Chapter 9
Pericytes in Ischemic Stroke

Turgay Dalkara, Luis Alarcon-Martinez, and Muge Yemisci

Abstract Recent stroke research has shifted the focus to the microvasculature from neuron-centric views. It is increasingly recognized that a successful neuroprotection is not feasible without microvascular protection. On the other hand, recent studies on pericytes, long-neglected cells on microvessels have provided insight into the regulation of microcirculation. Pericytes play an essential role in matching the metabolic demand of nervous tissue with the blood flow in addition to regulating the development and maintenance of the blood–brain barrier (BBB), leukocyte trafficking across the BBB and angiogenesis. Pericytes appears to be highly vulnerable to injury. Ischemic injury to pericytes on cerebral microvasculature unfavorably impacts the stroke-induced tissue damage and brain edema by disrupting microvascular blood flow and BBB integrity. Strongly supporting this, clinical imaging studies show that tissue reperfusion is not always obtained after recanalization. Therefore, prevention of pericyte dysfunction may improve the outcome of recanalization therapies by promoting microcirculatory reperfusion and preventing hemorrhage and edema. In the peri-infarct tissue, pericytes are detached from microvessels and promote angiogenesis and neurogenesis, and hence positively effect stroke outcome. Expectedly, we will learn more about the place of pericytes in CNS pathologies including stroke and devise approaches to treat them in the next decades.

T. Dalkara (✉)
Department of Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Institute of Neurological Sciences and Psychiatry, Hacettepe University, Ankara, Turkey

Department of Radiology, Massachusetts General Hospital, Harvard University, Boston, MA, USA
e-mail: tdalkara@hacettepe.edu.tr

L. Alarcon-Martinez
Department of Neuroscience and Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Université de Montréal, Montréal, QC, Canada

M. Yemisci
Department of Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Institute of Neurological Sciences and Psychiatry, Hacettepe University, Ankara, Turkey

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Introduction: Pericytes and Stroke

Pericytes play a role in matching the metabolic demand of nervous tissue with the blood flow in addition to regulating the development and maintenance of the blood–brain barrier (BBB) (Abbott et al. 2006; Armulik et al. 2011; Attwell et al. 2010; Daneman et al. 2010; Iadecola 2004), leukocyte trafficking across the BBB (Proebstl et al. 2012; Dohgu and Banks 2013; Stark et al. 2013; Leick et al. 2014) and angiogenesis (Ozerdem and Stallcup 2003; Gerhardt and Betsholtz 2003; Díaz-Flores et al. 2009). Ischemic injury to pericytes on cerebral microvasculature unfavorably impacts the stroke-induced tissue damage and brain edema by disrupting microvascular blood flow and BBB integrity, whereas ischemia-triggered signaling in pericytes on the vasculature within the peri-infarct area positively effect stroke outcome by promoting post-stroke angiogenesis and neurogenesis (Ozerdem and Stallcup 2003; Gerhardt and Betsholtz 2003; Zhang et al. 2012).

CNS Pericytes

Pericytes are present on almost all microvessels in the body; however, their density is highest in the CNS and retina in accordance with their role in fine regulation of the microcirculatory blood flow and maintenance of the blood–brain/retina barrier (Frank et al. 1987; Shepro and Morel 1993; Winkler et al. 2011a; Armulik et al. 2011). Pericytes are located on pre-capillary arterioles, capillaries, and post-capillary venules (Sims 1986; Dore-Duffy and Cleary 2011a; Armulik et al. 2011) (Fig. 9.1a, b). Unlike smooth muscle cells (SMCs), pericytes are embedded within two layers of basement membrane (Shepro and Morel 1993). Adjoining membranes of the neighboring pericytes are interconnected with gap junctions, serving as a communication pathway along the microvascular wall (Peppiatt et al. 2006; Hamilton et al. 2010). Pericytes extend processes around microvessels, which are largely circumferential at the arteriole side of the microvascular bed and at branching points, more longitudinal in the middle of the capillary bed, and have a stellate morphology at the venular side (Fig. 9.1b) (Hartmann et al. 2015). Pericytes are structurally plastic cells (Berthiaume et al. 2018) and their morphology and protein expression vary along the course of microvasculature, presumably to accommodate differing functions (Nehls and Drenckhahn 1991; Armulik et al. 2011; Dore-Duffy and Cleary 2011a; Hill et al. 2015; Hartmann et al. 2015; Jung et al. 2018). Pericytes are heterogeneous in their origin (Dias Moura Prazeres et al. 2017). Several transitional forms are observed along the vascular bed at various developmental stages or after pathological stimuli (Sims 1986; Dore-Duffy and Cleary 2011a; Armulik

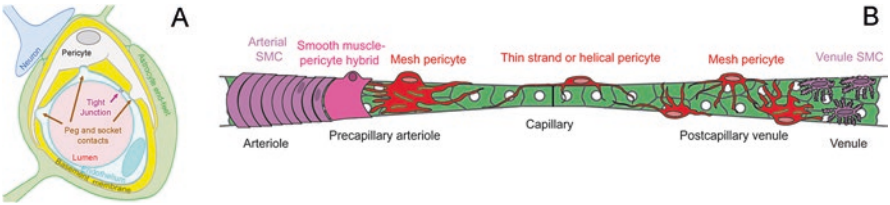

Fig. 9.1 Neurovascular unit and pericytes. (**a**) The neurovascular unit is composed of the endothelia and tight junctions between them, pericytes, the basal lamina encircling endothelia and pericytes, and astrocyte endfeet surrounding the microvessel. Note the peg and socket type contacts between endothelia and pericytes (Reproduced from Dalkara and Alarcon-Martinez 2015 with permission). (**b**) Pericyte processes are highly varied with shapes ranging from thin singular strands that run parallel to the microvasculature to more complex mesh processes that enwrap the entire vessel lumen. Pericytes located closer to the arteriolar end of the microcirculation exhibit more circular processes that may be essential to their contractile function (Reproduced from Hartmann et al. 2015 with permission)

et al. 2011; Sharma et al. 2012). The transition from smooth muscle cells to pericytes is not sharp. Smooth muscle-pericyte "hybrid" cells precede the prearteriolar pericytes having mesh-like circular processes (Sims 1986; Hartmann et al. 2015). Pericytes that give out more circumferential processes express more α-SMA, when assessed either with immunohistochemistry of brain sections ex vivo (Nehls and Drenckhahn 1991) or in mice cortex expressing reporter dyes under control of the α-SMA promoter in vivo (Hill et al. 2015; Hartmann et al. 2015). Detection of α-SMA in pericytes has been a controversial issue because of the some technical drawbacks that were missed in the methods used. First of all, It should be noted that reporter dyes expressed under the control of α-SMA promoter are membrane-bound, and therefore basically label the pericyte membrane, whereas immunohistochemistry directly detects the α-SMA protein (mainly in the cytoplasm of the soma and processes). However, the detection of the small pool of α-SMA in their relatively short processes by immunohistochemistry requires rapid fixation before α-SMA depolymerises during tissue processing (Alarcon-Martinez et al. 2018) (Fig. 9.2), whereas low level of α-SMA expression could be difficult to visualize due to dispersion of the limited amount of reporter fluorescent protein diffused over the large surface area of the pericyte membrane (Hill et al. 2015). Of note, α-SMA expression in pericytes is readily induced by tissue injury such as trauma, ischemia and injections (Dore-Duffy et al. 2000; Bai et al. 2018; Alarcon-Martinez et al. 2018).

Pericytes Regulate Microcirculatory Blood Flow in CNS and Retina

Functional hyperemia is an essential phenomenon in CNS by which oxygen and nutrients are supplied to tissue in accordance with metabolic demand generated by neuronal activity (Attwell et al. 2010). This tight pairing between the neural firing and blood flow, named neurovascular coupling, is provided by the neurovascular

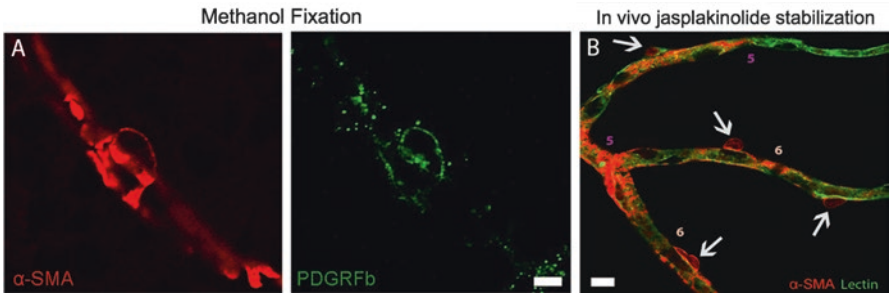

Fig. 9.2 Capillary pericytes express α-SMA. (**a**) Rapid fixation with methanol allowed visualization of α-SMA expression (red) in a deeper plexus capillary pericyte by preventing depolymerization of a small pool of α-SMA during tissue processing. This mural cell was also immunopositive for the pericyte marker PDGFRβ (green). Scale bar: 5 μm. (**b**) Stabilization of F-actin with intravitreal injection of Jasplakinolide before sacrificing the mouse disclosed α-SMA immunolabeling (red) on a sixth order retinal capillary visualized with lectin (green). Arrows point to pericyte somas and numbers indicate the branch order. Scale bar: 10 μm. (Reproduced from Alarcon-Martinez et al. 2018 with permission)

unit composed of neurons, astrocytes, vascular endothelia, smooth muscle cells, and pericytes (Iadecola 2004; Attwell et al. 2010; Dalkara and Alarcon-Martinez 2015). The neurovascular unit shows structural and functional differences between capillaries, which are covered by pericytes that respond to local activity in immediate vicinity and the intra-parenchymal arteries/arterioles, which are wrapped by smooth muscle cells and regulated by signals coming from a larger cohort of neurons as well as distal microvasculature (Hall et al. 2014; Dalkara and Alarcon-Martinez 2015; Biesecker et al. 2016; Mishra et al. 2016; Kisler et al. 2017). Both smooth muscle cells and pericytes contain α-SMA; a contractile protein that mediates the vascular diameter changes associated with neurovascular coupling (Herman and D'Amore 1985; Kelley et al. 1987; Hall et al. 2014; Alarcon-Martinez et al. 2018). The capability of pericytes to change vascular diameter in response to neural activation has been debated because blood flow is regulated at the level of arterioles in the peripheral circulation where a very focal flow regulation is not required unlike the CNS and retina. Failure of some laboratories to detect α-SMA in capillary pericytes strengthened these reservations. However, it has recently been proposed that this failure was caused by rapid depolymerization of small pool of α-SMA in capillary pericytes during slow tissue fixation with paraformaldehyde because rapid fixation methods disclosed that even small pericytes on high order capillaries expressed α-SMA (Alarcon-Martinez et al. 2018) as originally shown by Herman and D'Amore (Herman and D'Amore 1985). By using short interfering RNA, Alarcon-Martinez et al. readily suppressed α-SMA expression in distal capillary pericytes, but not in upstream larger microvessels where α-SMA is more abundant, supporting the view that the histological detection of the small pool of α-SMA in capillary pericytes is challenging compared to the α-SMA-rich pericytes on pre-capillary arterioles and vascular smooth muscle cells (Alarcon-Martinez et al. 2018). Indeed, as reviewed in detail by Díaz-Flores et al. (2009), the pericyte contractility is supported by several lines of evidence including their characteristic morphology with

processes that envelop the microvessels as well as ultrastructural and immunohistochemical demonstration of contractile proteins (Wallow and Burnside 1980; Herman and D'Amore 1985; Joyce et al. 1985a, b; Fujimoto and Singer 1987; Kelley et al. 1987; Das et al. 1988; Nehls and Drenckhahn 1991; Shepro and Morel 1993; Allt and Lawrenson 2001; Bandopadhyay et al. 2001) in addition to the presence of receptors for vasoactive mediators on their surface (Peppiatt et al. 2006; Puro 2007; Hamilton et al. 2010). In vitro studies on cerebellar, cerebral, and retinal slices or on isolated microvessels or cultured pericytes and recent in vivo studies have disclosed that pericytes are indeed capable of contracting or dilating in response to vasoactive mediators and physiological stimuli (Herman and D'Amore 1985; Kelley et al. 1987; Peppiatt et al. 2006; Puro 2007; Fernandez-Klett et al. 2010; Hall et al. 2014; Biesecker et al. 2016; Mishra et al. 2016; Kisler et al. 2017). A recent in vivo study showed that cortical capillaries dilated before arterioles during sensory stimulation, supporting the view that microvascular blood flow in the CNS is regulated by pericytes in response to the very focal demand originating from a small group of nearby cells as a final step of flow regulation after the arterioles, which serve a larger cohort of cells (Hall et al. 2014). This flow regulation with fine spatial resolution may be essential for tissues with high functional specialization such as the brain and retina. However, it should be noted that all microvascular pericytes are not contractile and proportion of the contractile ones may vary with the tissue, species and developmental stage as well as along the arteriovenous axis (Krueger and Bechmann 2010; Fernández-Klett and Priller 2015; Hill et al. 2015).

When Rouget discovered the pericytes in 1873, he proposed that they might have contractile capability and regulate microcirculatory blood flow because of their shape and position on microvessels (Rouget 1873). This hypothesis has been supported and elaborated by many in vitro and in vivo studies over the years. The capillary diameter changes during metabolic demand were recently proposed to be mediated by astrocytic calcium increase through ATP-gated channels and pericyte relaxation on release of PGE2 from astrocyte endfeet (Mishra et al. 2016). In line with the findings from CNS, Biesecker et al. showed that calcium signaling in Müller cell endfeet was sufficient to evoke capillary but not arteriole dilation in the retina (Biesecker et al. 2016). Kisler et al. showed that transgenic mice with a decreased number of pericytes had a deficient neurovascular coupling, reinforcing the importance of pericytes in blood flow regulation (Kisler et al. 2017). Moreover, during ischemia, it was shown in situ that pericytes constricted capillaries by calcium-induced α-SMA contraction, impairing microcirculatory re-flow after recanalization (Yemisci et al. 2009; Hall et al. 2014) (Fig. 9.3). Hill et al. observed that most of the mural cells on the first 4 order capillaries expressed α-SMA and contracted in response to physiological stimuli or ischemia; however, they named these cells as smooth muscle cells because they expressed α-SMA and reserved the name pericyte for only strand-like mural cells lacking α-SMA (Hill et al. 2015). An opinion article entitled "What is a pericyte?" discusses this unconventional definition of pericyte and point to the fact that Hill et al.'s findings in fact confirm previous reports demonstrating pericyte contractility under physiological and ischemic conditions, once pericytes are defined as first described by Zimmerman in 1923 (Attwell et al. 2016).

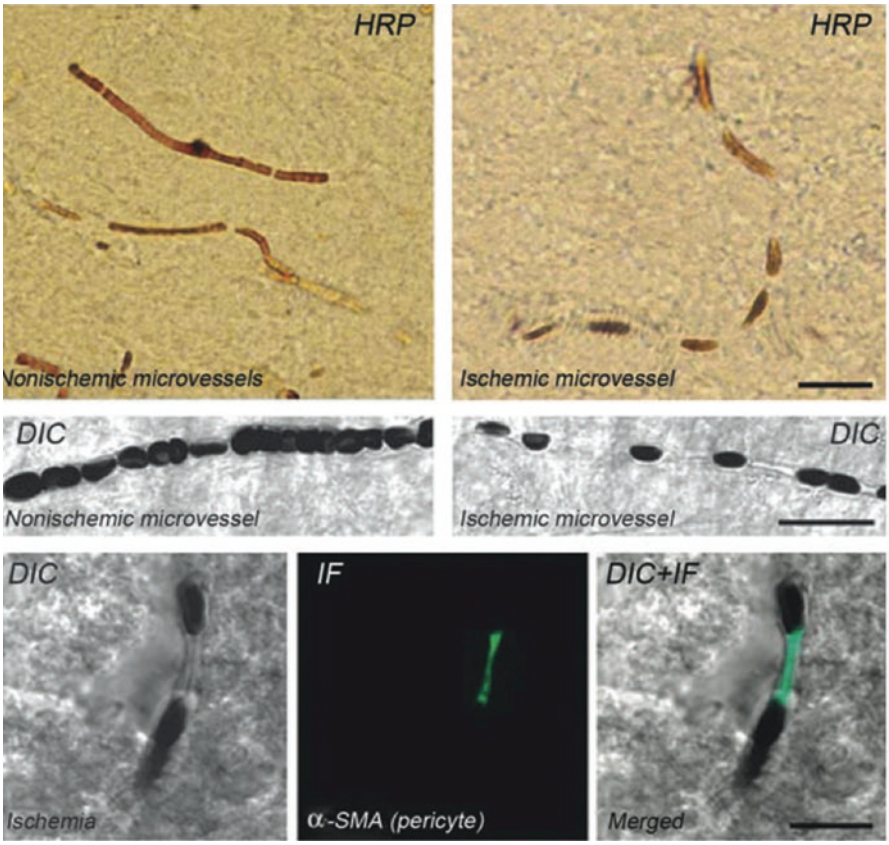

Fig. 9.3 Ischemia causes persistent pericyte contraction, which is not restored after complete recanalization of the occluded artery. Mice were subjected to 2 h of proximal MCA occlusion and intravenously injected with horseradish peroxidase (HRP) before decapitation 6 h after reopening of the MCA. HRP-filled microvessels exhibited sausage-like segmental constrictions in ischemic areas on brain sections (upper row). The differential interference contrast (DIC) microscopy images illustrate frequent interruptions in the erythrocyte column in an ischemic capillary contrary to a continuous row of erythrocytes flowing through an intact capillary (middle row). The constricted segments colocalized with α-smooth muscle actin (α-SMA) immunoreactive pericytes (bottom row). IF denotes immunofluorescence. Scale bar for upper and middle row, 20 μm; bottom row 10 μm (Reproduced from Yemisci et al. 2009 with permission)

Pericytes Are Vulnerable to Ischemic Injury

Pericyte contractility is regulated by intracellular Ca^{2+} concentrations (Kamouchi et al. 2004; Hamilton et al. 2010). The energy loss triggered by acute cerebral ischemia disrupts Ca^{2+} homeostasis and leads to an uncontrolled rise in intracellular Ca^{2+} in these metabolically dynamic cells (Hamilton et al. 2010). Calcium overload is likely to be potentiated by reactive oxygen species (ROS) (Kamouchi et al. 2007; Nakamura et al. 2009) coming from multiple sources during ischemia-reperfusion,

including mitochondria in pericytes, astrocyte endfeet and endothelia (Gürsoy-Ozdemir et al. 2004, 2012) and, ROS generating enzymes on the microvascular wall. Pericytes express high quantities of a major superoxide-producing enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) (Manea et al. 2005; Kuroda et al. 2014; Nishimura et al. 2016). This enzyme was shown to be upregulated in microvascular pericytes of the peri-infarct region in a MCAo stroke model, which contributed activation of metalloproteinase-9 and BBB breakdown (Vallet et al. 2005; Nishimura et al. 2016). Reactive oxygen and nitrogen species, and their reaction product, the potent oxidant peroxynitrite are intensely generated on the microvascular wall during ischemia and reperfusion, and constrict microvessels by contracting pericytes (Chan 1996; Yemisci et al. 2009; Gursoy-Ozdemir et al. 2012). Importantly, pericytes on microvessels remain contracted after recanalization of the occluded artery; therefore, the microcirculatory flow cannot completely be restored. The impaired reperfusion despite recanalization, known as the 'no-reflow' phenomenon, negatively affects post-stroke tissue survival (Hallenbeck et al. 1986; del Zoppo et al. 1991a; Yemisci et al. 2009; del Zoppo et al. 2011; Dziennis et al. 2015) (Figs. 9.3 and 9.4). Therefore, the experimental evidence still warrants pursuit of this goal (Diener et al. 2008; Amaro and Chamorro 2011; Gursoy-Ozdemir et al. 2012; Taskiran-Sag et al. 2018) despite failure of an antioxidant agent in clinical trials (Diener et al. 2008).

Incomplete Microcirculatory Reflow After Recanalization

An impaired tissue reperfusion after recanalization of an occluded artery or restoration of blood flow following circulatory collapse was first noted more than half a century ago and named as no-reflow phenomenon (Ames et al. 1968; Crowell and Olsson 1972). Unfortunately, some later studies measuring capillary patency with serum flow claimed that all capillaries were reperfused after restoration of blood flow (Theilen et al. 1993; Li et al. 1998). Recent studies with modern imaging techniques recording from intact mice brain clearly illustrated that fluorescently labeled serum continued to flow at the periphery of clogged capillaries (though slowly), creating the illusion that capillaries remained patent when only serum was monitored (Yemisci et al. 2009; Hill et al. 2015). Fortunately, interest in no-reflow phenomenon was re-kindled with studies on post-ischemic microcirculatory failure caused by leukocytes, platelets, fibrin and, recently, by pericytes (Hallenbeck et al. 1986; del Zoppo et al. 1991a; 2011; Zhang et al. 1999; Yemisci et al. 2009; Hall et al. 2014).

The impaired reflow emerges as a function of the duration and severity of ischemia, which varies between brain regions. Ten to twenty minutes of global ischemia is sufficient to induce no-reflow. For focal ischemia, proximal MCA occlusion in the mouse induces nodal microvascular constrictions that generally do not recover after recanalization starting 1 h after ischemia and affecting half of the microvessels within 2 h (Yemisci et al. 2009; Hill et al. 2015) (Fig. 9.3). Capillary constrictions

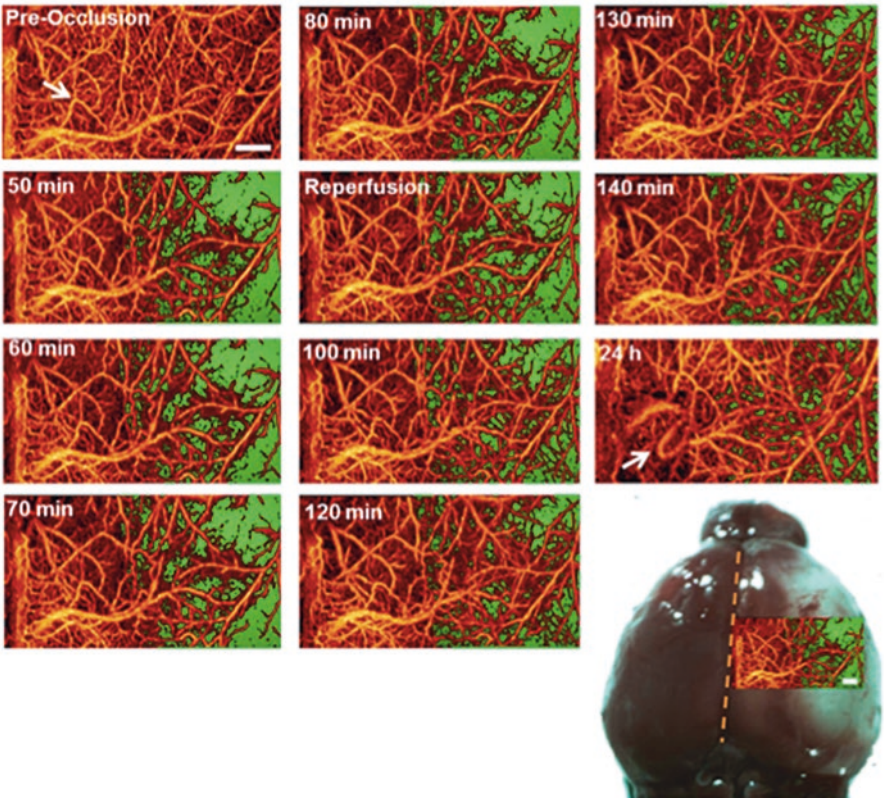

Fig. 9.4 Incomplete microcirculatory reflow after recanalization. Dynamic imaging of cortical blood flow using optical microangiography during 90-minute proximal MCA occlusion followed by recanalization illustrates the lack of microcirculatory blood flow in the MCA territory (the green area) during occlusion and its partial recovery after recanalization (incomplete microcirculatory reperfusion) in the mouse. Consecutive images are shown at 10-min intervals. Image size is 2.2×4.4 mm^2. The image in the lower right is the optical microangiography image taken at 50 min overlaid on the 24 h infarct analysis by histological staining as the area of pallor. Scale bar = 500 μm (Reproduced from Dziennis et al. 2015 with permission)

emerge earlier when focal ischemia was induced by photothrombosis, perhaps due to an additional injury induced by this method (Underly et al. 2017). Microvessel lumina at the constricted segments are filled with entrapped erythrocytes (RBCs), leukocytes, and fibrin-platelet deposits (Little et al. 1976; Hallenbeck et al. 1986; Garcia et al. 1994; Zhang et al. 1999; Morris et al. 2000; Belayev et al. 2002). RBCs are the predominant cell type in aggregates possibly because they are the most prevalent cells in circulation. In addition to the constricted segments observed at the arteriolar end of microcirculation and capillaries, leukocytes adhered to postcapillary venules for entering to the parenchyma also induce luminal aggregates together with fibrin and platelets (Belayev et al. 2002; Zhang et al. 1999; del Zoppo et al. 1991b; Ritter et al. 2000).

Experimental data strongly suggest that incomplete restoration of the microcirculatory blood flow negatively impacts tissue recovery even if reopening of the occluded artery is achieved within the time window when there is still salvageable penumbral tissue (Yemisci et al. 2009; del Zoppo et al. 2011; McCabe et al. 2018). Recent clinical data from large prospective studies that examined recanalization as well as tissue reperfusion concluded that reperfusion was essential to achieve good functional recovery such that a satisfactory reperfusion was 4 times stronger in predicting the outcome than recanalization or collateral status (Eilaghi et al. 2013; Cho et al. 2015; Catanese et al. 2017). Interestingly, reperfusion provided through collaterals was also associated with good clinical outcome even when recanalization could not be attained (Makris et al. 2019). Anti-thrombotic agents and genetic manipulations reducing microvascular clogging by inhibiting leukocyte adherence, platelet activation, or fibrin-platelet interactions have been shown to restore microcirculation and improve stroke outcome in animal models (Hallenbeck et al. 1986; Mori et al. 1992; Choudhri et al. 1998; Belayev et al. 2002; Ishikawa et al. 2005). Current guidelines, however, do not recommend anti-thrombotic medication use in patients undergoing recanalization therapies because of increased risk of hemorrhage (Powers et al. 2018). Interestingly, adenosine-squalene nanoparticles have been shown to improve microcirculation by relaxing contracted pericytes during ischemia in mouse stroke models (Gaudin et al. 2014) (Fig. 9.5). Radiolabeling studies disclosed that adenosine nanoparticles did not enter the brain parenchyma but provided neuroprotection by improving microcirculation with slowly released adenosine in endothelia. Importantly, the neuroprotection was also obtained with other BBB-impermeable agents such as L-N^5-(1-iminoethyl)-ornithine (L-NIO) and 2-sulfo-phenyl-N-tert-butyl nitrone (S-PBN), strongly supporting the idea that restoring microvascular patency can alone improve stroke outcome independently of parenchymal mechanisms (Yemisci et al. 2009; Gaudin et al. 2014). A recent study by simultaneously imaging ROS formation in the parenchyma and vasculature, demonstrated that S-PBN, a BBB-impermeable analog of the ROS scavenger PBN provided neuroprotection by improving microcirculatory reperfusion and then secondarily reducing parenchymal ROS formation without entering parenchyma (Taskiran-Sag et al. 2018). Consequently, restitution of the microcirculatory reperfusion emerges as an exciting target to improve the success rate of recanalization (Dalkara and Arsava 2012) and neuroprotection therapies (Gursoy-Ozdemir et al. 2012).

In the past, ischemia-induced capillary constrictions were thought to be caused by swollen astrocyte endfeet around microvessels (Little et al. 1976; Garcia et al. 1994). However, this idea is hard to reconcile with the nodal character of constrictions because the endfeet homogenously encircle capillaries, hence, should lead to an even narrowing of the lumen. The pericyte contraction-induced segmental constrictions fit better with these observations as pericytes are intermittently spaced along the microvessels (Yemisci et al. 2009; Dore-Duffy and Cleary 2011b; Hall et al. 2014; Alarcon-Martinez et al. 2018) (Figs. 9.2b and 9.3). Nomenclature disagreements in naming capillary mural cells notwithstanding, the important point for the stroke pathophysiology is that contractile cells on brain microvessels impede

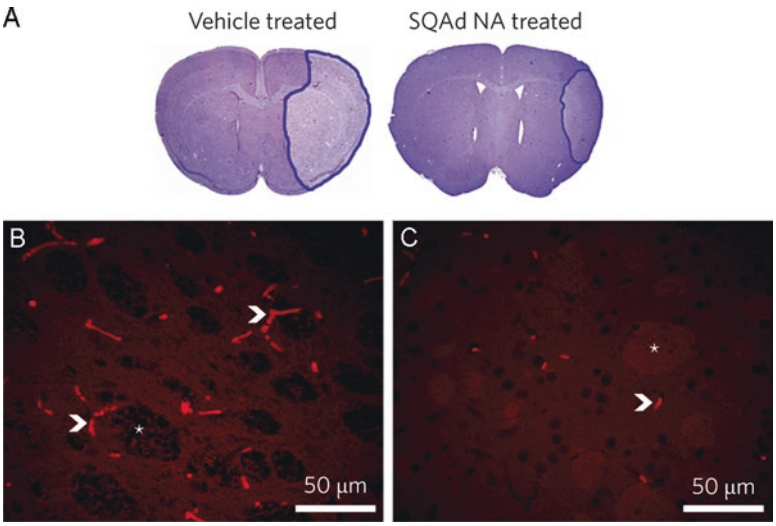

Fig. 9.5 Systemic administration of squalenoyl-adenosine (SQAd) nano-assemblies (NAs) provides significant neuroprotection in a mouse model of focal cerebral ischemia. (**a**) Infarct areas in control and treated mice subjected to transient (2 h MCAo and 22 h reperfusion) focal cerebral ischemia were identified by reduced Nissl staining under a light microscope (magnification ×10). (**b**) In untreated mice, capillaries in the ischemic brain were filled with trapped erythrocytes, whose hemoglobin was rendered fluorescent by treating brain sections with NaBH4 (**b**, red, arrowheads) 6 h after re-opening of the MCA following 2 h of occlusion, whereas the majority of capillaries were not clogged in SQAd nano-assemblies-treated mice (**c**). Unlike adenosine infusion, slow release from squalenoyladenosine nanoparticles did not cause cardiotoxicity or hypotension in the mouse model used. (Reproduced from Gaudin et al. 2014 with permission)

reperfusion after ischemia and unfavorably impact the outcome of recanalization. It should be noted that even small decreases in capillary radius caused by subtle pericyte contractions can lead to erythrocyte entrapments because capillary luminal size hardly allows passage of RBCs (Yemisci et al. 2009; Hamilton et al. 2010) (Fig. 9.3 middle row). Entrapped erythrocytes trigger platelet and fibrin aggregation by impeding passage of blood cells (Zhang et al. 1999; del Zoppo and Hamann 2011). The failure of erythrocyte circulation within some of the microvessels will lead to accelerated passage through the patent ones causing inefficient O_2 extraction (shunting). Modeling studies suggest that this increased heterogeneity of RBC transit times through patent capillaries (due to varying degrees of capillary resistances) can catastrophically reduce O_2 delivery to the tissue struggling to recover from ischemia-induced perturbations (Jespersen and Østergaard 2012). Since the plasma flow in constricted capillaries is relatively less restricted compared to RBC flux, glucose supply to some parts of the tissue may exceed O_2 supply and stimulate anaerobic glycolysis, hence, lactic acidosis (Yemisci et al. 2009; Dalkara et al. 2011) (please see supplementary movies mmc 5–7 in Hill et al. 2015). Current MR or CT techniques measure perfusion by detecting passage of contrast agent but not RBCs through the ischemic tissue, therefore, might be overestimating tissue

oxygenation. However, since plasma flow is related to capillary resistance, perfusion parameters based on the transit time may still be used to assess the microcirculatory reperfusion and its disturbances (Engedal et al. 2017). Distal embolization and re-occlusion are not uncommonly encountered during thrombolysis or endovascular therapies (Alexandrov and Grotta 2002; Janjua et al. 2008); however, microcirculatory failure appears as an independent factor than flow reduction due to occlusion at proximal sites and predicts tissue to be infarcted in recanalized as well as non-recanalized patients (Engedal et al. 2017). In line with these findings, our group recently showed that the presence of microcirculatory failure distal to the thrombus prior to attempting recanalization is an unfavorable prognostic factor for a satisfactory reperfusion and clinical outcome in acute ischemic stroke patients treated with clot retrievers (Arsava et al. 2018).

Pericytes and Post-Stroke BBB Leakiness

The BBB is fundamental for normal functioning of the CNS. The sealed endothelial cells by tight junction proteins, astrocyte endfeet, and extracellular matrix form the main physical barrier between the blood and CNS parenchyma. A close communication between the pericytes and endothelia as well as astrocytes is required for development and functioning of the BBB (Armulik et al. 2010; Daneman et al. 2010). Pericytes regulate the expression of tight junction proteins and inhibit transendothelial vesicular transport and immune cell extravasation into CNS (Armulik et al. 2010; Daneman et al. 2010; Sweeney et al. 2016). Thereby, pericytes play a critical role in vascular stability at the microcirculatory level such that the number of pericytes per endothelial cell and the surface area of the vascular wall covered by pericytes determine the relative permeability of capillaries (Winkler et al. 2011b; Armulik et al. 2011). Accordingly, pericyte dysfunction as well as deficiency causes increased BBB permeability (Armulik et al. 2010, 2011; Daneman et al. 2010; Winkler et al. 2011a)

Injury to pericytes during acute ischemia contributes to BBB breakdown, hence brain edema in the ischemic territory in addition to impairing microcirculation (Simard et al. 2007; Underly et al. 2017). Death of the damaged pericytes may further aggravate BBB breakdown at later hours along with other factors such as MMP activation (Hall et al. 2014; Underly et al. 2017; Neuhaus et al. 2017). However, in the peri-infarct areas, pericytes were shown to migrate from microvessels within 1 h following ischemia. This migration may be protective by providing guidance for peri-infarct angiogenesis, but also be detrimental as it could increase microvascular permeability by disrupting the interaction of pericytes and tight junctions (Kamouchi et al. 2011; Liu et al. 2012). In the long run, however, post-stroke angiogenesis and neurogenesis in peri-infarct area plays an important role in stroke outcome (Wang et al. 2004; Ergul et al. 2012; Zhang et al. 2012; Cai et al. 2017).

Diabetic patients are prone to cerebral hemorrhage. This could be due to dysfunctional microvascular pericytes, as suggested for diabetic retinopathy manifested

by retinal edema and hemorrhage (Wardlaw et al. 2009; Willard and Herman 2012; Desilles et al. 2013). Increased BBB permeability predisposes to intraparenchymal hemorrhage in about 5–6% of patients receiving recanalization treatments (Donnan et al. 2011). Diabetes is considered a risk factor for thrombolysis-related hemorrhage and adversely influences post-stroke recovery. These negative effects of diabetes are considered to be a reflection of microvascular dysfunction, and pericytes might play a central role in this. Most of the knowledge about diabetes and pericyte dysfunction comes from the observations in diabetic retinopathy (Ergul et al. 2014). The loss of pericyte coverage around retinal endothelial cells in diabetes has been shown to trigger pathological angiogenesis, endothelial cell apoptosis, and plasma leakage (Prakash et al. 2013; Ergul et al. 2014, 2015). Although the effects of diabetes on brain pericytes are not fully known, a decreased pericyte density has been reported within the cerebral microcirculation as well (Prakash et al. 2012). Experimental stroke in diabetic animals has led to an increase in hemorrhagic transformation after ischemia in diabetic mice (Ergul et al. 2007) and impairment in vascular repair mechanisms critical for neovascularization and angiogenesis (Prakash et al. 2013).

Interestingly, pericyte loss is increasingly reported for conditions that are risk factors for stroke, such as aging, hypertension as well as diabetes, the impact of which on stroke outcome needs to be clarified with future research (Østergaard et al. 2016). Cerebral pericytes in hypertensive animals show irregular profiles, associated with fragmentation of their processes and thickening in their basement membranes (Suzuki et al. 2003). These changes are reportedly led to decreased endothelial coverage by pericytes, capillary thrombotic occlusion, and luminal collapse (Suzuki et al. 2003). Capillary dysfunction induced by the above cerebrovascular disease risk factors has also been proposed to contribute to the risk of subsequent stroke and cognitive decline (Østergaard et al. 2016).

Post-Stroke Angioneurogenesis and Pericytes

Pericytes are essential, especially, for the early phase of neovascularization (angiogenic sprouting) (Ozerdem and Stallcup 2003; Gerhardt and Betsholtz 2003). Pericytes and endothelial cells communicate with each other for regulation of angiogenesis (Ozerdem and Stallcup 2003; Gerhardt and Betsholtz 2003; Díaz-Flores et al. 2009) (Fig. 9.6). Platelet-derived growth factor-β (PDGFβ), transforming growth factor-β (TGFβ), notch, angiopoietin and sphingosine-1-phosphate signaling, and the vascular endothelial grow factor and its receptor-2 (VEGF/VEGFR2) mediate this crosstalk (Gaengel et al. 2009; Armulik et al. 2011). Through those signaling pathways, pericyte may drive angiogenesis after stroke (Kokovay et al. 2006; Dore-Duffy et al. 2007; Beck and Plate 2009; Ergul et al. 2012; Zechariah et al. 2013a, b; Cai et al. 2017). First, endothelial cells start to proliferate and give off vessel sprouts 12–24 h after brain ischemia, leading to formation of new vessels in the peri-infarct region 3 days after ischemic injury

(Hayashi et al. 2003; Chopp et al. 2007; Beck and Plate 2009). Following a similar time course, the PDGFRβ expression is upregulated in pericytes, which increase in number and start migrating from the microvessel wall to the newly formed vessel sprouts to foster their maturation after ischemic injury (Takahashi et al. 1997; Dore-Duffy et al. 2000; Marti et al. 2000; Renner et al. 2003; Arimura et al. 2012; Dulmovits and Herman 2012). Renner et al. found that PDGFRβ increased in pericytes 48 hours after permanent ischemia (Renner et al. 2003). This upregulation of PDGFRβ in pericytes is proposed to be promoted by ischemia-induced increase in the basic fibroblast growth factor (bFGF) (Nakamura et al. 2016). Similarly, NG2+ or PDGFRβ+ pericytes are reportedly increased in peri-infarct areas 1–3 weeks after transient MCA occlusion (Fernández-Klett et al. 2013; Yang et al. 2013). A proportion of locally proliferating pericytes give rise to microglial cells (Özen et al. 2014). Corroborating these studies, conditional knockout of PDGFβ/PDGFRβ signaling in adult mice that have normally developed brain vasculature led to larger infarcts than controls when subjected to focal cerebral ischemia (Shen et al. 2012). Similarly, Zechariah et al. showed that pericytes did not appropriately cover the brain

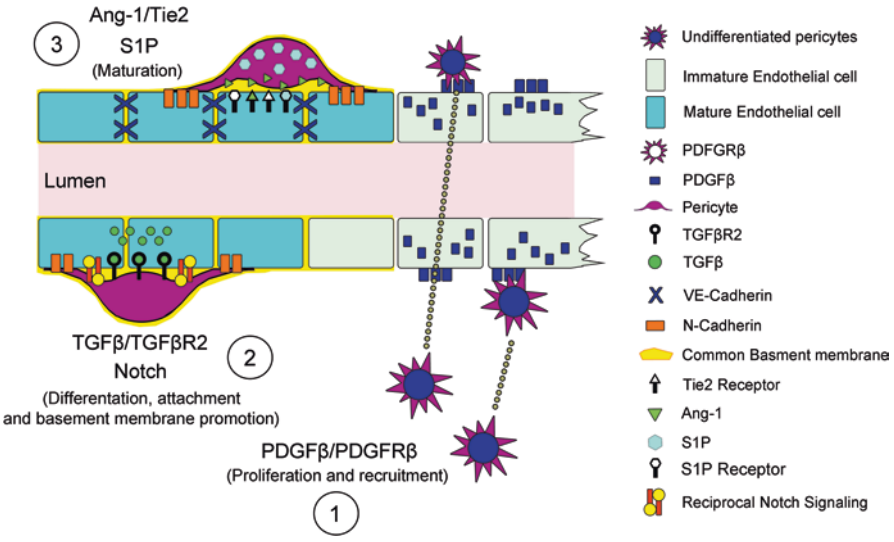

Fig. 9.6 Role of pericytes in angiogenesis. The interaction between PDGFβ secreted by the endothelium and its receptor localized on pericytes (PDGFRβ) is essential for recruitment of undifferentiated mesenchymal cells/pericytes to newly formed vessels. Once pericytes are at the vascular wall, reciprocal Notch signaling between the endothelia and pericytes as well as interactions between TGFβ secreted by endothelial cells and its receptor TGFβR2 located at pericytes differentiate mural cells and attach them to the newly formed vessels. The TGFβ/TGFβR2 interaction also promotes formation of the common basement membrane and stabilizes newly formed vessels by inhibiting endothelial proliferation. Ang-1, which is secreted by pericytes, activates its endothelial receptor Tie2 and promotes blood–brain barrier formation. Finally, S1P, whose receptor is abundantly expressed on pericytes down regulates genes related to vascular permeability and promotes both endothelial-endothelial (VE-cadherin) and pericyte-endothelial cell (N-cadherin) interconnections (Reproduced from Dalkara and Alarcon-Martinez 2015 with permission)

capillaries in hyperlipidemic mice exposed to ischemia, and this was associated with attenuation of post-stroke angiogenesis (Zechariah et al. 2013b). Moreover, after ischemic stroke, brain pericytes may also start to express other angiogenic signaling mediators. For instance, during hypoxia, pericytes begin to secrete VEGF (Dore-Duffy et al. 2007), which activates VEGFR2 in endothelial cells (Greenberg and Jin 2005; Beck and Plate 2009) and promote neovascularization in the peri-infarct region (Marti et al. 2000). Additionally, TGFβ, which is expressed in endothelial cells and pericytes (Gaengel et al. 2009), increased significantly in capillaries of the ischemic areas (Haqqani et al. 2005). Finally, following an ischemic injury, an upregulation of the angiopoietin and sphingosine-1-phosphate signaling pathway in the peri-infarct capillaries has been reported (Lin et al. 2000; Zhang and Chopp 2002).

Kokovay et al. showed that, following brain ischemia, bone marrow-derived cells with a pericytic phenotype and expressing angiogenic factors as VEGF and TGF-b were recruited to cerebral capillaries (Kokovay et al. 2006). Angiogenesis is also essential to promote neurogenesis after stroke (Palmer et al. 2000; Kamouchi et al. 2012; Nih et al. 2012). In fact, newly formed neurons are located near to the remodeled vessels (Okano et al. 2007), probably because vascular cells recruit and form a niche for neural stem cells (Palmer et al. 2000; Licht and Keshet 2015). Since pericytes express factors that can induce neurogenesis as well as angiogenesis, they may also be involved in post-stroke neurogenesis not only as neuroblast recruiters but also as a source of neural stem cells (Parent et al. 2002; Wang et al. 2004; Dore-Duffy and Cleary 2011b). After acute ischemic stroke in rodents, neurogenesis is activated within the subventricular zone (Parent et al. 2002). Recently, Wang et al. showed that PDGFR signaling was essential for the recruitment of neuroblasts formed at the subventricular zone to the infarct area after ischemic stroke (Wang et al. 2017). In line with this, in vitro studies have shown that the brain-derived pericytes have potential to differentiate into neurons in response to trophic factors (Dore-Duffy et al. 2006; Paul et al. 2012; Karow et al. 2012; Karow 2013). It has been reported that pericytes extracted from ischemic mouse brain regions expressed various stem cell markers or essential factors for reprogramming such as c-myc, Klf4, and Sox2 (Nakagomi et al. 2015). Similarly, Nakata et al. found that, after transient brain ischemia/reperfusion injury in the mouse, PDGFRβ+ pericytes were located within injured areas and commenced to expressed neural stem cell markers as nestin and immature neuronal markers as doublecortin (Nakata et al. 2017). In accordance with this, culture experiments showed that human brain pericytes under oxygen/glucose deprivation expressed not only pericyte markers as PDGFRb, NG2, or α-SMA but also Sox2 or Klf4 (Nakagomi et al. 2015). After examining post-stroke human brain tissue, Tatebayashi et al. also found the presence of nestin+ cells localized near blood vessels and co-expressing the pericytic markers α-SMA and NG2 (Tatebayashi et al. 2017). Finally, pericytes obtained from ischemic MCA tissue of adult animals or pericytes cultured under ischemic conditions also showed capability to differentiate to cells of neural as well as vascular lineage (Nakagomi et al. 2015).

Role of Pericytes in CADASIL

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the *NOTCH3* gene (Joutel et al. 1996). The protein encoded by the *NOTCH3* gene is expressed in pericytes and vascular SMCs. Studies in Notch3 transgenic mice expressing one of the human mutations showed that Notch3 aggregated around microvascular pericytes, leading to pericyte loss or reduced coverage of capillaries (Gu et al. 2012; Ghosh et al. 2015). These changes were associated with decrease in BBB impermeability and neurovascular dysfunction. In line with these findings, pericyte loss was also detected in skin and muscle biopsies of CADASIL patients (Dziewulska and Lewandowska 2012).

Future Trends and Directions

Recent research has clearly documented the important role of pericytes on microvascular physiology, especially in the brain and retina. Significant clues to the roles played by pericytes under several pathological conditions such as stroke, diabetic retinopathy, Alzheimer's disease, CADASIL have also been identified, creating novel targets for neuroprotection and restoring microvascular health. Expectedly, we will learn more about the place of pericytes in CNS pathologies and devise approaches to treat them in the next decades. It seems that it will be an exciting time for researchers interested in pericytes and microvasculature in health and disease.

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