserves BBB integrity via facilitating phagocytosis of the MVs.[146] Pericytes are a source of MGF-E8 under PDGF-BB stimulation.[147] A subset of pericytes may function as phagocytes in brain injury as noted above.[15] Therefore, it is highly likely that beneficial effects of MGF-E8 are partly attributed to pericytes. It appears paradoxical that pericytes can generate both MVs and MV-scavenger proteins. Temporal and spatial sequences may be present context-dependently between the two processes. Pericyte-derived MVs and MGF-E8 may work synergistically to guarantee a balance between producing particles and cleaning debris. Pathologies that reinforce one side may tilt the scale, and disrupt CNS homeostasis. Although the interactions between the MVs and pericytes presently are enigmatic, they may bear

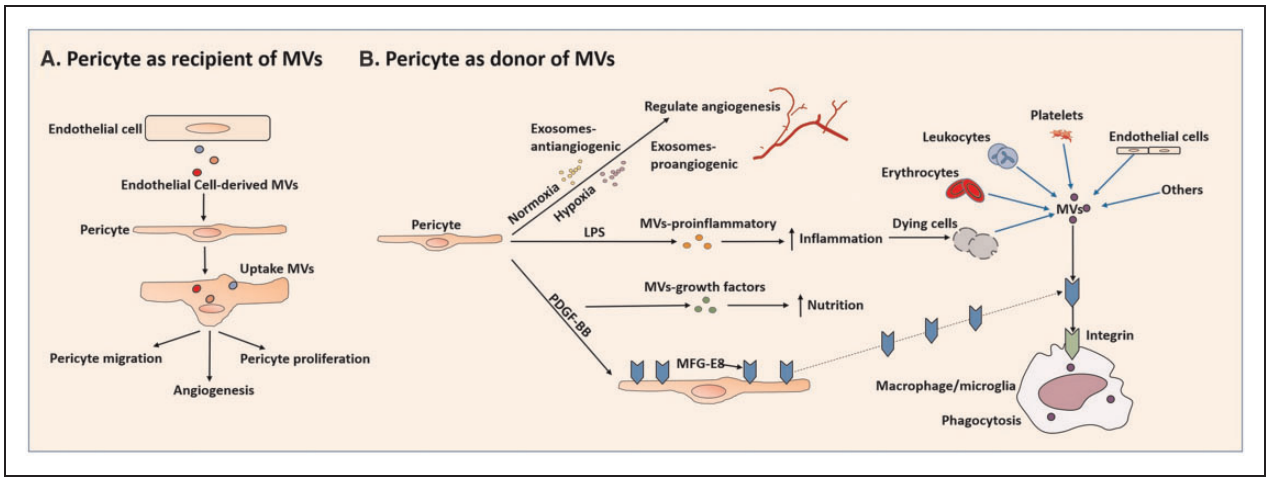


**Figure 6.** The relation between pericytes and EVs: Pericytes can be donors or recipients of EVs. (a) As a recipient, pericytes may receive EVs from multiple cell sources. Pericytes can engulf various ECs-derived EVs that contain miRs, and then migrate, proliferate, and promote angiogenesis. (b) As a donor, pericytes secrete vesicles context-dependently. Under normoxic conditions, pericytes may secrete exosomes that contain antiangiogenic factors (Exosomes-antiangiogenic) to maintain vascular stability. In hypoxia, pericytes secrete exosomes with proangiogenic factors (exosomes-proangiogenic). Stimulated by PDGF-BB, pericyte-derived MVs carry growth factors (MVs-growth factors), whereas inflamed by LPS, pericyte-derived MVs contain proinflammatory cytokines (MVs-proinflammatory). Conversely, pericytes may negatively regulate the transportation of EVs. Stimulated by PDGF-BB, pericytes secrete scavenger MGF-E8 that promotes phagocytes engulfment of MVs produced by cells that are coated with phosphatidylserines.

enormous therapeutic potential for certain CNS pathologies.

## Approaches to studying the pericytes

Progress in experimental tools allows more extensive and thorough aspects of pericytes to be unveiled. Here, we briefly review these approaches that have been used in the recent studies of pericytes.

## Genetic engineering technique

With the development of Cre-recombination techniques, pericyte-deficient transgenic mice permit tracking pericyte development from embryonic to aging periods.[65,66] Identification of the target genes specific to the pericytes at retinal angiogenic microvessels may offer a clearer picture of pericyte activities in angiogenesis with less noise from the angiogenic-quiescent pericytes.[35,36] The offspring of the transgenic mice designed to mimic CNS diseases and pericyte-deficient transgenic mice may help investigate the roles of pericytes in CNS pathological scenarios.[106] In combination with two-photon microscopy and 3D techniques, transgenic mice carrying fluorescent reporter markers are available to visualize the pericyte behavior in live animals.[17] Additionally, optogenetic techniques by using light and genetically encoded light-sensitive proteins in novel transgenic mouse lines may permit highly selective activation of pericytes that can be employed to distinguishe the role of pericytes in CBF regulation from other cells in NVU.

## Single-cell RNA sequence

The RNA sequence of individual cells isolated from the brain of transgenic reporter mice provide genome-wide quantitative transcriptomes, and thus facilitate insight into functional differences between individual cells.[148] The transcriptomes of pericytes, fibroblast-like cells and ECs can identify their respective phenotypic evolution along the arteriovenous axis (database of the website http://betsholtzlab.org/VascularSingleCells/database.html),[22] and identify novel capillary pericyte markers as well as the subcellular location of pericyte-enriched genes and related genomic network.[6] The single-cell RNA sequence can also be used to compare the genes of pericytes among organs, which may help evaluate pericytes from a systemic view.[149]

## Future perspective and conclusion

There are a number of important aspects of pericytes that warrant further investigation: (1) Stem cell potential of pericytes. In vitro and in vivo study shows that pericytes have stem-cell like potential and express markers for mesenchymal stem cells (MSC).[150] However, this view has been challenged by a recent in vivo loss-of-function study. Guimaraes-Camboa et al.[9] found that pericytes do not behave as MSCs or as

tissue-resident progenitors in multiple organs, suggesting the pericyte plasticity observed in previous studies may result from the manipulation of cells ex vivo.[9] (2) The role of pericytes on post-capillary venules remains enigmatic. Investigations on venular pericytes in identification, physiology, pathology are warranted. (3) The role of pericytes in peripheral organs. In addition to brain, pericytes are also necessary for heart, gut, and kidney function.[14] Systemic study of pericytes may help clarify the relationships of inflammatory response, blood flow regulation, and coagulopathy between brain and peripheral organs. (4) Therapies that maximize the benefits and minimize the toxicity of pericytes may provide therapeutic opportunities not only for CNS diseases but also for peripheral comorbidities.

To summarize, pericytes are indispensable for the normal development and the maturation of the CNS. Dysfunction of pericytes greatly aggravates CNS pathologies. With further advances in bio-techniques, additional functions of pericytes as well as their untoward effects can be investigated.

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## Authors' contributions

ZZ: manuscript writing; MC and JC: manuscript writing, final approval of manuscript, financial support.

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