


Roles of Pericytes in Stroke Pathogenesis

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Abstract

Stroke is a cerebrovascular disorder that affects many people worldwide. In addition to the well-established functions of astrocytes and microglia in stroke pathogenesis, pericytes also play an important role in stroke progression and recovery. As perivascular multi-potent cells and an important component of the blood–brain barrier (BBB), pericytes have been shown to exert a large variety of functions, including serving as stem/progenitor cells and maintaining BBB integrity. Here in this review, we summarize the roles of pericytes in stroke pathogenesis, with a focus on their effects in cerebral blood flow, BBB integrity, angiogenesis, immune responses, scar formation and fibrosis.

Keywords

stroke, hemorrhage, ischemia, pericytes, blood–brain barrier

Introduction

Stroke is the 5th leading cause of death and is a leading cause of long-term disability in the United States¹. Based on the type of injury (occlusion or rupture of blood vessels), it is broadly categorized into ischemic stroke and hemorrhagic stroke. When stroke occurs, a cascade of molecular and cellular events take place, which eventually result in cerebral blood flow interruption, blood–brain barrier (BBB) breakdown, inflammation, glial cell activation, vascular malformation, and neuronal death^{2–4}. Accumulating evidence suggests that astrocytes, microglia, and inflammatory leukocytes play critical roles in the pathogenesis of stroke^{5–7}. Recent studies demonstrate that pericytes also affect stroke pathology and contribute to disease progression and recovery.

Pericytes are perivascular multi-potent cells located on the abluminal side of capillaries. In the central nervous system (CNS), pericytes have the highest density with an endothelial-to-pericyte ratio estimated to be 1:1 ~ 3:1^{8,9}. This high density/coverage of pericytes has been shown to be crucial for the maintenance of BBB integrity^{10–14}. Another unique feature of CNS pericytes is that they are derived from neural crest cells, whereas pericytes from peripheral organs are mainly derived from the mesothelium^{10,15–17}. The different embryonic origins of pericytes suggest that CNS and peripheral pericytes may have distinct biological functions. There is evidence showing that CNS pericytes can exert a large variety of functions¹⁰, including regulation of cerebral blood flow, maintenance of BBB integrity, modulation of angiogenesis and inflammation, and serving as stem/progenitor cells. These functions are

dependent on appropriate interactions and signaling between pericytes and other cells at the BBB, especially endothelial cells and astrocytes.

Although pericytes are embedded in the basement membrane, which separates them and other cells, pericytes and endothelial cells do form numerous direct contacts, including peg-socket contact, adhesion plaques, N-cadherin junctions, and gap junctions¹⁰. In addition, a large variety of signaling cascades, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF- β), and Wnt pathways, are involved in pericyte–endothelium interaction^{9,11,18–24}. Defective interactions/signaling between these cells lead to various pathological conditions in humans, including diabetic retinopathy, tumor angiogenesis, ectopic calcification, dementia syndrome, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and stroke¹⁰. It has been shown that PDGF secreted by endothelial cells attracts pericytes and mediates their attachment and coverage on newly

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Table 1. Functions of Pericytes in Stroke.

Functions	Stroke types	Roles	References
Cerebral blood flow control	Ischemic stroke	Capillary constriction	22,35,36,37
BBB maintenance	Intracerebral hemorrhage	Regulation of vessel stability and vascular permeability	11,12,25
	Intracranial hemorrhage	Regulation of contractile capability	38,39
	Ischemic stroke	Regulation of endothelial cell permeability	40–42
Angiogenesis	Ischemic stroke	Pericyte survival	43,44
		Modulation of BBB integrity and VEGF/Nox4/ROS expression	45–49
		Revascularization	23,46,50
	Hemorrhagic stroke	Blood vessel stabilization	47,51,52
		Disruption of vascular integrity	53,54
Immunological properties	Ischemic Stroke	Regulation of basement membrane formation	55
		Vascular development	56
	Ischemic Stroke	Vessel stabilization	57
		Differentiating into microglia-like cells and performing microglia-like functions	58,59,60,61
Scar formation and fibrosis	Ischemic stroke	Reprogramming into NPCs	60,62
		Neuroprotection	21
		Promoting fibrosis and regulating fibronectin deposition	63,64

BBB: blood–brain barrier; NPCs: neural precursor cells ; ROS: reactive oxygen species; VEGF: vascular endothelial growth factor.

formed blood vessels^{11,25}. The recruitment of pericytes is closely associated with the barrier function of newly formed vessels. In vitro studies demonstrated that pericytes induced the synthesis of tight junction proteins through the release of angiopoietin-1²⁶, suggesting that pericyte–endothelium interaction promotes BBB integrity by reducing paracellular leakage. Contrary to this report, reduced pericyte recruitment/density failed to affect the expression of tight junction proteins in vivo^{12,27}. Instead, it induces BBB leakage by up-regulating endothelial expression of genes known to increase vascular permeability, such as plasmalemmal vesicle-associated protein-1 (PLVAP)^{12,27–29}. PLVAP, which regulates vesicle trafficking, is highly expressed in permeable/peripheral vessels or in CNS vessels with pathological breakdown of BBB. These results suggest that pericyte–endothelium interaction also contributes to BBB integrity by suppressing the intracellular pathway (transcytosis)²⁷.

Another cell type that actively communicates with pericytes is astrocytes. During vessel formation, astrocytes are also recruited to newly formed blood vessels, although it occurs later than pericyte recruitment^{30,31}. It has been shown that tight junctions form before astrocytic endfeet wrap endothelial cells and pericytes³⁰, suggesting that direct interaction with astrocytes is not required for tight junction formation. Astrocytes, however, can regulate tight junction integrity via Wnt and hedgehog signaling pathways^{32,33}. In addition, astrocytes also contribute to BBB barrier property at the vessel–neuron interface through a group of unique channels and transporters that are exclusively expressed at their endfeet, including aquaporin 4 (Aqp4) and Kir4.1³⁴. Pericytes, which are sandwiched between astrocytes and endothelial cells, have been speculated to modulate astrocyte functions. Consistent with this hypothesis, expression of

polarized astrocytic endfoot markers was significantly reduced in pericyte-deficient mice¹¹. Together, these results suggest that, by interacting with both endothelial cells and astrocytes, pericytes actively regulate the formation and maintenance of BBB integrity.

Here in this review, we summarize recent findings on the biological roles of pericytes in the pathogenesis of both ischemic and hemorrhagic stroke. Specifically, the functions of pericytes in cerebral blood flow, BBB integrity, angiogenesis, immune responses, scar formation and fibrosis are discussed. The functions and related signaling pathways of pericytes in both types of stroke are summarized in Tables 1 and 2, respectively.

Pericytes and Cerebral Blood Flow in Stroke

Whether pericytes regulate cerebral blood flow remains controversial. On one hand, there is evidence supporting that pericytes contribute to blood flow. For example, an early electron microscopy study revealed filamentous structures in the cytoplasm of pericytes, similar to myofilaments in smooth muscle cells (SMCs)⁷⁴. Later, actin- and myosin-like filaments were identified in rat brain pericytes⁷⁵. In addition, various contractile proteins, such as α -smooth muscle actin (SMA), were found in brain pericytes in culture^{22,76,77}, suggesting that pericytes are able to contract. Consistent with these studies, rat brain pericytes have been shown to contract in response to intracellular Ca^{2+} in vitro⁷⁸. Recently, using an innovative iCelligence electrical impedance system, cultured brain pericytes have been shown to contract and relax in response to endothelin-1 and adenosine, respectively³⁵. Using whole retina and brain slices, Dr. Atwell's group reported that electrical stimulation and

Table 2. Signaling Pathways related to Pericyte Functions in Stroke.

Signaling pathways	Stroke types	Functions	References
PDGF-BB/ PDGFR β	Ischemic stroke	Neuroprotection, angiogenesis and vascular remodeling, pericyte recruitment, scar formation and fibrosis regulation	11,12,21,25,65,66
	Hemorrhagic stroke	BBB maintenance	11,12,20,25
Notch	CADASIL	Regulation of pericyte survival and angiogenesis	55,43,44
	Neonatal intraventricular hemorrhage	Pericyte recruitment, Pericyte–endothelium interaction, BBB maintenance	55,67
Canonical Wnt/ β -catenin	Ischemic stroke	BBB maintenance	18
	Hemorrhagic stroke	Angiogenesis regulation	18
TGF- β /TGF β R2	Neonatal Intraventricular hemorrhage	Maintenance of vascular stability/BBB integrity	67,68
	Ischemic stroke	Angiogenesis regulation and vessel stabilization	69,70
VEGF-A/ VEGFR2	Ischemic stroke and hypoxia	Angiogenesis induction and tight junction stabilization	71–73
	Ischemic stroke	ECM protein deposition and BBB protection	10,19
Ang/Tie2	Ischemic stroke	ECM protein deposition and BBB protection	10,19
aPKC-CBP	Ischemic stroke	Vascular remodeling and motor recovery	62

aPKC: atypical protein kinase C; BBB: blood–brain barrier; CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CBP: Creb binding protein; ECM: extracellular matrix; PDGF: platelet-derived growth factor; PDGFR β : beta-type platelet-derived growth factor receptor; TGF: transforming growth factor; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor.

chemicals (adenosine triphosphate and noradrenaline) induced capillary constriction by pericytes, whereas glutamate reversed noradrenaline-induced constriction⁷⁹. Furthermore, the same group elegantly showed that glutamate-induced pericyte relaxation was mediated by prostaglandin E2 and nitric oxide, and that capillary dilation occurred before arteriole dilation in response to whisker stimulation-induced increase of blood flow³⁶. Together, these results strongly indicate that pericytes initiate blood flow control and actively regulate cerebral blood flow in physiological conditions.

In addition, pericyte constriction is also observed in pathological conditions, such as stroke. It has been shown that pericyte contraction entraps erythrocytes at the capillary constriction sites during stroke, which obstructs microcirculation³⁷. Similarly, Hall and colleagues reported that pericytes constricted capillaries and died quickly *in vivo* after ischemia³⁶. They further demonstrated that the death of pericytes in rigor produced a long-lasting constriction of the capillaries, leading to prolonged reduction of cerebral blood flow even when arterial flow was restored³⁶. These pathological changes of pericytes after stroke are replicated in the iCelligence electrical impedance system. Specifically, chemical ischemia induced profound and irreversible pericyte contraction before their death in this *in vitro* system³⁵. These results suggest that pericyte constriction and death contribute to the pathogenesis of stroke via regulating cerebral blood flow. Further mechanistic study revealed that pericyte death was mediated in part by glutamate and was not reduced by free radical scavenging³⁶. In contrast to this report, suppressing oxidative-nitrate stress has been found to alleviate ischemia and reperfusion-induced

pericyte contraction and positively affect tissue survival³⁷. Additionally, administration of free radical scavenger edaravone also reduced infarct volume through preventing pericyte contraction and promoting pericyte proliferation in a rat model of focal cerebral ischemia⁸⁰. The exact molecular mechanism underlying pericyte constriction and death needs further investigation.

On the other hand, there are also studies showing that pericytes cannot constrict and do not regulate cerebral blood flow. Using various transgenic mouse lines and two-photon microscopy, Hill and colleagues failed to detect SMA expression in pericytes in both mice and humans⁸¹. They also showed that optogenetic-, whisker stimulation-, and cortical spreading depolarization-induced changes in vessel diameter and blood flow occurred in SMC-covered microvessels but not pericyte-covered capillaries⁸¹. Furthermore, using a transient middle cerebral artery occlusion (MCAO) model, they found that SMC, rather than pericyte constriction, caused hypo-perfusion, leading to distal microvascular occlusion⁸¹. Together, these data suggest that pre-capillary SMCs rather than pericytes are responsible for blood flow regulation in physiological and pathological conditions. This discrepancy may be partially explained by the difficulty in distinguishing pericytes from pre-capillary SMCs due to their similar biochemical, structural, and functional properties.

Compared with ischemic stroke, blood flow is less studied in intracerebral hemorrhage. Although perihematoma tissue constantly undergoes edematous and metabolic changes^{82–84}, reduced blood flow with increased oxygen extraction fraction has been reported in perihematoma regions^{85,86}. Whether pericytes play a role in the reduced perihematoma blood flow remains unknown. Addressing

this question will enrich our understanding of pericyte biology/function in hemorrhagic stroke.

Pericytes and BBB During Stroke

The BBB is a dynamic structure located at the interface of the CNS and circulation system. By actively regulating material exchange between these two systems, it functions to maintain CNS homeostasis^{87,88}. Not only is BBB breakdown a consequence of stroke, it also exacerbates stroke outcome^{89,90}. Pericytes, as a major component of the BBB^{10,87}, have been hypothesized to contribute to stroke pathogenesis via regulating BBB integrity.

Accumulating evidence shows that reduced pericyte coverage on blood vessels compromises vascular integrity and causes hemorrhagic stroke. During development and/or recovery after injury, pericytes are recruited to newly formed immature blood vessels mainly via the PDGFB–PDGFR β axis^{25,91–93}. Pericyte recruitment and subsequent coverage on endothelial cells reduce vascular leakage and stabilize these vessels^{10–12,25,94,95}. Mutations in either PDGFB or PDGFR β diminish pericyte recruitment^{11,12,92,93}, resulting in severe BBB disruption and massive hemorrhage^{11,12,20,25}. In addition, Notch3 mutation led to substantially reduced pericyte numbers, BBB disruption, and intraventricular hemorrhage in a zebrafish model⁹⁶. Similarly, reduced pericyte coverage and arteriovenous malformations were observed in Notch1^{+/–}Notch3^{–/–} mice⁵⁵. These findings strongly indicate that pericyte coverage on blood vessels is important for the maintenance of vascular integrity, and that loss of pericytes causes hemorrhagic stroke.

In addition, abnormal differentiation of pericytes/SMCs has also been linked to BBB disruption and intracerebral hemorrhage. Previous work from our laboratory showed that loss of astrocyte-derived laminin induced aberrant differentiation of brain pericytes and compromised the maturation of vascular SMCs, leading to BBB disruption and intracerebral hemorrhage^{38,39}. Additionally, forkhead transcription factor 2 (FOXF2), a gene specifically expressed in pericytes and SMCs, has been found to associate with a higher risk of stroke⁴⁰. Inactivation of FOXF2 at both embryonic and adult stages results in BBB breakdown and intracranial hemorrhage in mice due to defects in the differentiation of pericytes and/or SMCs^{41,42}. These data suggest that abnormal pericyte/SMC differentiation plays a causative role in BBB breakdown and intracerebral hemorrhage.

Although there is no direct causal relationship between pericyte dysfunction and ischemic stroke, pericytes may contribute to ischemia pathogenesis indirectly via their effect on vascular integrity. For example, mutations in Notch3 gene, which is crucial for the survival of pericytes⁴³, are linked to CADASIL⁴⁴, a hereditary stroke disorder. There is also evidence showing that pericytes can modulate BBB integrity and thus ischemic injury through VEGF. Using sodium cyanide (NaCN) treatment as an *in vitro* ischemic model, it has been shown that NaCN substantially

increases VEGF expression in brain pericytes, and that conditioned medium from NaCN-treated pericytes disrupts vascular integrity in an *in vitro* BBB model⁴⁵. Consistent with this report, VEGF has been found to induce BBB leakage in ischemic brain⁴⁶. It should be noted that, however, there is also evidence suggesting that prolonged exposure to VEGF enhances post-ischemic BBB integrity⁴⁷. This discrepancy could be due to different dosage, treatment strategy, and timing. In addition, pericytes can also affect BBB integrity and thus ischemia progression via reactive oxygen species (ROS). A previous study showed that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase NOX4, an enzymatic source of ROS production, was highly up-regulated in the peri-infarct region in MCAO model⁴⁸. Recently, it has been reported that pericytes are a main cellular source of NOX4 and its expression in pericytes is greatly enhanced in peri-infarct areas after MCAO⁴⁹. Further study revealed that overexpression of NOX4 in pericytes induced BBB breakdown by up-regulating metalloproteinase-9⁴⁹, highlighting an important role of ROS in BBB integrity.

Angiogenic Property of Pericytes in Stroke

Angiogenesis, the generation of new blood vessels from existing vasculature, is an important process that occurs in both normal and pathological conditions⁹⁷. Using a variety of signaling pathways, including PDGFB–PDGFR β , angiogenic endothelial cells recruit pericytes^{10,91–93,98}, which stabilize newly formed blood vessels^{10,25,94,95}. Stroke is a brain vascular disease caused by occlusion and/or rupture of blood vessels. Revascularization in injured brain regions promotes stroke recovery^{23,46,50,99}. Given the critical role of pericytes in angiogenesis^{24,100–102}, it has been speculated that pericytes may promote stroke recovery via modulating angiogenesis.

In ischemic stroke, various reports support this hypothesis. First, many important angiogenic factors, such as VEGF and TGF- β , have been found in pericytes^{24,100,101}, and their expression is significantly altered in various types of ischemic stroke in rodents^{103,104}. Next, it has been shown that transplantation of saphenous vein-derived pericyte progenitor cells to a mouse model of myocardial infarct exerts a beneficial role, which is mediated by their pro-angiogenic, pro-survival, and anti-fibrotic activities¹⁰⁵. Similarly, bone marrow-derived pericytes also contribute to revascularization after ischemia. It has been reported that transplanted bone marrow-derived cells differentiate into microglia and pericytes in mouse brain after ischemia⁵¹. These bone marrow-derived pericytes express high levels of VEGF and TGF- β ⁵¹, suggesting that they are involved in ischemia-induced angiogenesis and blood vessel stabilization. Furthermore, recombinant human VEGF has been demonstrated to increase capillary density and pericyte coverage, improve cerebral energy state and blood flow, and reduce brain infarction size in MCAO model⁴⁷. Consistent with this finding, VEGF receptor-inhibition promotes cell death, reduces

endothelial cell proliferation, and worsens injury in a neonatal stroke model⁵². Together, these observations suggest that pericytes play a beneficial role in ischemic stroke via promoting angiogenesis.

It should be noted, however, that there is also evidence showing that angiogenesis may play a detrimental role in ischemic stroke. For instance, elevated VEGF expression in ischemia has been associated with uncoupling of endothelial cell-cell junctions and increased vascular permeability & edema⁵³. Consistent with this report, VEGF antagonism reduced edema formation and tissue damage following MCAO in mouse brain⁵⁴. In addition, signs of enhanced angiogenesis, including exacerbated endothelial cell activation and retinal hyper-vascularization, were observed in Notch1^{+/-}Notch3^{-/-} mice⁵⁵, a model of hereditary stroke disorder CADASIL. Furthermore, increased risk of ischemic stroke is associated with both type 1 and type 2 diabetes¹⁰⁶, a disease characterized by persistent and uncontrolled angiogenesis¹⁰⁷. Together, these studies suggest that the angiogenic property of pericytes may exert a dual role in ischemic stroke, depending on animal models, injury types, and timing.

Unlike in ischemic stroke, the angiogenic effect of pericytes is less studied in hemorrhagic stroke. Increased proliferation of pericytes and endothelial cells has been reported in mice with mural cell-specific deletion of Foxc1, which develop late-gestation cerebral micro-hemorrhages⁵⁶. Additionally, excessive angiogenesis and enhanced sensitivity to angiogenic stimuli were observed in hereditary hemorrhagic telangiectasia⁵⁷, a genetic disease characterized by arteriovenous malformations and brain hemorrhage. Furthermore, type 1 but not type 2 diabetes is associated with elevated risk of hemorrhagic stroke¹⁰⁶. These findings demonstrate a positive correlation between angiogenesis and hemorrhagic stroke. The exact role of the angiogenic effect of pericytes in hemorrhagic stroke, however, remains largely unclear.

Immunological Property of Pericytes in Stroke

Pericytes participate in CNS defense by exhibiting both innate and adaptive immune responses. Numerous studies conducted in both rodents and humans have shown that pericytes are able to respond to pro-inflammatory signals and release anti-inflammatory cytokines/chemokines. For instance, mouse brain pericytes constitutively produce chemokines and cytokines, including granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1 α , IL-6, monocyte chemoattractant protein-1 (MCP-1) and nitric oxide, under normal conditions^{108,109}. In lipopolysaccharide-induced inflammation, not only do the expression levels of these chemokines and cytokines change, many new factors (e.g. IL-5 and RANTES) are also induced^{108,109}. A recent study identified two different pericyte populations in the brain based on CD90 expression¹¹⁰. An *in vitro* study

showed that, when compared with CD90⁻ pericytes, CD90⁺ pericytes expressed lower levels of pericyte markers (e.g. SMA and PDGFR β) and extracellular matrix proteins, had higher basal proliferation, and exhibited a reduced pro-inflammatory response¹¹⁰. Consistent with previous reports^{10,16,111-119}, these results suggest that pericytes are a heterogeneous population, and that different subpopulations may have distinct biological functions.

There is also evidence showing that pericytes, like classical antigen-presenting cells (e.g. dendritic cells and macrophages), can express cell surface proteins, essential for the acquired immune system to recognize foreign molecules. For example, primary rat CNS pericytes constitutively express low levels of intercellular adhesion molecule-1 and major histocompatibility complex (MHC) class I molecules¹²⁰. Upon induction by interferon (IFN)- γ , they also express MHC class II molecules, which enable them to present antigens to primed syngeneic T-lymphocytes¹²¹. Like CNS pericytes, placenta- and human pluripotent stem cell-derived pericytes constitutively express MHC class I and costimulatory molecules under basal culture conditions¹²². Upon IFN- γ treatment, they start to express MHC class II molecules¹²². In addition, like microglia/macrophages, pericytes also exhibit phagocytic activity. It has been reported that human brain pericytes phagocytose erythrocytes in brain trauma¹²³. Consistent with this finding, cultured rat pericytes expressed macrophage markers ED-2 and CD11b, and phagocytosed fluorochrome-conjugated polystyrene beads and antibody-coated zymosan¹²⁰, suggesting both Fc receptor-independent and -dependent phagocytic activity. Together, these studies support that pericytes have immune cell-like properties and are able to regulate immune responses.

Stroke is a neurological condition that involves a local inflammatory reaction and a plethora of immune responses in the brain. It has been speculated that pericytes might transform into microglia/macrophage-like cells in response to CNS injuries, including stroke⁵⁸. Recently, a few studies provide evidence that brain pericytes acquire a microglia-like phenotype after ischemic stroke. First, it has been shown that RGS5 (regulator of G-protein signaling 5)-expressing cells, which are predominantly pericytes¹²⁴⁻¹²⁶, leave blood vessel wall, proliferate and give rise to CD11b⁺ and galectin-3⁺ microglia-like cells after ischemic injury in rodents⁵⁹. Second, under oxygen glucose deprivation, which mimics ischemic injury *in vitro*, human brain pericytes acquire stemness and differentiate into cells of various lineages, including microglia⁶⁰. Similarly, stemness is also detected in pericytes isolated from ischemic regions of the mouse brain^{60,62}, suggesting that ischemia induces multi-potency in pericytes. Additionally, Iba1⁺ microglia also express PDGFR β in ischemic brains⁶¹, suggesting that some microglia may be derived from multi-potent pericytes after ischemia. Consistent with these findings, PDGFR β ⁺ pericytes isolated from ischemic but not non-ischemic brains differentiated into microglia-like cells and obtained phagocytic activity⁶¹. Together, these studies strongly suggest that

pericytes can differentiate into microglia-like cells and exert microglia-like functions under ischemic conditions.

Whether pericytes are able to differentiate into microglia-like cells and exert microglia-like functions after hemorrhagic stroke remains unclear. Future studies should focus on investigating pericyte's stemness in hemorrhagic stroke and other pathological conditions.

Pericytes and Scar Formation and Fibrosis in Stroke

Upon CNS injury, glial cells become activated and contribute to the formation of a glial scar around the injury site by depositing chondroitin sulfate proteoglycans, including neurocan and phosphacan^{127–131}. Scar tissue functions to prevent the spreading of toxic substances in the CNS^{132,133}. However, excessive or long-lasting scar formation inhibits axon regeneration and stalls the recovery process, leading to fibrosis^{133–135}.

Recent studies show that pericytes also contribute to scar formation and organ fibrosis. In a spinal cord injury model, pericytes are categorized into type A and type B based on glutamate aspartate transporter (GLAST) expression, and GLAST⁺ type A pericytes are identified as the major cell type that forms a glial scar¹³⁶. In addition, pericytes are also classified into type I (Nestin-GFP⁺/NG2-DsRed⁺) and type II (Nestin-GFP⁺/NG2-DsRed⁺), which generate adipocytes/fibroblasts and neural/myogenic cells, respectively^{137,138}. Type I pericytes have been shown to accumulate and participate in scar formation in multiple organs after injury, including the kidney, myocardium, spinal cord, and brain¹³⁹. In ischemic injury, PDGFRβ⁺ pericytes have been shown to trigger fibrotic responses in both kidney and CNS^{63,140}. Compared with the controls, PDGFRβ^{+/-} mice demonstrated decreased fibrosis with reduced fibronectin deposition in the ischemic area and enlarged infarct volume, suggesting that PDGFRβ signaling-induced fibronectin production is indispensable for the repair processes after ischemic stroke⁶³. This finding is consistent with the neuroprotective role of fibronectin in CNS injury^{141,142}. Currently, it is unclear whether and how pericytes affect scar formation and fibrosis after hemorrhagic stroke. Answers to these questions may lead to innovative pericyte-based therapies for stroke.

Therapeutic Implications and Future Directions

With the increase of knowledge in pericyte biology/function, therapeutic strategies utilizing/targeting pericytes have been developed and tested. For example, in an acute myocardial infarction model, transplantation of human microvascular pericytes (CD146⁺CD34⁻CD45⁻CD56⁻ cells) has been shown to attenuate ventricular dilatation, improve cardiac contractility, reduce myocardial fibrosis, and significantly diminish infiltration of host inflammatory cells at the injury site¹⁴². More importantly, these pericytes demonstrated a much better therapeutic effect when compared with CD56⁺

myogenic progenitor cells¹⁴². Based on the finding that human brain pericytes can be reprogrammed to neuronal cells by co-expression of SOX2 and MASH1¹⁴³, it is logical to hypothesize that neuronal reprogramming of pericytes may replace degenerated/damaged neurons and have a therapeutic potential¹⁴³. In addition, drugs targeting pericytes or modulating their activities also show promising results in treating stroke. In an experimental intracerebral hemorrhage model, administration of recombinant ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin type I motif, member 13) led to enhanced pericyte coverage, attenuated BBB leakage, reduced inflammation markers, ameliorated cerebral edema, diminished hematoma volume, and improved neurological functions¹⁴⁴. In an ischemic model, Cilostazol, an antiplatelet drug, showed a neuroprotective effect by preventing pathological detachment of astrocytic endfeet¹⁴⁵. The same drug also exerted a beneficial role in a collagenase-induced hemorrhage model by enhancing pericyte coverage in the brain¹⁴⁶. Additionally, the free radical scavenger edaravone has been shown to ameliorate brain damage after MCAO via increasing pericyte proliferation and their coverage around endothelial cells⁸⁰.

Although significant progresses have been made on how pericytes regulate stroke pathogenesis, a few critical questions need further investigation. First, no pericyte-specific markers are available currently, although various markers have been used to identify pericytes¹⁰. It should be noted that the 'pericyte' populations described in most studies also contain other cells. For example, PDGFRβ⁺ cells include both pericytes and SMCs^{10,25,147}, whereas RGS5 labels pericytes, SMCs and possibly cardiomyocytes^{10,125,126}. Next, pericytes are a heterogeneous population¹⁰. It is speculated that different subtypes of pericytes may exert distinct roles in stroke. The marker expression and function of these subpopulations remain largely unknown at present. Future work should focus on identifying pericyte-specific & subtype-specific markers and characterizing subpopulations of pericytes. Third, compared with ischemic stroke, hemorrhagic stroke is relatively less studied. The biological function and therapeutic potential of pericytes in hemorrhagic stroke remain largely elusive. Understanding this information will enable us to study pericytes in a cell type- and subtype-specific manner, which will promote the development of innovative and effective treatments for stroke.

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