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Pericytes in Brain injury and Repair after Ischemic Stroke

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Abstract

Pericytes are functional components of the neurovascular unit (NVU). They provide support to other NVU components and maintain normal physiological functions of the blood-brain barrier (BBB). The brain ischemia and reperfusion result in pathological alterations in pericytes. The intimate anatomical and functional interactions between pericytes and other NVU components play pivotal roles in the progression of stroke pathology. In this review, we depict the biology and functions of pericytes in the normal brain and discuss their effects in brain injury and repair after ischemia/reperfusion. Since ischemic stroke occurs mostly in elderly people, we also review age-related changes in pericytes and how these changes predispose aged brains to ischemic/reperfusion injury. Strategies targeting pericytes responses after ischemia and reperfusion may provide new therapies for ischemic stroke.

Keywords

Pericyte; Ischemic stroke; Neurovascular unit; Blood-brain barrier

Introduction

Ischemic stroke is a cerebrovascular disease caused by the sudden obstruction of a blood vessel and the loss of blood supply in related brain areas [1]. Effective treatment for ischemic stroke is rather limited and almost exclusively based on the recanalization of the occluded vessels [2–4]. However, it becomes increasingly clear that effects of recanalization on stroke outcomes are greatly influenced by the functions of different components in the neurovascular unit (NVU). The NVU is the fundamental structural and functional unit in the central nervous system (CNS). It is composed of endothelial cells (ECs), pericytes, basal lamina, astrocytes, peri-capillary microglia, and neurons [5]. Pericytes are a group of contractile cells that are located between ECs, astrocytes, and neurons and almost entirely embedded within the basal lamina [6]. Pericytes provide support to other NVU components

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and maintain the normal physiological functions of blood-brain barrier (BBB) [7–8]. Moreover, accumulating evidences suggest the importance of pericytes in the pathogenesis of cerebrovascular diseases, including stroke. This review focuses on the biology and functions of pericytes and discusses their effects in brain injury and repair after brain ischemia/reperfusion. Since ischemic stroke occurs mostly in elderly people, we also reviewed the age-related changes in pericytes and how these changes predispose the aged brain to ischemic/reperfusion injury. Finally, we summarize current breakthroughs in therapeutic strategies that target at pericyte responses after stroke.

Origin and Distribution of Pericytes

Pericytes are widely distributed throughout the body. Around 80% of microvessels (arterioles, capillaries and venules) in our body are covered by pericytes. In particular, the CNS has the highest density of pericytes [9]. Brain pericytes are derived from different embryonic sources during development. According to the chick-quail chimera studies, pericytes in the forebrain originate from neural crest cells, while pericytes in the midbrain, brainstem, and spinal cord come from mesoderm mesenchymal stem cells (MSCs) [10]. Despite their distinct origins, brain pericytes express some common markers, including platelet-derived growth factor receptor-β (PDGFR-β), α-smooth muscle actin (α-SMA), and regulator of G protein signalling-5 (RGS-5). Additionally, pericytes express some immunological markers such as fragment-crytallizaable receptor (FcR), CD4, CD11b, major histocompatibility complex (MHC) class I and II, and desmin [11]. It is noted that most markers used in pericyte studies are not pericyte-specific. For example, PDGFR-β is also expressed on endothelial precursor cells [12] α-SMA is an important functional component in smooth muscle cells [13]. The unspecificity of pericyte markers makes it difficult to distinguish pericyts from other vascular cells and immune cells, which impairs the accuracy of pericyte research. It was recently discovered that potassium channel kir6.1 was only expressed in brain pericytes and might be used as a pericyte-specific marker [11]. Further studies are warranted to validate the specificity of this marker.

CNS pericytes can be divided into three subtypes based on their location, morphology, and protein expression (Figure 1): (1) Arterial pericytes are located at the arteriole end of capillary beds. These pericytes have many circumferential processes to surround capillaries and express high levels of α-SMA. They play a vital role in regulating cerebral blood flow. (2) Capillary pericytes reside in the middle of capillary beds. They give out more longitudinal processes and express less α-SMA. Functionally, these pericytes are important for maintaining BBB integrity. (3) Venule pericytes are located at the venule end of capillaries. This subtype of pericytes has a stellate morphology and modulates the infiltration of peripheral immune cells into brain paranchyma [14]. All of these three subtypes of pericytes play critical roles in normal brains and in post-stroke brain injuries and functional recovery.

Physiological Functions of Pericytes in the Normal Brain

Pericytes display a variety of functions in the NVU (Table 1). As main component of the NVU, pericytes provide support to other NVU members and help to maintain normal

functions of the NVU. In addition, the contractility of pericytes can control blood flow in CNS microvessels. Pericytes also possess immunological functions and participate in immune responses in the brain [15]. The stemness of brain pericytes is recently recognized and regarded as a mechanism of CNS renewal.

1. Maintaining Blood Brain Barrier Integrity

The BBB is composed of ECs, pericytes, astrocytes, and the extracellular matrix (ECM). BBB maintains brain homeostasis by controlling the passage of substances from the blood into the brain [16]. It allows essential metabolites, such as oxygen and glucose, but not non-CNS cells, various foreign substances, and microbes to pass from the blood to the brain. The breakdown of the BBB is an important pathological change in a variety of CNS diseases [17]. As main component of BBB, pericytes not only exert barrier function by themselves but also promote physiological functions of other BBB components, including endothelial cells, basal lamina, and astrocyte [7–8, 18]. (Figure 2)

(1) Pericytes-Endothelial Cells Interaction—Pericytes are located on the abluminal surface of ECs and extend long primary processes along the axis of microvessels. These primary processes then give rise to secondary and tertiary processes, which preferentially wrap around tight junctions (TJ) between neighboring ECs [19] [20]. Pericytes and ECs are continuously separated by basal lamina. However, the basal lamina between pericytes and ECs have some hole-like structures, which allow EC-pericyte communication and interaction in a peg-socket manner [21]. Adhesion and gap junctions are found in the peg-socket contacts between ECs and pericytes. Adhesion junctions are formed by cadherins and catenins, which connect to the cytoskeletons of these two cell types and mechanically anchor them together. Gap junctions are mainly comprised of connexins (connexin-37, 40, 43) and allow molecular transfer and information exchange between ECs and pericytes [19]. These close interactions enable pericytes to provide structural and nutritional support to ECs and to enhance the barrier function of the BBB. In pericyte-deficient mice, BBB permeability was greatly increased, strongly supporting the importance of pericytes in BBB integrity [9]. In vitro studies further confirmed that coculture with brain pericytes or pericyte-conditioned media could increase the trans-endothelial electrical resistance (TEER) of ECs and enhance their barrier functions [22–23]. Specifically, pericytes play critical roles to promote TJ formation between ECs. Although the recruitment of both pericytes and astrocytes is important in the formation of TJ between ECs, pericyte-EC communication seems to be more important than astrocytes. Direct cell-cell contact between pericytes and ECs alone, without the prominent ensheathment of astrocyte endfeet, is enough for TJ induction in ECs [24]. Further studies suggest that angiopoietin-1 (Ang-1) secreted by pericytes mediates TJ induction through the activation of Tie-2, which is an Ang-1 receptor on ECs [23, 25].

(2) Influence of Pericytes on Basal Lamina—Microvasculature basal lamina plays a critical role in ischemic brain injury [26]. Pericytes are nearly completely embraced in the basal lamina and actively participate in the formation and degradation of extracellular matrix (ECM) proteins in the basal lamina. On one hand, pericytes produce degrading proteases, including matrix metalloproteinase (MMP)-2 and MMP-9 [27], to enhance ECM degradation in the early stage of angiogenesis [10]. MMP production also enables pericytes

to detach from basal lamina and migrate to newly formed microvasculature. On the other hand, pericytes express potent inhibitors of different matrix metalloproteinase (MMP), such as tissue inhibitor of metalloproteinase-3 (TIMP-3), during vessel stabilization to inhibit the degradation of ECM and facilitate vessel maturation. In addition, pericytes contribute to the formation of basal lamina by secreting ECM proteins, including laminin and fibronectin [10]. Elucidating the mechanisms that control of the expression of these proteins in pericytes could help us gain insight into basal lamina dynamic and may shed new light on BBB protection during CNS insults, including ischemic stroke.

(3) Pericytes-astrocytes Interaction—Astrocytes ensheath microvessels with their endfeet [28] and contribute to the normal functions of the BBB [28]. Under physiological conditions, pericytes and astrocytes play synergistic roles to maintain BBB integrity. As mentioned above, they both contribute to TJ induction, although the effects of pericytes seem to be more important than that of astrocytes [23–24]. Additionally, they both facilitate the function of ATP-binding cassette transporters in ECs, enhancing both barrier and transportation functions of ECs [29]. Astrocyte-pericyte interaction is also important in neovascularization. Both astrocytes and pericytes have been shown to suppress endothelial proliferation. They together induce the proper localization of barrier proteins and the lumen polarization of ECs. In an in-gel matrix vascular model, it was found that ECs formed a capillary-like structure, while astrocytes and pericytes suppressed the degradation of these new capillary structures. Importantly, the co-existence of astrocytes and pericytes more efficiently enhanced the formation of new vessels than astrocytes alone [30]. Furthermore, both astrocytes [31] and pericytes [32] play vital roles in the neurovascular coupling. Neuron-released glutamate has been shown to activate metabotropic glutamate receptors on astrocytes, which subsequently increase the expression of nuclear factor of activated T-cells, cytoplasmic 3 (NFATc3) in pericytes. NFATc3 is a transcriptional factor that is known to regulate vascular cell contractility and therefore may play a critical role in the regulation of blood flow [33].

The mechanisms underlying the synergetic effects of astrocytes and pericytes on BBB functions remain elusive. However, several possible mechanisms have been suggested. For example, one study pointed out the importance of aquaporin-4 (AQP-4) in the crosstalk between pericytes and astrocytes. AQP-4 is a molecule located on the luminal side of astrocyte endfeet. It is responsible for the maintenance of water homeostasis in the CNS. It was recently found that AQP-4 anchor to perivascular astrocytic endfoot membrane with the assistance of pericytes. Astrocytic endfeet adjacent to pericytes have higher expression of AQP-4 than those directly attached to ECs [18]. The mechanisms for how astrocytes regulate pericytes' activity have also been reported. For example, laminin in basal lamina, which is specifically secreted by astrocytes, affects activation of pericytes. Astrocytic laminin binds to integrin α2 on pericytes and prevents pericytes from transforming from BBB stabilizing status to contractile status [34]. The exact mechanism for astrocytes and pericytes interaction awaits further elucidation.

2. Phagocytosis and other immunological functions of pericytes

As a BBB component, pericytes encounter numerous neuro-toxins and injurious factors under physiological and pathological conditions. Pericytes are endowed with macrophage-like phagocytotic ability to eliminate these harmful factors. There are two kinds of brain pericytes, namely granular pericytes and filamentous pericytes, in microvessels under electron microscopy [35]. These two populations of pericytes can be distinguished by the presence of lysosome-like granules in the cytoplasm [36]. Granular pericytes, which compose about 95% of the entire cerebral pericyte population [36], may serve as scavenger cells in the brain.

Many pattern recognition receptors (PRRs) such as toll-like receptor 4 (TLR4), scavenger receptor (SR), and Fc receptors have all been shown to be expressed on brain microvascular pericytes, which enable these cells to recognize invading or abnormal antigens. With robust expression of acid phosphatase in lysosomes, pericytes are able to digest intaken antigens [37]. In the case of CNS insults, pericytes are able to sense signals of danger, engulf and process abnormally released antigens, present the processed antigens to other immune cells, and promote the subsequent immune responses.

In CNS inflammation, brain pericytes play both immunoactive and immunosuppressive roles. An in vitro study showed that retinal pericytes could profoundly inhibit proliferation and cytokine production of activated T cells during inflammation, thus protecting ECs from inflammation-mediated apoptosis [38]. Significantly upregulated intercellular adhesion molecule-1 (ICAM-1) and leukocyte infiltration are found in pericyte deficient mice (pdgfrβ −/−), indicating that pericytes can prevent leukocyte invasion into the brain. During development, the immunosuppressive effect of pericytes is thought to be relevant to immune quiescence of CNS [39]. In contrast, the pro-inflammatory properties of pericytes are also prominent. Pericytes continuously express low levels of adhesion molecules (e.g. ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1)), which enable them to sense danger [6] and are associated with leukocyte recruitment in immune reaction [21]. Besides, pericytes secrete other immunoactive molecules, including interleukin (IL)-9, IL-10, IL 12, IL-13, IL17, tumor necrosis factor-α (TNF-α), and interferon-gamma (IFN-γ), under physiological conditions. In response to immune challenge, pericytes produce large amounts of reactive oxygen species (ROS), nitric oxide (NO), and other inflammatory cytokines [11, 21]. According to a recent study, pericytes at the venule end of the capillary play a major role in regulating peripheral immune cells infiltration in response to neurovascular insult [14]. Such bidirectional anti-inflammatory and pro-inflammatory effects of pericytes suggest that pericytes could prevent unnecessary immune reactions in physiological conditions while always being ready to fight intrusive dangers.

3. Blood Flow Control

The CNS requires accurate energy supply which demands precise blood flow SMCs are the major blood flow controller. Pathological alterations of SMCs have been reported as a contributor to the disruption of cerebral blood flow after ischemic insult [40]. In terminal vessels where SMCs no longer exist, fine regulation of blood supply is achieved by pericytes. Pericytes have potential contractility. They express α-SMA, tropomyosin, desmin,

and other contraction related proteins [41–42]. These structures react to voltage-gated calcium currents and calcium-activated chloride currents, which result from depolarization of pericytes. Among them, α-SMA is the main contracting protein in pericytes. Levels of α-SMA in pericytes vary according to their location. It was found that pericytes at the arteriole end of capillaries express the most abundant amount of α-SMA [20]. The expression of α-SMA could be regulated by different factors. For example, transforming growth factor-β (TGF-β) increases α-SMA expression [43], while fibroblast growth factor (FGF) can antagonize this effect of TGF-β [44]. Modulating the expression of α-SMA may become a therapeutic strategy for ischemic stroke by regulating pericyte contractility and microvessel blood flow.

In physiological conditions, pericytes contract or dilate according to the energy demands of nervous tissue. Hall et, al. demonstrated that neuronal activity and neurotransmitter glutamate could dilate pericytes and thus increase capillary diameter. This process was proved to be mediated by postaglandin (PG) E2 in a NO-dependent manner [36]. Apart from PGE2, signals of increased energy use, including lactate, adenosine, and low pH, can also relax pericytes. As a marker of sped-up metabolism, increased reactive oxygen metabolites (ROMs) can also dilate pericytes when the exposure time is short (10 min), while long time exposure (30 min) to ROMs, which is related to danger and harm, contracts pericytes [8]. In contrast, surplus energy could contract pericytes to reduce blood flow. For example, increased level of adenosine triphosphate (ATP) induces pericyte contraction via P2×7 receptors and uridine triphosphate-activated receptors [45].

It is well accepted that blood flow is regulated by the autonomic nervous system. Studies have reported that in terminal vessels without SMC, the autonomic nervous system participates in the control of blood flow through pericyte contractility [46–47]. Choroidal pericytes are found to be reactive to autonomic neurotransmitters, such as tyrosine hydroxylase (TH), vasoactive intestinal polypeptide (VIP), and choline acetyl transferase (ChAT). Other substances that induce pericytes contraction include endothelin-1 (ET-1) [47], thromboxane A2 [48], and angiopoietin (Ang)-2 [8]. Discovering more vasoactive substances that target pericytes could help to understand contractile physiology of pericytes and inspire therapeutic researches on pericyte contractility.

Despite a large body of evidence supporting the contractility and blood flow regulation capability of pericytes, there is still a debate on the cerebral blood flow controller at terminal capillary beds. A recent study claimed that arteriole SMCs, not pericytes, take the responsibility of blood flow regulation physiologically and pathologically in ischemic stroke. [49] However, it was pointed out that the pre-capillary SMCs and pericytes on the arteriole end have similar morphology and share many molecular markers except α-SMA. It is unknown whether they represent two distinct cell types or a single cell type with different phenotypes. Therefore, the identification of novel markers that distinguish between SMCs and pericytes in the cerebral vascular tree is critical to confirm the roles of these two cell populations in the healthy brain and under pathological conditions [50].

4. Stem cell function

In mature adult brains, pericytes maintain low rate of turnover. Pathological insult can increase the proliferation rate of pericytes in pre-existing pools around brain vessels and recruit pericyte progenitor cells from bone marrow [51]. Pericytes are not only self-renewable but also have the capacity to differentiate into other types of CNS cells. Pericytes share some similarities with mesenchymal stem cells (MSCs). Both of these two cell types express similar pattern of immunological markers (e.g. CD44, CD73, CD90, CD105, Sca-1, CD9, CD45, and CD11b) and are able to differentiate into other types of cells. They can both regulate the homeostasis of neighboring cells [11]. Brain pericytes also display neural stem cell features. They express vimentin and nestin, which are thought to be specific intermediate filaments of neural progenitor cells. Brain pericytes are able to differentiate into neurons and astrocytes as well as oligodendrocytes [6]. Pericytes, with their multi-differentiation potency, have been reported to be involved in neurogenesis after transient ischemic brain injury [52]. It is possible that pericytes could be used as a cell-based therapy to promote tissue restoration after stroke or other brain injuries.

Pathological Alterations of Pericytes in Ischemic Stroke and the Following Reperfusion Injury

As shown above, pericytes play vital roles in maintaining physiological functions of the CNS. In ischemic stroke, pericytes respond to ischemia promptly and are involved in various pathological and repair processes. The following part of this review summarizes the findings about pericytes behavior during ischemic stroke and the following reperfusion.

1. Dilation and constriction

Physiological hypoxia, which occurs during high levels of metabolism, induces dilation of pericytes. Consistently, in stroke, short time ischemia seems to relax pericytes in order to restore blood flow and nutrition supply. Several mediators, including platelet-derived growth factor (PDGF)-β, adenosine, and NO are recognized to be responsible for pericytes contractility control. PDGF-β can activate various ion channels such as nonspecific cation channels, chloride channels, and ATP sensitive potassium channels and regulate contractility of pericytes according to metabolic status. Specifically, PDGF-β is able to contract pericytes when circulation supply is ample and dilate pericytes in case of hypoperfusion. The level of PDGF-β is elevated during ischemia [53]. Increased endothelial PDGF-β dilates pericytes in an effort to increase microvessel diameter and increase blood supply [53]. Another mediator of pericytes contractility is adenosine. Adenosine activates ATP-sensitive potassium channels (K(ATP)). Under normoxic condition, adenosine receptors, both the high affinity A1 and the low affinity A2a, mediate opening of K(ATP). Opening of K(ATP) results in hyperpolarization of pericytes and dilation of microvessels. Under hypoxic condition, adenosine may activate A2a receptors, resulting in the opening of K(ATP) and the subsequent dilation of pericytes. It has been reported that adenosine accumulates significantly in the ischemic brain and acts as a potent vasodilator [54–55]. NO production is also elevated under hypoperfusion. The NO/guanylate cyclase pathway is known to mediate dilation of pericytes, which becomes more pronounced in hypoxia than normoxia [56–57].

Interestingly, despite the release of all these factors to dilate pericytes, contraction of pericytes has been observed in ischemic stroke models, which indicates that sustained ischemia might reverse the phenotype of pericytes from dilation to contraction. Pericyte contraction could induce capillary constriction and hence obstruct blood flow. Indeed, pericyte contractility is thought to at least partially contribute to microcirculation no-reflow phenomenon in ischemia [58].

Pericyte constriction is more prominent during reperfusion after ischemic stroke. It is known that reperfusion may bring even greater damage to the injured tissue than ischemia itself. Paradox of oxygen, calcium, and pH change in ischemia-reperfusion process all contribute to production of free radicals, development of oxidative stress, and other toxic factors. Among them, oxidative stress is a potent constriction inducer of pericytes and appears to be an especially prominent injurious mediator in ischemia-reperfusion injury. In vitro studies verify that pericytes are sensitive to even low concentration of reactive oxygen species and are much more fragile than ECs. Exposure of cultured retinal pericytes to hydrogen peroxide (H2O2) or ultraviolet radiation, a free radical generating system, led to translocation of myosin heavy chain from cytosol to the cytoskeleton and the following contraction of pericytes. This could be rescued by the elimination of free radicals or oxidative stress [59]. Yemisci M et, al. reported in vivo contractility change of pericytes during reperfusion in ischemic stroke. Pericytes contraction occurred since 1h after reperfusion in the middle cerebral arterial occlusion (MCAO) model of stroke. It was proved that pericyte contraction was induced by oxidative and nitrative radicals to suppress oxidative and nitrative stress before reperfusion relieved pericyte contraction [58]. This study suggests the importance of pericytes contractility in stroke pathology and indicates that sustained contraction of pericytes might be the reason for adverse outcome of thrombolysis therapy in stoke.

2. Mediating Leukocyte Infiltration

Ischemic injury can be aggravated by inflammatory responses featured by recruitment and transmigration of immune cells from peripheral circulation to ischemic or peri-ischemic tissue [60–61]. Accumulating evidences confirm the critical role of pericytes in regulating leukocyte adhesion and migration. Pericytes express ICAM-1, which interacts with its intergrin ligands on leukocytes and thus guide leukocyte migration through gaps between adjacent pericytes during ischemia [62–63]. Pericytes at the venular end of capillary beds show a prominent role in regulating neutrophils infiltration. Postcapillary pericytes can inhibit neutrophils transmigration by pericytes-derived matrix [20]. Under inflammatory stimulation, the gaps between adjacent pericytes become notably enlarged, which facilitate transmigration of neutrophil [64]. Neutrophils prefer gaps with high expression of ICAM-1 and keratinocyte-derived chemokine as their entry points in breaching the venular wall. Consistently, the ICAM-1 blockade slows down infiltration of pericytes. All of these studies suggest the importance of pericytes in leukocyte trafficking into the inflamed brain.

3. Increased Phagocytosis

Granular pericytes containing lysosome-like granules can serve as scavenger cells in an injured brain [65]. In stroke-prone spontaneously hypertensive rats, granular pericytes were activated and proliferated [35]. The accumulation of granular pericytes was also detected in

atheroma injection induced ischemic brain as soon as 2h after exposure [66]. These granular pericytes were capable of accumulating lipid components of the injected atheroma, suggesting a phagocytotic property of granular pericytes. In addition, with the capacity of multi-potent differentiation, pericytes may differentiate into microglia after ischemic stroke. [67–68] These phagocytotic pericytes and the microglia derived from multi-potent pericytes can help to eliminate dead and damaged cells in ischemic areas, which in turn alleviates local inflammation and reduces secondary tissue damage.

4. Detachment and migration

It was found that pericyte is the first cell type that reacts to brain hypoxia. In ischemic stroke, pericytes change from quiescent flat state into ameboid morphology and express RGS5, which is an activated marker of the cells. Pericytes were found to separate from basal lamina as early as 1h after stroke in rat permanent MCAO model [69]. Following detachment, pericytes migrate toward the hypoperfusion lesion, which could be observed as early as 2h after hypoperfusion insult [70] and peaked at 7d after ischemia [68]. Detachment and migration of pericytes are associated with their secretion of MMPs. Pericytes have been shown to be an important source of MMP-9 elevation in the basal lamina after ischemic stroke [71]. Pericyte secretion of MMP-9 could be stimulated by TNF-α [72], which is an important inflammation modulator during ischemic stroke. By migrating, pericytes escape from injurious destiny, guide angiogenesis in the hypoperfusion site, and direct vascular maturation. They also help clear up neural debris and could differentiate into NVU components so as to restore neurological functions [7]. On the other hand, pericyte detachment could lead to disruption of BBB integrity [69, 73]. For example, reduced pericyte coverage in microvessels, which is a hallmark of diabetic retinopathy, induced decreased BBB stability and subsequent pathological change [74]. Therefore, migration of pericytes seems to be a double-edged sword in ischemic stroke. Balancing the advantages and disadvantages could benefit the final outcome of pericyte detachment.

5. Cell Preservation

Pericytes exert protective effects to the surviving cells within NVU in a paracrine manner during ischemic stroke. They enhance endothelial barrier function and provide trophic factors to neurons as well as other types of cells.

(1) Neuron—Pericytes express a variety of neurotrophic factors. Glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) secreted by pericytes both facilitate neuronal and axonal regeneration [22]. Pericytes can also express nerve growth factor (NGF) and neurotrophin-3 (NT-3), both of which are neuro-protective in response to the stimulation of endothelial-derived PDGF-β [53]. Moreover, increased production of NT-3 by pericytes during ischemia could activate astrocytes, raise astrocytic secretion of NGF, and thus indirectly enhance neuro-protection in the peri-ischemic area [75]. In addition to releasing neurotrophic factors, pericytes were also discovered to be a potential source of the perivascular NSCs that generate neuronal cells following transient ischemia/reperfusion [52].

(2) Endothelial Cells—Since ischemic stroke is essentially a vascular disease, endothelial cells on the luminal aspect of vessels are the first to be injured [76]. Preserving endothelial cells and their functions are key tasks in stroke therapeutic research. Pericytes could enhance TJ between endothelial cells, protect endothelial cells from necrosis, and promote angiogenesis in ischemic stroke.

Ang-1 and GDNF are found to be responsible for the TJ enhancement effect of pericytes. Pericytes are important sources of Ang-1 in CNS. An in vitro study found that under both normal and hypoxic conditions, the expression of TJ proteins ZO-1 and occludin in endothelial cells was enhanced by pericyte conditioned medium (PCM), while pretreating PCM with Ang-1 neutralizing antibodies abolished the increased expression of the TJ proteins [77]. Pericyte-derived GDNF could also up-regulate TJ protein claudin-5 expression in endothelial cells and increase TEER value in in vitro BBB models [22].

Vascular endothelial growth factor (VEGF) is another pericyte-derived factor that protects endothelial cells and preserves their functions in the ischemic brain. VEGF administration before MCAO significantly increased capillary density, decreased infarct volume, and up-regulated pericytes coverage of endothelial cells [78]. Pericytes could also secrete TGF-β1, which enhanced VEGF receptor 1 (VEGFR1) expression on endothelial cells and subsequently improved endothelial cells survival in ischemic retinopathy [43].

6. Angiogenesis

In ischemic stroke, hypoperfusion resulting from vascular occlusion leads to tissue necrosis. Re-flow of the occluded vessels is a fundamental solution of the insult [4, 79]. However, the phenomenon of reperfusion-no-reflow in microvasculature is common. Therefore, angiogenesis, which opens new pathways of blood flow to the hypoperfusion area, is crucial for tissue preservation and restoration [80]. Angiogenesis includes proliferation of vessel composing cells, recruitment of pericytes, coverage of endothelial tube by pericytes, and maturation of neo-vessels. Pericytes function as vital modulators in angiogenesis. Various signaling pathways, including VEGF signaling, PDGF-β/PDGFR-β system, Ang/Tie system, and RGS5 signaling in pericytes, are involved in the angiogenesis after ischemic stroke (Figure 3).

(1) VEGF—The VEGF family, including placenta growth factor (PlGF), VEGF-B, and VEGF-C, serves as mitogen to both pericytes and endothelial cells. Both pericytes and endothelial cells produce VEGF, modulating angiogenesis in autocrine and paracrine manners [81]. In normoxia, the rank order of mRNA content in pericytes is VEGF-C > VEGF-A > VEGF-B > PlGF and PlGF> VEGF-B > VEGF-C > VEGF-A in endothelial cells [82]. There are two forms of VEGF receptors (VEGFR), namely kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase 1 (FLT1). Endothelial cells have both types of VEGFRs while pericytes express only FLT1 [81]. During hypoxia, pericyte VEGF production increases [83], which is consistent with the upregulation of FLT1 expression [84]. VEGF binding to FLT1 on pericytes results in a delayed proliferation of pericytes at around 7d after hypoxia but has little effect on their initial growth [84]. Neutralizing VEGF function results in low synthesis of DNA in pericytes and endothelial

cells [81]. Besides, VEGF induces up-regulation of Ang2 expression in endothelial cells and Ang1 and Tie2 (Ang1 receptor) in pericytes, which all mediate further angiogenesis and the following vascular maturation [85].

(2) PDGF-β /PDGFR-β—PDGFR-β is the most widely used marker for pericytes. Its interaction with PDGF-β, a functional isoform of PDGF secreted by endothelial cells, contributes to the angiogenesis process. In ischemia, PDGFR-β was found to be induced specifically in peri-infarct pericytes [53]. Meanwhile, peri-infarct endothelial cells could produce more PDGF-β. Activation of PDGFR-β by PDGF-β induces phosphorylation of Akt in pericytes, resulting in anti-apoptotic response and cell proliferation, which are the first steps of angiogenesis [53]. On the other hand, PDGF-β /PDGFR-β signaling was found to be indispensable for migration of pericytes to the newly formed endothelial tubes and the formation of the BBB, which are vital steps for microvascular maturation [53]. Therefore, it is obvious that the PDGF-β/PDGFR-β system is involved in various steps in angiogenesis, playing a positive role in the process while providing neuroprotection.

(3) Ang/Tie—The Ang/Tie system modulates angiogenesis bi-directionally. Interaction between Ang1 and Tie2 receptor is involved in pericyte recruitment, capillary maturation, and reduction of leakiness in newly formed microvessels. Meanwhile, Ang2 serves as a natural antagonist to Ang1/Tie2 signaling [86]. Ang2 signaling works on proliferation of endothelial cells and prevention of pericytes recruitment, thus impeding neovascular maturation [87]. In pericytes, expression of Ang 1 and Tie 2 both increase with hypoxia or VEGF stimulation, but Ang2 expression remains unaltered. While both hypoxia and VEGF can induce upregulation of Ang2 expression in endothelial cells, neither Ang1 nor Tie2 respond to the two angiogenestic stimuli [85]. These effects altogether increase proliferation of endothelial cells and pericytes and guarantee neo-vascular maturation at the same time. Balance of Ang1 and Ang2 signaling is obviously important for proliferation of pericytes and endothelial cells as well as the maturation of new vessels. Regulation of the balance in different periods of angiogenesis could promote the beneficial process, which is a potential therapeutic strategy for ischemic stroke.

(4) RGS5—RGS-5 is identified as an angiogenestic marker of pericytes. During neovascularization, expression of RGS-5 in pericytes is elevated in an HIF-1-α dependent manner [88]. Downregulating RGS-5 expression impedes proliferation of pericytes and leads to pericytes maturation [88]. Therefore, RGS-5 is also considered an activated marker of pericytes. The underlying mechanism of RGS5 signaling in pericytes is still under research. Revealing the mechanism should help us to further understand angiogenestic mediation process in pericytes and promote angiogenesis relevant therapeutic research in ischemic stroke.

(5) Other pathways associated with pericytes proliferation and recruitment—Growth of pericytes results from proliferation of pericytes in pre-existing pericyte pools and recruitment of pericyte progenitor cells from bone marrow [51]. It has been suggested that proliferation of pericytes is partially positively associated with Notch-3 signaling according to a study on zebrafish [89]. NG2 is also proved to be related to the proliferating profile of

pericytes [90]. In tumor relevant study, PGI2 agonist was found to positively modulate capillary maturation by increasing the number of pericytes [91].

Pericytes can not only grow by proliferating but also by recruitment from periphery. Bone marrow-derived cells (BMDCs) were found to contribute to revascularization after ischemia in a murine MCAO model of stroke and present pericyte features. They infiltrated the brain parenchyma, peaking at 7d after stroke and lasting at least for 14d [92]. The fusion of BMDCs with mature pericytes seems to be critical for BMDCs to obtain the pericyte profile after ischemia [93].

Angiogenesis is a precise process that requires not only positive regulation but also negative signals to maintain an adequate extent of neovasculation. Bone morphogenetic protein (BMP)-4, which is secreted by pericytes, exerts an antiangiogenic effect and co-regulates angiogenesis with VEGF. During hypoxia, chordin like-1 (CHL-1) is induced in pericytes in a HIF-1 dependent manner and serves as an anti-angiogenic mediator. The effect of CHL-1 is synergetic with BMP-4 [94]. Ninjurin1 (Ninj1) is another inhibitor of angiogenesis during hypoxia. Hypoperfusion can induce Ninj1 expression in pericytes, which can reduce expression of VEGF and Ang1 In a matrix gel based co-culture system comprising of pericytes and thoracic aorta, the sprouting of endothelial tubes was suppressed by the existence of pericytes. However, when Ninj1 was blocked, the suppressive effect was abolished [95]. Endostatin, which is derived from the C-terminal of extracellular protein collagen XVIII, is also a potent anti-angiogenic factor. Both pericytes and endothelial cells express collagen XVIII, which gives rise to endostatin and thus modulates angiogenetic process. During hypoxia, endostatin production is downregulated at the translational level in both pericytes and endothelial cells, which promotes angiogenesis in the pathological process [96]. Balancing the functions of pro-angiogenesis modulators and anti-angiogenesis mediators released by pericytes would be critical for any therapeutic strategies targeting at post-stroke angiogenesis.

7. Multipotent differentiation

Regeneration of NVU components serves as a reconstructive mechanism in neuropathology. Ischemia can induce the multi-potential differentiation capacity of pericytes. Pericytes isolated from ischemic regions of mouse or human brains reveal mesenchymal multi-lineage developmental properties when cultured under oxygen/glucose-deprived environment. They can differentiate into basic components of the NVU, including vascular cells, and glial cells [97, 67]. Moreover, hypoxic condition is propitious for other pleotropic cells (e.g. adipose and bone marrow progenitor cells) to adopt pericytic characteristics [98]. Intriguingly, pericytes could be reprogrammed into neurons by transducing the neurogenic transcription factors *sox2* and *ascl1* [99]. Reprogramming of pericytes into neurons raises a hope for neurogenesis in CNS diseases. However, some studies documented that although the pericytes derived from ischemic brain were able to differentiate into neuronal cells *in vitro*, they predominantly differentiated into glial cells rather than neuronal lineage *in vivo*.[100] Fate mapping study also showed that Glast^{+} pericyte subpopulation in the spinal cord forms a glial scar after injury.[101] Therefore, pericytes, although can differentiate into different neurovascular cells, may not be able to reconstruct NVU under pathological conditions. It is

therefore critical to understanding the factors that control the fate of pericyte differentiation. Although the exact mechanisms remain elusive, several cytokines and factors, including interleukin-6 and ciliary neurotrophic factor have been shown to be important in promoting differentiation of precursor cells into a glial lineage.[102–103] Strategies that modulate the expression of these factors might be promising for accomplishing NVU reconstruction during CNS regeneration and repair.

Pericytes Alterations Caused by Aging

Since ischemic stroke is common in the elderly population, alterations of pericytes along with the aging process may be critical for stroke outcomes. Most studies agree that the number of pericytes decreases with age. Interestingly, it has been found that in either PDGFR-β+/− or PDGFR-βF7/F7 transgenic mice that progressively lose their brain pericytes with age, the BBB breakdown also develops in an age-dependent manner [104]. A similar conclusion was drawn from Notch3 mutant mice [105]. These studies suggest the contribution of pericyte aging to the impairment of the BBB integrity. However, other studies argue that the number of pericytes may not change in aged population. Comparable numbers of pericytes were detected in the brains of aged and young rhesus monkeys [106]. By comparing the number of pericytes in the parietal cortex (Par1 region) of young (3–4 months old) and aged (32–36 months old) Wistar rats, Peinado et, al. found that the number of pericytes increased by 22% with age, which was most significant in layer II-IV and V [107]. There is also a debate on the coverage rate of pericytes of endothelial cells with aging. Some studies demonstrated that pericyte coverage of endothelial cells decreased with age [108] while others showed the opposite [109]. These contradictory results could be explained by different animal models and different age stage used in experiments. More studies with consistent models and observing ages are required.

Age-related ultra-structural alterations in pericytes have been reported. Inclusions and vacuoles of pericytes are found to accumulate with age [108]. It is proposed that inclusions in pericytes are due to their phagocytotic activity, which may serve as a defense against potential age-associated leakage of the BBB [110]. The accumulation of these inclusions and vacuoles may because of low metabolism rate and dysfunction of organelles such as lysosomes. In addition, there is evidence showing that pericytes empty the contents of their inclusions and vacuoles directly into capillaries [106]. Since it is widely accepted that capillary thickness increases with age [109], pericytes may face difficulty in emptying the contents, which leads to accumulation of particles. Increased α-SMA expression as well as altered length and orientation of desmin filaments are also found in aged pericytes, while the diameter of microvessels seems to be increased [108]. Disordered expression of these contractile proteins together with the dilation of microvessels could result from pericyte sclerosis. Pericyte sclerosis may lower blood flow control capacity of pericytes. Moreover, when pericytes encounter oxidative stress, which is common in aged individuals, the constriction of pericytes may increase in intensity and thus lead to significant hypoperfusion.

Functional impairments of aged pericytes have also been reported. Age-dependent vascular malfunction was observed in pericyte-deficient mice. Moreover, these vascular changes precede neuronal degenerative changes and neuroinflammatory responses, suggesting that

age-dependent loss of pericytes might be a contributing factor for vascular-mediated neurodegeneration [104].

The mechanisms of pericyte impairment during aging are still under investigation. Injurious factors from the microenvironment within the CNS may be responsible for pathological changes in pericytes. For example, oxidative stress increases during aging and could impair pericyte functions. Amyloid-β deposition, which is significant in the elderly and is a characteristic pathological change of Alzheimer's disease, is proven to be harmful to pericytes by forming fibrils at the cell surface [111]. Accumulation of other metabolic wastes could also damage pericyte structure and functions. Therefore, developing elimination methods for these harmful factors could help to preserve pericytes, thus protecting the CNS from disorders and degeneration during aging.

Some age-related metabolic disorders, such as hyperlipidemia and diabetes mellitus, influence pericyte function and may worsen the outcome of ischemic stroke. Hyperlipidemia can dose-dependently attenuate VEGF induced capillary formation and pericyte coverage to neo-vasculature, thus inhibiting the restoration of blood flow in peri-infarct regions [112]. At physiological concentration, insulin can protect pericytes from death in ischemia-reperfusion injury [5]. However, after the onset of diabetes, insulin fails to protect pericytes, partially resulting in diabetic retinopathy (DR) [113]. This evidence indicates that treatments targeting hyperlipidemia and diabetes are beneficial for ischemic injury via protecting pericytes.

Therapeutic Strategies Targeting at Pericyte Responses after Ischemic Stroke

Since pericytes play important roles under physiological conditions and mediate vital pathological processes in ischemic stroke, there is an upsurge of interest in developing therapeutic strategies for ischemic stroke targeting pericytes. Direct transplantation of pericytes, medication treatment preserving pericyte function, and other possible therapeutic strategies are all possible new solutions for ischemic stroke.

Several medicines have been reported to preserve pericyte functions after ischemic brain injury. For example, the widely used antiplatelet drug cilotazol has been shown to prevent the detachment of pericytes and astrocyte endfeet from microvessels in spontaneous hypertensive rats with spontaneous cerebral infarctions. Cilotazol could enhance pericyte proliferation while inhibiting their production of MMP9 and increase VEGFR2 expression in endothelial cells. As a result, cilotazol preserves BBB integrity and facilitates angiogenesis [114]. As mentioned above, oxidative stress and free radicals serve as main toxic factors to pericytes in reperfusion injury. Free radical scavenger edaravone has been proven to promote pericyte proliferation and increase the pericyte coverage of endothelial cells. Moreover, edaravone reduces production of MMP9 by pericytes, thus attenuating BBB destruction during reperfusion injury [115].

Hypothermia is thought to be protective to ischemic stroke [116]. Evidences show that hypothermia can delay migration of pericytes from microvessels [69]. As mentioned in

previous sections, pericytes migration toward lesion site is important for post-ischemia angiogenesis, though early detachment of pericytes from microvessels may lead to BBB leakage. Therefore, postponing the migration of pericytes until other neuro-protective mechanisms become effective might be one of the mechanisms for the beneficial effect of hypothermia.

Directly transplanting pericytes into ischemic areas is a possible strategy to promote tissue survival or NVU reconstruction. However, the barrier function of the BBB may prevent the penetration of pericytes progenitor cells into the ischemic brain. A recent study showed that opening the BBB with the help of mannitol could enable the penetration of intra-arterially transferred pericyte progenitor cells into the ischemic brain. Unfortunately, the therapeutic effect of transferred pericytes was not evaluated in the study [117]. To date, the curative effect of pericyte transplantation in ischemic stroke has not been reported. Nevertheless, injecting pericytes derived from leg veins has been proved to be beneficial to myocardial infarction. The injected pericytes relocated to ischemic areas and promoted angiogenesis as well as cardio-myocyte survival [65]. Further studies are warranted to evaluate the effect of pericyte progenitor cell transplantation into ischemic brains.

Summary

In this review, we summarize physiological features of pericytes and their responses toward ischemic brain injuries. Overall, pericytes are much more than supportive cells to endothelial cells. They are important functional components in the BBB and NVU. They display significant alterations during ischemia and reperfusion and actively participated in brain injury, cell preservation, and brain repair. Strategies targeting pericyte responses after ischemia and reperfusion may provide new therapies for ischemic stroke.

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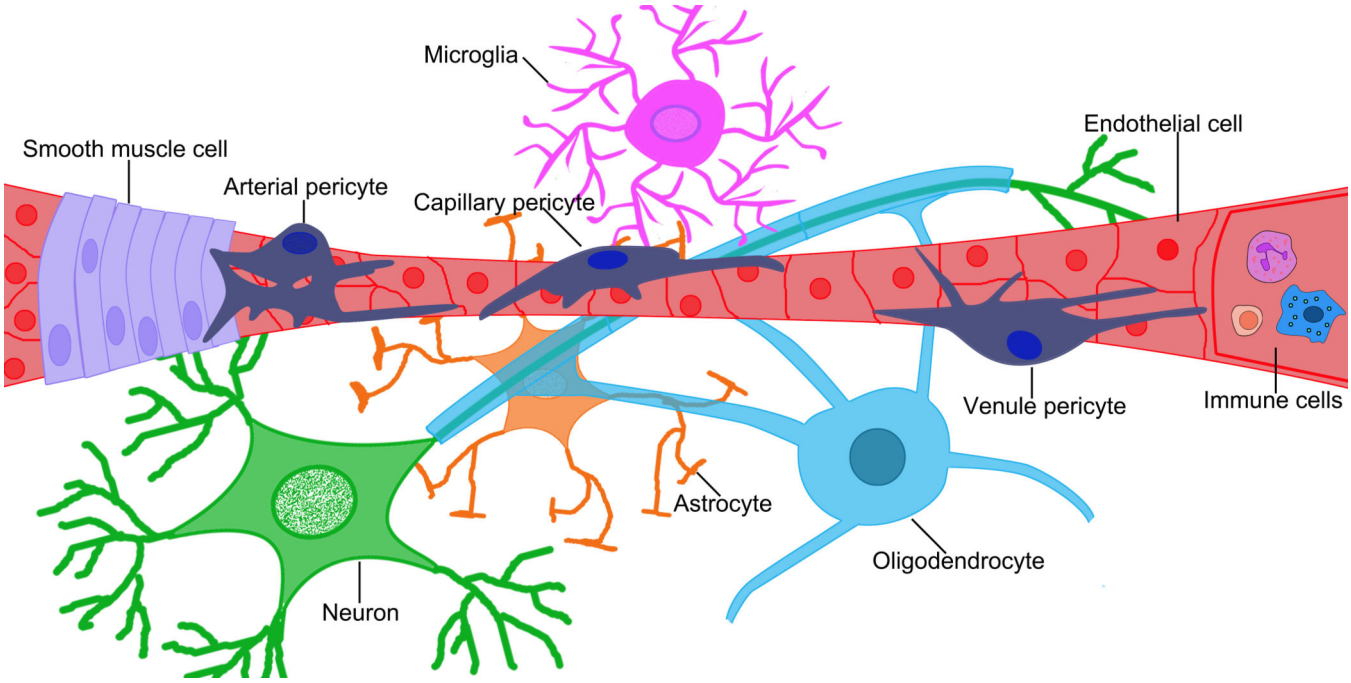

Figure 1. Pericyte classification based on its location, morphology and protein expression

Arterial pericytes are located at the arteriole end of the capillary bed. These pericytes have many circumferential processes to surround capillaries and express high levels of α-smooth muscle actin (α-SMA). Capillary pericytes reside in the middle of the capillary bed. They give out more longitudinal processes and express less α-SMA. Venule pericytes are located at the venule end of the capillary. This subtype of pericytes has a stellate morphology and modulates the infiltration of peripheral immune cells into brain paranchyma.

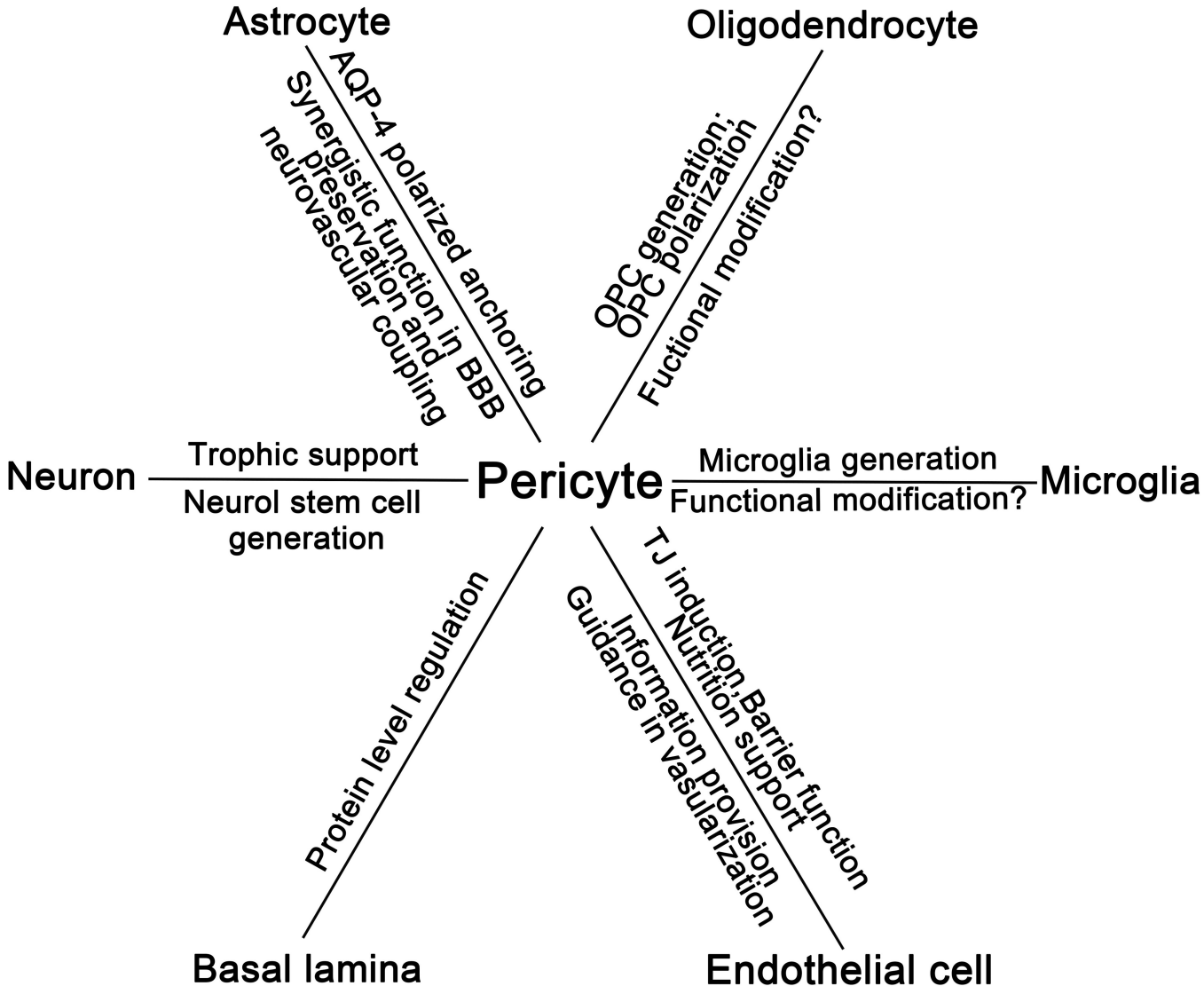

Figure 2. Interactions between pericyte and other neurovascular unit (NVU) components

As a main component of NVU, pericytes not only exert barrier function by themselves but also promote physiological functions of other BBB components, including endothelial cells, basal lamina, astrocytes and neurons.

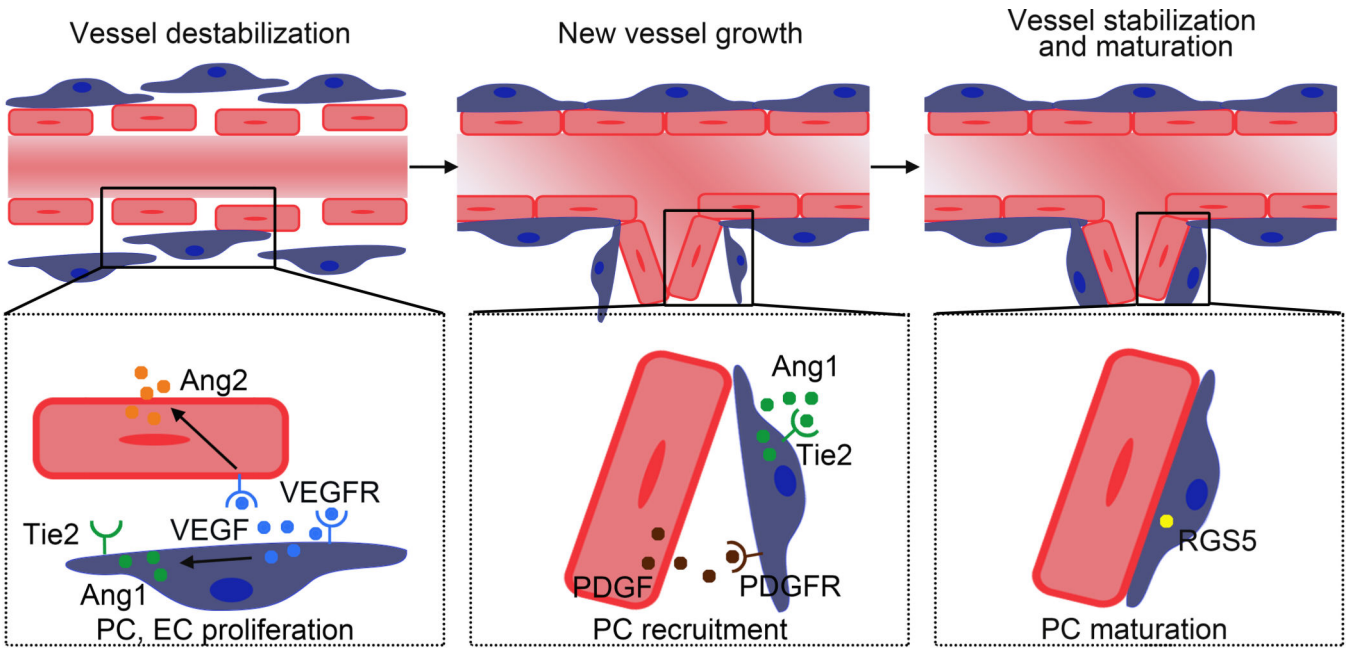

Figure 3. Pericyte-derived angiogenic factors and related signaling pathways after ischemic stroke

During ischemia, several signaling pathways are activated and each of them is involved in various steps in angiogenesis. VEGF binds to its receptor on pericytes and endothelial cells and results in proliferation of these cells. Besides, hypoxia and VEGF signaling induce up-regulation of Ang2 expression in endothelial cells and up-regulation of Ang1/Tie2 (Ang1 receptor) in pericytes, which all mediate further proliferation of endothelial cells and pericytes and the following vascular formation. PDGFR-β is found to be induced specifically in peri-infarct pericytes. Meanwhile, peri-infarct endothelial cells could produce more PDGF-β. Activation of PDGFR-β by PDGF-β in pericytes is found to be indispensable for migration of pericytes to the newly formed endothelial tubes, which is a vital step for neovasculation. RG5 is important for pericyte maturation. EC, endothelial cells; PC, pericyte; PDGF: platelet-derived growth factor; PDGFR: PDGF receptor; VEGF: Vascular endothelial growth factor; VEGFR: VEGF receptor;

Table 1

Functions of pericytes in the central nervous system.

Aspects	Roles
BBB maintenance	**(1)** Barrier functions: prevent leukocyte infiltration [39]. **(2)** Interaction with endothelial cells: assist endothelial function, induce TJ formation [19–25]. **(3)** Interaction with basal lamina: regulate lamina protein level with MMPs and TIMP-3 secretion [10, 26–27]. **(4)** Interaction with astrocytes: mediate AQP-4 polarization [18], synergistically maintain BBB integrity.
Phagocytosis and immunological mediation	**(1)** clear hazard, metabolic debris of cells or organelles from CNS component [16]. **(2)** Sense danger [6, 37]. **(3)** Eliminate harmful or injurious factors [37]. **(4)** Serve as antigen-presenting cell [37]. **(5)** Immuno-suppressing function [38]. **(6)** Immuno-promoting function [11, 14, 21, 39].
Cerebral blood flow control	**(1)** Express contractile proteins [20]. **(2)** Regulate cerebral blood flow according to metabolism level [8, 36, 45].
Pluripotency	Differentiate into SMC, fibroblast, endothelial cells, astrocytes, microglial and even neurons in different induction [6, 51–52].