17 Emerging Mechanism of Cell Death Caused by Stroke: A Role of Neurovascular Unit

Ryo Ohtomo and Ken Arai

Abstract

Stroke is one of the leading causes of death and even the survivors suffer from severe aftereffects. Although effective treatments have long been awaited, early therapeutic approaches focused on neuronal death had been insufficient due to the heterogeneous etiology of stroke. From the fact that brain function along with dysfunction arise from integrated interactions between a network of cellular components, conceptual structural unit, so-called "neurovascular unit" was proposed as a new paradigm for the investigation of stroke. Since then, variety of cell–cell and cell–extracellular matrix interactions have been discovered, which lead us to profound understanding of the pathophysiology of stroke. Besides neuronal damage, pathophysiology of stroke also consists of glial activation and transformation, vascular and blood–brain barrier alteration, and inflammatory reactions. Recent investigation shows that mediators of these reactions are not only detrimental but also could turn out to be beneficial for neurovascular repair in the chronic phase of the disease. In this chapter, we briefly overview the mechanisms of cell–cell interactions within the neurovascular unit under the normal conditions, and then discuss the crosstalk between different cell types during the acute and chronic phases of stroke.

17.1 Introduction

Stroke is one of the leading causes of death around the world. Even if stroke patients survive, they often suffer from devastating neurological deficits needing sufficient rehabilitation and medication for secondary prevention. For this reason, increasing number of stroke patients has been one of the main reasons of swelling medical expenses in developed countries for decades.

Until the late 1990s, various experiments had been carried out for the breakthrough of this situation, and their results brought us profound understanding of the pathophysiology of stroke. However, neuroprotective drugs that were developed based on the findings of these

R. Ohtomo · K. Arai (✉)
Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA
e-mail: rohtomo@mgh.harvard.edu; karai@mgh.harvard.edu

S.-H. Lee (ed.), *Stroke Revisited: Pathophysiology of Stroke*, Stroke Revisited,
https://doi.org/10.1007/978-981-10-1430-7_17

studies came up empty-handed in human trials. Under such circumstance, first meeting of Stroke Progress Review Group was convened by National Institute of Neurological Disorders and Stroke in 2001. In this workshop, based on the complex pathophysiology of stroke which involves multiple cell–cell interactions, failure of clinical trials was attributed to the narrowness of therapeutic target limited to neuronal death. Scientists emphasized that purely focusing on neurons is not sufficient, since brain function along with dysfunction arise from integrated interactions between a network of cellular components such as neurons, glia, and cerebral endothelium. This conceptual structural unit as a new paradigm for investigation of the central nervous system (CNS) was proposed as "neurovascular unit (NVU)" [1] (Fig. 17.1).

17.2 The Neurovascular Unit (NVU) and Its Components

The NVU is responsible for the regulation of blood flow through the vascular system. Large arteries on the surface of the brain separate into smaller arteries and arterioles known as pial arteries. Pial arteries are innervated by nerves from autonomic and sensory ganglia sending signals for constriction and dilation. These signals are considered to mediate global changes in cerebral blood flow (CBF).

In the parenchyma, the arterioles become closely associated with astrocytes, which play an important role in regulating diameter of the arterioles. As vessels continue to run deeper into the brain, they lose their smooth muscle cell and pia mater coverage, and gain pericytes between the vascular endothelial cells and astrocyte end-feet.

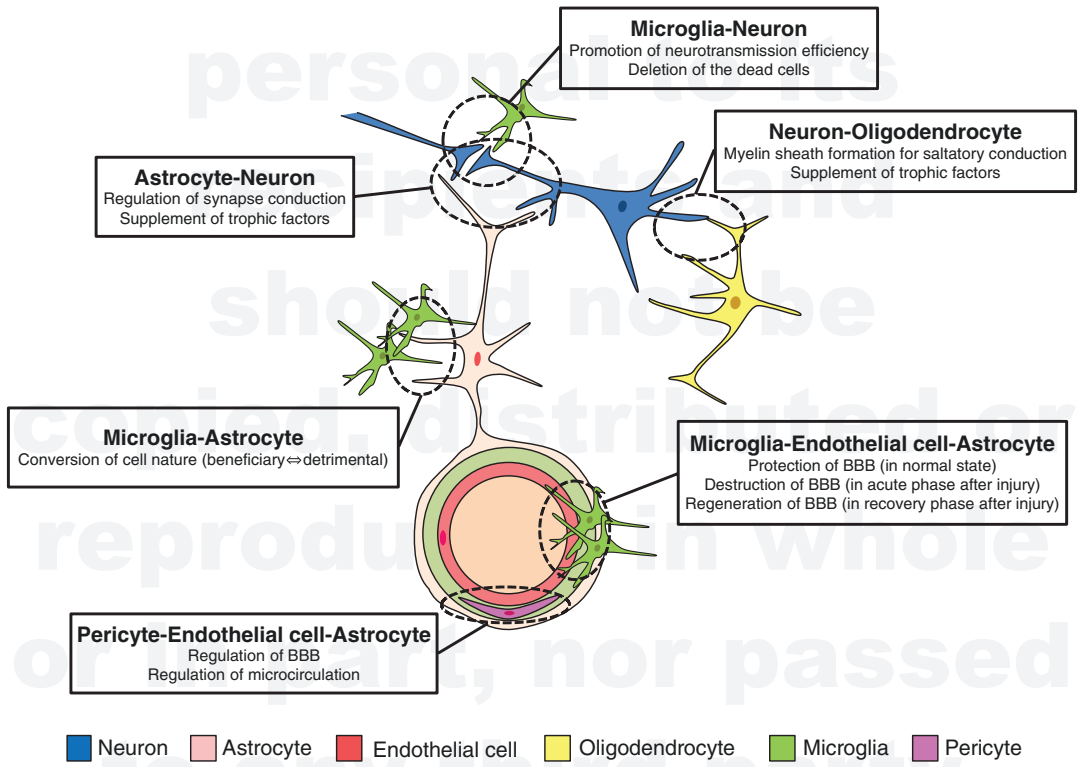

Fig. 17.1 Schematic of the neurovascular unit: Neuron, astrocyte, cerebral endothelium, oligodendrocyte, microglia, and pericyte compose the neurovascular unit. Cell–cell interactions between the NVU components are critical to maintain the brain function

Astrocytic end-feet occupy a larger surface area of the vasculature than neural processes at this depth, consistent with the major role of astrocytes in the regulation of vessel diameter. Along the length of the vasculature, neuronal and astrocyte processes contact with other NVU components, which regulate the function of the whole unit. In this section, we briefly describe individual components of the NVU and their roles inside the NVU.

17.2.1 Neurons

Neuronal processes physically contact with the vasculature, and mediate a local increase in CBF in response to the increased metabolic demand of the neurons. This mechanism is known as functional hyperemia. Vasoconstriction and dilation are thought to be conducted by the contractility of smooth muscle cells of the arterioles, and capillary pericytes responding to vasoactive substances released by neurons and astrocytes during synaptic activity such as metabolites of cyclooxygenase-2 [2], cytochrome P450 epoxygenases [3], acetylcholine [4], corticotropin-releasing factor [5], neuropeptide Y [6] nitric oxide [7], somatostatin [8], and vasoactive intestinal polypeptide [9]. Neurons are assumed to regulate regional CBF depending on the area of the brain and populations of neurons nourished by the vasculature, the presence of glial cells that mediate local stimuli, the magnitude and duration of neuronal activity, and the effects of brain insult that may influence any of the above factors. Association of neuronal processes with vasculature of the brain is crucial for the nascent stage of blood–brain barrier (BBB) as well as its maintenance. Vascular endothelial growth factor (VEGF) signaling is thought to regulate vascular patterning during development [10], and neural progenitor cells contribute to stabilizing the incipient vascular network [11]. It is speculated that neuronal activities continue to maintain the vascular network even after the developmental stage by supporting astrocytes which are the main regulators of cerebrovascular permeability [12].

17.2.2 Vascular Endothelial Cells

Vascular endothelial cells are the core anatomical component of the BBB, protecting the brain by limiting transcellular and paracellular transportation of pathogens and deleterious factors from cerebral vessels. No fenestrae are seen in vascular endothelial cells of the brain and transcytosis occurs at very low rate [13]. Tight junctions and adherens junctions formed between adjacent endothelial cells build a physical barrier that blocks paracellular diffusion of ions and molecules. Tight junctions are composed of combinations of integral membrane proteins such as claudins and occludins and cytoplasmic accessory proteins including ZO-1, ZO-2, ZO3, and cingulin that link these transmembrane proteins to the cytoskeleton of actin [14]. This builds up tight inter-endothelial seal with maximum of 1800 ohms/cm^2 trans-endothelial electrical resistance in vivo [15]. Besides physical barrier, vascular endothelial cells form a transport interface between the blood and the brain. The luminal as well as abluminal membranes of endothelial cells contain polarized transporters, receptors, ion channels, and metabolite-degrading enzymes, so that molecules such as amino acids, electrolytes, glucose, and nucleosides can be delivered to the brain from the blood, and efflux metabolite waste products and solutes to the opposite direction [16]. Endothelial cells nourish neighboring neurons by supporting the development of axons [17], by protecting them from stress, and by providing niche for supporting neural stem cells. This cell–cell signaling called "neurovascular niche," between the endothelial cells and neuronal precursor cells mediate and sustain ongoing neurogenesis and angiogenesis in adult brains [18]. Endothelial cells are also known to be supportive for oligodendrocyte lineage cells. Endothelial cells and oligodendrocyte precursor cells (OPCs) are speculated to provide an "oligovascular niche," wherein endothelium-derived growth factors facilitate the proliferation of OPCs [19]. This support is attenuated under certain pathologic conditions such as cerebral ischemia and traumatic brain injuries [20].

17.2.3 Astrocytes

Astrocytes are found throughout the brain constituting nearly half of brain cell population. They exhibit heterogeneous morphology that varies depending on cell populations they interact with. Traditionally, astrocytes have been considered to physically, biochemically, and metabolically support cells of the CNS. However, recent studies have revealed variety of its functions regarding the regulation of NVU. Individual astrocytes play an important role on the formation, function, and elimination of the synapses. They extend numerous processes to several neurons which can result in the formation of 140,000 synapses [21]. Synaptic formations and pre-/postsynaptic functions are promoted by transmitters secreted from astrocytes. Astrocytes possess many receptors like neurons. This enables neurotransmitters to activate calcium-based signaling cascades in astrocytes to release active substances which act back to neurons to regulate their activities. Each astrocyte has its own spatial domain which will not overlap with other astrocytes. However, they are closely interconnected with neighboring astrocytes by gap junctions for the promotion of long ranged signaling [22]. In the context of the NVU, astrocytes tune vascular tone and CBF through their fine processes that form close liaison with blood vessels and synapses [23]. When neuronal activity is enhanced, nearby astrocytes send signals about the need for a regional increase of CBF to blood vessels, directly through gap junctions or indirectly by releasing soluble factors. As mentioned previously, astrocytes regulate BBB. Scar-forming astrocytes play a pivotal role when the sealing of BBB injury is necessary. Historically, reactive astrocytes after brain injury were considered as detrimental, but can become beneficial under certain conditions. Reactive astrocytes produce pro-inflammatory cytokines and astroglial scar that are likely to inhibit axon regeneration. However, they can also support neurons through upregulation of the genes that induces synaptogenesis [24] or by the secretion of trophic factors. When reactive astrocytes were conditionally knocked down in mice, enlargement of the lesion with more inflammatory responses were observed after brain trauma [25]. Additionally, reactive astrocytes are reported release tissue-type plasminogen activator which promoted neuronal dendrite formation [26]. The dual function of reactive astrocytes after brain injury remains mostly unknown and awaits elucidation. As they are highly secretory in nature, astrocytes are also known to influence oligodendrocyte linage cells either positively or negatively by releasing multiple trophic factors. End-feet of the astrocytes cover 99% of the abluminal vessels, and express high levels of water channel proteins (aquaporin-4), which are assumed to play pivotal role for perivascular clearance mechanism called the “glymphatic system” [27].

17.2.4 Pericytes

Pericytes are mural cells buried within the basement membrane which surrounds the vessels. They extend thin processes along the (precapillary) arterioles, capillaries, and (postcapillary) venules [28]. Morphology of the pericytes is known to change according to their position in the vascular bed. They show diverse functions such as formation and maintenance of the vessels, clearance of cellular debris, and CBF regulation. Density of pericytes and population of the pericyte-covered endothelial cells are also known to vary among the organs. CNS has higher coverage of pericytes compared to other organs, covering approximately 30% of the abluminal surface. In areas without basement membrane, pericytes are able to communicate directly with endothelial cells through gap junctions and with other pericytes through peg-and-socket contacts [29]. Transduction cascades that include angiopoietin, Notch, PDGF-B, sphingosine-1 phosphate, and transforming growth factor (TGF)-β, are responsible for the functional coupling between pericytes and endothelial cells [30]. Angiogenic actions of pericytes are triggered by the expression of several matrix metalloproteinases (MMPs) and urokinase plasminogen activator receptor that enhance extracellular matrix degradation. These reactions remove mechanical inhibition of endothelial cell migration, and facilitate

the release of matrix-sequestered angiogenic factors [31]. Pericytes are known to secrete tissue inhibitor of metalloproteinase 3, which protects basement membrane proteins from degradation during the vessel stabilization phase. Maintenance of BBB is also one of their important roles. In vivo experiment which used mice with PDGFRβ signaling deficiency (shows deficits in embryonic pericyte recruitment) showed that loss of pericytes resulted in the increased permeability of BBB [32].

17.2.5 Microglia

Microglia are the resident immune cells of the brain which constitute nearly 10% of CNS glia. Unlike astrocytes, ependymal cells, and oligodendrocytes, microglial cells are mesodermal in origin. During early development, myeloid precursors are seeded throughout the brain and develop into cells with high plasticity and mobile capability. In contrast to other glial cells, microglia are not electronically coupled with syncytial network and retain their own surveillance territory [33]. During their native resting state, microglia have small cell bodies with numerous long and highly branching processes. Random scanning by their processes rapidly leads them even to tiny ruptures in the blood vessels. When microglia transform into amoeboid morphology under pathological condition, they become highly phagocytic, and start producing and secreting numbers of cytokines with soluble factors. These processes are thought to be assisted by neighboring astrocytes which release purinoreceptor ligands [34]. Activated microglia have heterogenic phenotypes. Two famous phenotypes would be M1 and M2 microglia. When microglia are challenged by the invasion of pathogens, M1 phenotype releases inflammatory mediators such as interferon (IFN)-γ, interleukin (IL)-1β and tumor necrosis factor (TNF)-α along with phagocytosis. On the other hand, M2 phenotype releases anti-inflammatory factors such as insulin-like growth factor (IGF)-1, IL-4, and IL-10 to remove apoptotic cells or myelin debris. However, in vivo, there is a broad range of activated microglial phenotypes that reflect the specific stimulus and the status of the surrounding cells that compose the NVU. For example, it is known that expression of CD4, FcγRII, and TNF-α mRNA in hippocampal microglia is higher than those from the cerebellum, cerebral cortex, diencephalon, and tegmentum [35]. Expression of Neurotrophin-3 is known to localize in microglia within the cerebral cortex, globus pallidus, and medulla. Recent studies showed that while microglia associated with inflammation attenuate neurogenesis, microglia activated by certain T cell-derived cytokines may promote neurogenesis [36]. Modulation of microglial polarization, which presumably could serve as an effective therapeutic tool for stroke, is currently under investigation.

In the NVU, there are also perivascular microglia/macrophages that originate from residing microglia of the CNS and monocytes from bone marrow circulating the vessels. Perivascular microglia couple with tip cells on sprouting vessels to facilitate angiogenesis during developmental stage. In the adult brain, perivascular macrophages are known to derive from circulating monocytes, and fight against pathogens in the very front line [37]. Perivascular macrophages maintain contact with other types of cells composing the NVU, and the crosstalk between these cells presumably contributes to the NVU function and dysfunction. Recent studies utilizing two-photon laser scanning microscopy showed that under certain pathological condition, parenchymal microglia could migrate to form perivascular cuffs that lead to vascular degradation and progression of the disease [38].

17.2.6 Oligodendrocytes

Although the NVU is relatively a well-accepted conceptual model for profound understanding of the phenomena occurring during brain injuries in the gray matter, cell–cell interactions are very important for white matter as well. Oligodendrocyte is one of the major types of cell found in the white matter. Lipid-rich myelin produced by oligodendrocytes enwraps axons and

enable saltatory conduction of electrical impulses. Myelin-forming oligodendrocytes are derived from OPCs which originate from subventricular neuronal stem/progenitor cells [39]. In the adult brain, OPCs can be found within the whole brain, comprising 5–8% of all cell components. It is known that most of the myelination process occurs early in life, and continues at least into late adolescence. In certain regions of the CNS, ongoing myelination may also be seen in adults. Experiments indicate that myelin in adult CNS may show some plasticity when stimulated by alternations in neural activities. For example, successful learning of juggling in human was shown to be correlated with an increased fractional anisotropy within the white matter underneath the intraparietal sulcus, suggesting that increase in myelination could occur in adult brains [40]. Rat experiment has also shown that environment modification could result in a detectable increase of myelin in adulthood [41].

Since axons are myelinated by matured oligodendrocytes, interactions between oligodendrocytes and neurons have been broadly examined. Oligodendrocytes are known to send signal to neurons through myelin–axon interactions. However, axon loss without severe demyelination was observed in mouse models with dysfunctional oligodendrocyte, implicating that oligodendrocytes could also support axon survival without through myelin sheath [42, 43]. It has been recently demonstrated that oligodendrocytes may serve as a principal supplier of lactate, which is indispensable for energy support of the axons [44]. Additionally, trophic factors such as IGF-1 and glial cell-derived neurotrophic factor (GDNF) released from oligodendrocytes have been shown to facilitate survival of neurons and outgrowth of axons in vitro [45]. Meanwhile, axonal activities, axon-secreted molecules, and axonal surface ligands have been proposed to regulate differentiation and maturation processes of oligodendrocytes. Examples are: (1) Jagged ligands expressed in axons that send signal to OPCs through Notch pathway inhibiting their differentiation [46], and (2) PSA-NCAM or LINGO-1 which are also known as molecules that inhibit myelination [47, 48]. There is an experimental evidence that myelination is partly triggered by electrical activities of the axons in developmental stage. Study using a mouse model of remyelination has shown that OPCs derived from the subventricular zone were observed to receive synaptic input of remyelination [49]. This result suggests possible involvement of neuronal activity in remyelination process as well.

As mentioned above, interaction between the endothelial cells and oligodendrocytes in the "oligovascular niche" after brain injury presumably triggers angiogenesis and oligodendrogenesis in the white matter of adult brain. MMP-9 released from oligodendrocytes is proposed to promote vascular remodeling during the chronic phase after white matter injury [50]. Also in demyelinating diseases such as vascular dementia, leukodystrophy, and multiple sclerosis, OPCs are speculated to attempt remyelination in degenerated areas during the chronic phase [51]. Although ability of endothelial cells to promote oligodendrogenesis remains unknown, they have shown potential to facilitate migration and proliferation of OPCs in cell culture studies.

17.2.7 Basement Membranes

A specialized extracellular matrix is made up from secreted proteins to form the basement membrane between endothelial cells and pericytes, and between astrocytes and pericytes. Since pericytes cover the vasculature discontinuously, astrocytes and endothelial cells share single basement membrane in the discontinued area. Proteomic studies of rodents show that composition of extracellular protein in brain vasculature differs from those found in the peripheral vessels. Basement membrane protein composition varies even between the large and small vessels within the brain, indicating functional heterogeneity of NVU throughout the brain [52]. Major proteins of the basement membranes include numbers isoforms of extracellular matrix proteins such as collagens, fibrillins, fibronectins, laminins, and vitronectin. Cytokines, growth factors, enzymes responsible for the degradation and processing of MMPs, and proteins like lectins that bind to

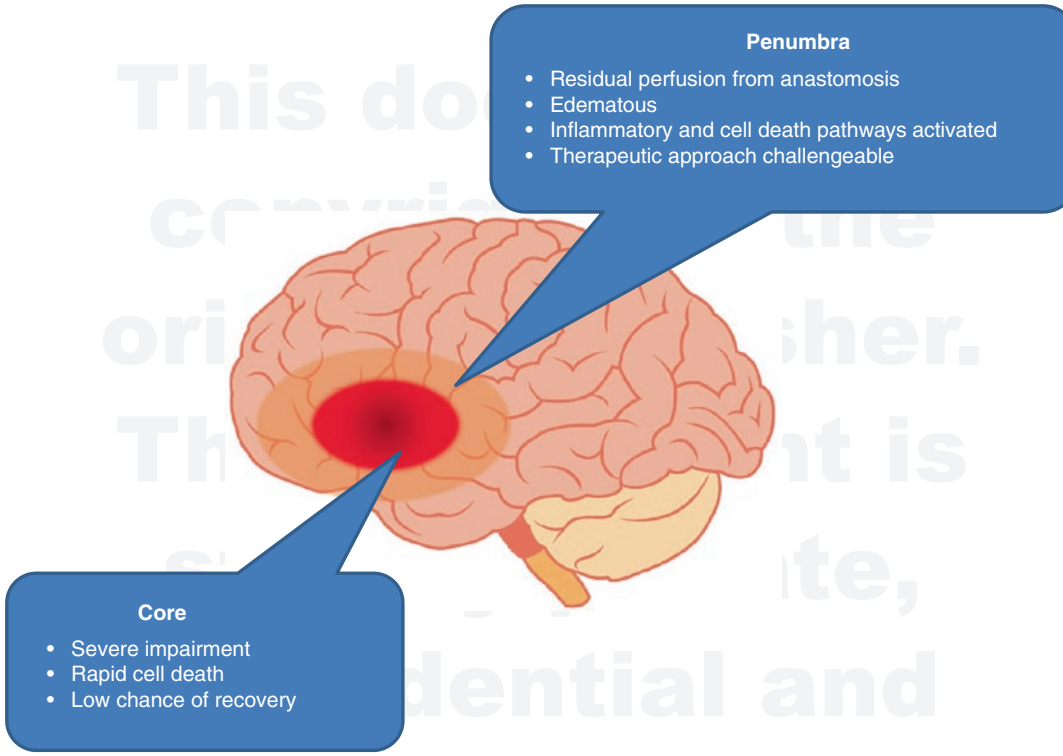

Fig. 17.2 Schematic of ischemic infarction: In the central core areas of stroke, blood flow deficits and/or hemorrhagic lesions are severe and brain cells die rapidly. On the other hand, in the peripheral areas (penumbra), it has been proposed that cell death, inflammation, and neurovascular perturbations proceed at a slower pace. Hence, theoretically the penumbra region can be a therapeutic target

extracellular matrix are also included. Extracellular matrix and protein components that support basement membrane are essential for the proper operation of NVU as they directly regulate signals from many activated receptors found on the cellular components of NVU.

17.3 Stroke Pathophysiology and NVU

Among the CNS diseases which have been studied in relation to the NVU, stroke is the most investigated type of disease, since its pathophysiology consists of fairly significant biphasic reactions of chemical mediators. In the acute phase after the onset of stroke, severe decline of CBF causes abrupt deprivation of nutrient supplies that leads to irreversible damage in the "core" of the affected area. During chronic phase, remodeling signal may occur within the semi-preserved area surrounding the "core" called "penumbra" (Fig. 17.2). Although precise mechanisms of post-stroke biphasic pathophysiology remain to be elucidated, in this section we will discuss some of the key phenomena that were identified to modulate the responses of NVU in acute and chronic phases of stroke.

17.3.1 NVU Dysfunction in Acute Phase of Stroke

In ischemic stroke, cells in the "core" of the lesion are exposed to rapid loss of adenosine triphosphate and energy stores due to severe decline of blood flow. As neuronal cell death within the "core" occurs within a few minutes, it is usually impossible to protect those neurons by medication. In hemorrhagic stroke, area surrounding the hema-

toma may also suffer from expanding edema and progressive inflammation, although molecular mechanisms are not well defined in comparison with ischemic stroke. Fundamental mechanism of neuronal death in the acute phase of stroke is complex. However, results of the experiments over the past three decades have implicated presumable involvement of excitotoxicity, oxidative stress, and, in some occasions, apoptotic-like pathways [53]. When the brain fails to generate sufficient adenosine triphosphate by reduction of CBF, energy failure and loss of ionic gradient occurs. Consequently, glutamate reuptake processes are impaired and excessive calcium entry and release are promoted by accumulation of glutamate. Degradation of cytoskeletal and enzymatic proteins by calcium-dependent proteases and synthases contribute to neuronal death. Calcium homeostasis abnormality also affects the neighboring cells by generating nitric oxide and peroxynitrite, which directly attack cells. When dysfunction of oxidative phosphorylation occurs in mitochondria, reactive oxygen free radicals are discharged to further eliminate cells by attacking nucleic acids, lipids, and proteins. Along with these pathways related to ions and free radicals, deleterious molecules such as caspases also accelerate cell death via apoptosis. Besides these mechanisms related to cell death, inflammatory cascade is triggered by upregulation of damage-associated molecular patterns (DAMPs) within few hours after the onset of stroke. HMGB1, heat shock proteins, and hyaluronic acid are included in DAMPs [54]. After being released from dead cells (passive pathway) and activated microglia and astrocytes (active pathway), DAMPs are captured by Toll-like receptors and other scavenger receptors which are broadly expressed in NVU component cells such as endothelial cells, microglia, and perivascular macrophages. Once this signaling is activated, mediators such as chemokines, cytokines, nitric oxide, and reactive oxygen species (ROS) are excreted from cells mentioned above leading to breakdown in BBB function [55]. DAMPs also upregulate adhesion molecules lining the endothelial cells such as ICAM-1, VCAM-1, and E-selectin, making it possible for circulating leukocytes to enter into brain parenchyma through loosened BBB. Homeostasis of BBB largely depends on interaction between endothelial cells, astrocyte, and extracellular matrix. Disruption of fibronectin, heparin sulfate proteoglycan, laminin, and type IV collagen break down signals between cells and extracellular matrix, and even between the cells that is needed for the function of NVU. Dysregulation of proteinases that contribute to proteolysis of extracellular matrix, and extracellular proteases occurs during the disease. Particularly, the MMP family has been known to cause neurovascular damage following stroke. Increased levels of MMPs were confirmed in both animal stroke models and stroke patients [56–60]. When excessive, MMPs take on deleterious activities by degradation of the extracellular matrix comprising the basal lamina, which damages the BBB directly. Inhibition of MMPs is found to reduce volume of infarction and edema in experimental models of stroke [61]. MMPs cause dysfunction of NVU also by inducing proteolysis of the extracellular matrix. This detaches cells from the extracellular matrix and leads to induction of anoikis [62]. These perturbations could be captured as failure of crosstalk between the NVU components. Lacking normal endothelial-astrocyte signaling may result in leakage of BBB. Signaling error between neurons and endothelium may interrupt hemodynamic coupling needed for active brain function. Improper neuron–glia signaling may affect release–reuptake kinetics of neurotransmitter and its transmission along the axons. Thus, focusing only on neuronal death will not be enough when thinking about therapeutic target for stroke. A truly effective therapy would be, if any, to protect all the functional interactions between the multiple types of cells which are the constituent of NVU. It is a disappointing fact that none of previous convincing discoveries toward the mechanisms of neuronal death during the acute phase of stroke have successfully provided benefits to stroke patients. Among all the translational barriers, heterogeneity of patients and very rapid cell–cell interaction after the onset of stroke make it difficult to suppress acute reactions efficiently. Therefore, recent studies have gradually shifted their focus to promoting recovery of the NVU in the chronic phase of stroke.

17.3.2 NVU Remodeling in Chronic Phase of Stroke

After the acute phase of stroke, patients present temporal recovery to some extent. Functional magnetic resonance imaging studies show that peri-infarcted regions retain plasticity [63], although cellular mechanisms underlying processes for recovery remain to be clearly elucidated.

Primary neurovascular responses during the recovery phase of stroke involve angiogenesis and neurogenesis. Since their molecular mechanisms are evolutionarily conserved, both phenomena share similar mediators and pathways. It is now a well-accepted fact that in adult brains, cell–cell signaling between the endothelium and neuronal precursor cells serves to mediate and to sustain pockets of active angiogenesis and neurogenesis. These interactions compose the "neurovascular niche," where soluble signals mediate crosstalk between the vascular and neuronal compartments. Endothelial cells in the brain release trophic factors to partly mediate this phenomenon. In subgranular and subventricular zones of the normal brain where neurogenesis is known to occur, the neurovascular niche mediates the complicated cell–cell signaling mechanism that takes place between endothelial cells and neural precursor cells. Maintenance of close relationship between angiogenesis and neurogenesis as mentioned above is essential for post-stroke recovery. When neuroblasts migrate through perivascular routes, along with the enhancement of vascular regeneration by promotion of neurogenesis, angiogenic stimulation adversely facilitates neurogenesis [64]. As rodent stroke model and human stroke samples show active angiogenesis within the peri-infarct regions [65, 66], recovery mechanism in the chronic phase of stroke is built on plasticity and remodeling processes controlled by mutually dependent neurovascular coupling that assemble various mediators and signals. Hence, our immediate goal for the treatment of stroke would be to discover medical therapies that can uplift endogenous signals and substrates needed for neurovascular remodeling, although how such approaches could be applied in daily clinical settings remains unclear. Notably, most of the candidate target molecules for stroke therapy act in biphasic manner during the course of stroke. As previously mentioned, in the early phase of stroke, MMPs trigger neurovascular dysfunction by breaking down BBB and inducing anoikis-like cell death. However, during the chronic recovery phase, the same mediators may become beneficial to neurovascular remodeling which includes angiogenesis and neurogenesis. In an experiment using mouse model of stroke, endothelial cells and glial cells were shown to proliferate in peri-infarcted areas secondary to MMP-9 elevation. What is more interesting is that mice with inhibited MMPs during the delayed phase resulted in worse outcomes [67]. Additionally, there was a co-localization of secondary MMP-9 signals with neuroblasts migrating from the subventricular zone, and here, inhibition of MMPs also blocked the transference of the neuroblasts toward the legion as well [68]. Mediators such as VEGF and HMGB1 can also play dual roles in neurovascular responses following stroke. While VEGF increases permeability of BBB in the acute phase, it facilitates angiogenesis and neurogenesis in the delayed recovery phase of stroke. HMBG1, which is normally present in the nucleus, is released in extracellular space under ischemic insult. As previously described, HMBG1 induces necrosis and accelerates the migration of destructive inflammatory cells during the acute phase of stroke. Conversely, during the delayed chronic phase, it mediates beneficial plasticity needed for recovery of the NVU. Dual role is also found in TNF-α, released from activated glial cells upon stimulation by the DAMP molecules. However, its dual mechanisms do not necessarily depend on timeline, and are thought to be affected by its target and secondary signals after the binding to corresponding receptors. Reactive oxygen species could be another example. Contrary to harmful events it may cause in the acute setting of stroke, it has been shown to mediate migra-

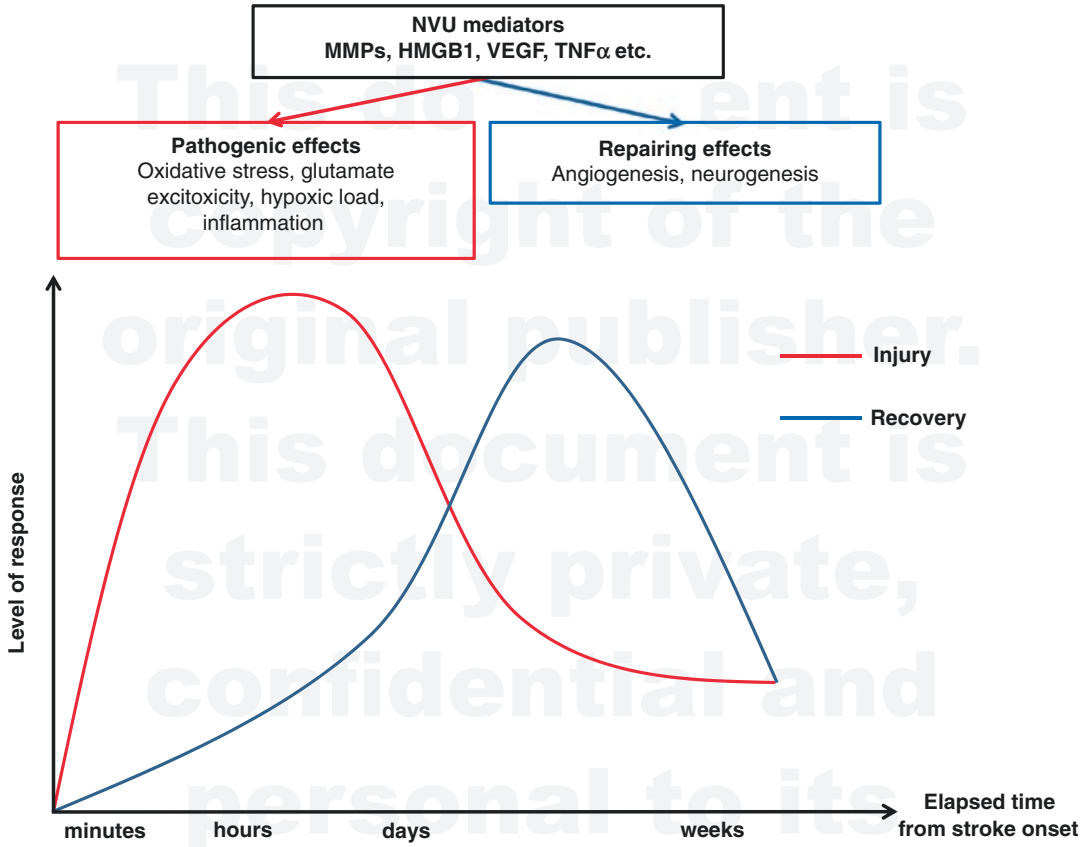

Fig. 17.3 Schematic to summarize the biphasic responses after stroke onset: In the acute phase, deleterious responses lead to NVU damage. On the other hand, remodeling signaling may emerge at the later time point. NVU mediators such as MMPs and VEGF would work in both phases, with opposite actions

tion of the OPCs essential for oligodendrogenesis [69]. Likewise, there are various mediators in the NVU with detrimental effects in the acute phase that turn out to be beneficial in the chronic phase (Fig. 17.3). Therefore, if we are to succeed in neurovascular protection of stroke patients, it is essential for us to consider when the acute phase of deleterious responses turns into recovering phase.

17.4 White Matter Injury

The concept of NVU has been mainly utilized for investigations of stroke pathophysiology occurring in the gray matter. However, since lacunar infarction is the most common type of ischemic stroke, knowledge about white matter damage is also clinically important for stroke treatment. In comparison with the cellular mechanisms of neurovascular coupling in the gray matter, pathophysiology of the white matter stroke still remains relatively unknown. As oligodendrocytes are one of the major component cells of the white matter, protection of oligodendrocyte lineage cells is of paramount importance when thinking about the treatment of stroke involving the white matter. From the notion that cell–cell trophic interactions are functioning in the white matter as well, the idea of NVU is now being applied to research of the white matter stroke. Neuronal axon, oligodendrocyte lineage cells (including myelinating oligodendrocytes and OPCs), endothelial cell, and astrocyte are the main component of the white matter. Astrocytes and endothelial cells collaborate to maintain BBB in white matter

as seen in the gray matter. Astrocytes are in close apposition to oligodendrocytes within the white matter, and interact directly via gap junctions for the maintenance of their functions [70, 71]. Soluble factors secreted from astrocytes are also reported to protect oligodendrocyte lineage cells from ischemic stress [72]. Needless to say, interaction between myelin and axon is critical for homeostasis of the white matter. Oligodendrocytes also maintain functional integrity and survival of axons through a myelin-independent manner by releasing trophic factors such as IGF-1 and GDNF. Just like the gray matter, activation of several deleterious factors and pathways takes place during the acute phase of stroke. Survival and normal functions of oligodendrocytes are affected by direct attack of MMPs to myelin components. Even if cells could avoid immediate death, metabolic dysfunction triggered by the assault would result in abnormal myelin replenishment and synthesis of myelin-related proteins, ultimately leading to impairment of myelin–axon coupling. Biphasic reactions of endogenous mediators are also known to occur in the course of white matter stroke, and several molecules are shown to work for repairing in the chronic phase. OPCs, which migrate from the subventricular zone to form myelin sheaths during development, are also distributed in the adult brain. It has been proved that some of these cells are guided to the lesion where remyelination is needed after the white matter injury [73]. Although precise molecular mechanism of migration after white matter stroke remains to be elucidated, oligovascular niche (corresponding to neurovascular niche within the gray matter) is speculated to play a crucial role in sustaining trophic interactions between the OPCs and vascular endothelial cells.

17.5 Conclusions

Ever since its birth, the concept of NVU has provided us with novel frameworks for the research of CNS diseases including stroke. Therapeutic target has shifted from “neuron-centric” to “neurons + surrounding atmosphere” where dynamic interactions of different cell types take part in function and dysfunction of the brain. Besides neuronal damage, pathophysiology of stroke also consists of: (1) glial activation and transformation, (2) vascular and BBB alteration, and (3) inflammatory reactions. These responses are detrimental in the acute phase, and sometimes turn out to be beneficial for neurovascular repair in the chronic phase, leading to the biphasic course of clinical manifestation. Investigating cellular mechanisms within the transitional zone between these two phases may give us a new hint for the development of effective treatments for stroke.

References

1. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci. 2003;4(5):399–415.
2. Niwa K, Araki E, Morham SG, Ross ME, Iadecola C. Cyclooxygenase-2 contributes to functional hyperemia in whisker-barrel cortex. J Neurosci. 2000;20(2):763–70.
3. Bhardwaj A, Northington FJ, Carhuapoma JR, Falck JR, Harder DR, Traystman RJ, et al. P-450 epoxygenase and NO synthase inhibitors reduce cerebral blood flow response to N-methyl-D-aspartate. Am J Physiol Heart Circ Physiol. 2000;279(4):H1616–24.
4. Scremin OU, Rovere AA, Raynald AC, Giardini A. Cholinergic control of blood flow in the cerebral cortex of the rat. Stroke. 1973;4(2):233–9.
5. De Michele M, Touzani O, Foster AC, Fieschi C, Sette G, McCulloch J. Corticotropin-releasing factor: effect on cerebral blood flow in physiologic and ischaemic conditions. Exp Brain Res. 2005;165(3):375–82.
6. Abounader R, Villemure JG, Hamel E. Characterization of neuropeptide Y (NPY) receptors in human cerebral arteries with selective agonists and the new Y1 antagonist BIBP 3226. Br J Pharmacol. 1995;116(4):2245–50.
7. Gotoh J, Kuang TY, Nakao Y, Cohen DM, Melzer P, Itoh Y, et al. Regional differences in mechanisms of cerebral circulatory response to neuronal activation. Am J Physiol Heart Circ Physiol. 2001;280(2):H821–9.
8. Cauli B, Tong XK, Rancillac A, Serluca N, Lambolez B, Rossier J, et al. Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways. J Neurosci. 2004;24(41):8940–9.
9. Yaksh TL, Wang JY, Go VL. Cortical vasodilatation produced by vasoactive intestinal polypeptide (VIP) and by physiological stimuli in the cat. J Cereb Blood Flow Metab. 1987;7(3):315–26.
10. Ruhrberg C, Bautch VL. Neurovascular development and links to disease. Cell Mol Life Sci. 2013;70(10):1675–84.

11. Ma S, Kwon HJ, Johng H, Zang K, Huang Z. Radial glial neural progenitors regulate nascent brain vascular network stabilization via inhibition of Wnt signaling. PLoS Biol. 2013;11(1):e1001469.
12. Lacoste B, Comin CH, Ben-Zvi A, Kaeser PS, Xu X, Costa Lda F, et al. Sensory-related neural activity regulates the structure of vascular networks in the cerebral cortex. Neuron. 2014;83(5):1117–30.
13. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci. 2004;5(5):347–60.
14. Stamatovic SM, Johnson AM, Keep RF, Andjelkovic AV. Junctional proteins of the blood-brain barrier: new insights into function and dysfunction. Tissue Barriers. 2016;4(1):e1154641.
15. Butt AM, Jones HC, Abbott NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. J Physiol. 1990;429:47–62.
16. Betz AL, Firth JA, Goldstein GW. Polarity of the blood-brain barrier: distribution of enzymes between the luminal and antiluminal membranes of brain capillary endothelial cells. Brain Res. 1980;192(1):17–28.
17. Makita T, Sucov HM, Gariepy CE, Yanagisawa M, Ginty DD. Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. Nature. 2008;452(7188):759–63.
18. Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci. 2006;26(50):13007–16.
19. Arai K, Lo EH. Oligovascular signaling in white matter stroke. Biol Pharm Bull. 2009;32(10):1639–44.
20. Arai K, Lo EH. An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. J Neurosci. 2009;29(14):4351–5.
21. Agulhon C, Petravicz J, McMullen AB, Sweger EJ, Minton SK, Taves SR, et al. What is the role of astrocyte calcium in neurophysiology? Neuron. 2008;59(6):932–46.
22. Nagy JI, Rash JE. Astrocyte and oligodendrocyte connexins of the glial syncytium in relation to astrocyte anatomical domains and spatial buffering. Cell Commun Adhes. 2003;10(4-6):401–6.
23. Iadecola C, Nedergaard M. Glial regulation of the cerebral microvasculature. Nat Neurosci. 2007;10(11):1369–76.
24. Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell. 2005;120(3):421–33.
25. Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. Essential protective roles of reactive astrocytes in traumatic brain injury. Brain. 2006;129(Pt 10):2761–72.
26. Xin H, Li Y, Shen LH, Liu X, Wang X, Zhang J, et al. Increasing tPA activity in astrocytes induced by multipotent mesenchymal stromal cells facilitate neurite outgrowth after stroke in the mouse. PLoS One. 2010;5(2):e9027.
27. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci Transl Med. 2012;4(147):147ra11.
28. Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. Nat Neurosci. 2016;19(6):771–83.
29. Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. Nat Neurosci. 2011;14(11):1398–405.
30. Gaengel K, Genove G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol. 2009;29(5):630–8.
31. Candelario-Jalil E, Yang Y, Rosenberg GA. Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia. Neuroscience. 2009;158(3):983–94.
32. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature. 2010;468(7323):562–6.
33. Graeber MB. Changing face of microglia. Science. 2010;330(6005):783–8.
34. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005;8(6):752–8.
35. Ren L, Lubrich B, Biber K, Gebicke-Haerter PJ. Differential expression of inflammatory mediators in rat microglia cultured from different brain regions. Brain Res Mol Brain Res. 1999;65(2):198–205.
36. Olah M, Ping G, De Haas AH, Brouwer N, Meerlo P, Van Der Zee EA, et al. Enhanced hippocampal neurogenesis in the absence of microglia T cell interaction and microglia activation in the murine running wheel model. Glia. 2009;57(10):1046–61.
37. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science. 1988;239(4837):290–2.
38. Bayerl SH, Niesner R, Cseresnyes Z, Radbruch H, Pohlan J, Brandenburg S, et al. Time lapse in vivo microscopy reveals distinct dynamics of microglia-tumor environment interactions-a new role for the tumor perivascular space as highway for trafficking microglia. Glia. 2016;64(7):1210–26.
39. Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci. 2006;26(30):7907–18.
40. Scholz J, Klein MC, Behrens TE, Johansen-Berg H. Training induces changes in white-matter architecture. Nat Neurosci. 2009;12(11):1370–1.
41. Juraska JM, Kopcik JR. Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. Brain Res. 1988;450(1-2):1–8.

42. Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, et al. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science. 1998;280(5369):1610–3.
43. Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, et al. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet. 2003;33(3):366–74.
44. Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature. 2012;487(7408):443–8.
45. Wilkins A, Majed H, Layfield R, Compston A, Chandran S. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. J Neurosci. 2003;23(12):4967–74.
46. Wang S, Sdrulla AD, Disibio G, Bush G, Nofziger D, Hicks C, et al. Notch receptor activation inhibits oligodendrocyte differentiation. Neuron. 1998;21(1):63–75.
47. Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, et al. Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. Proc Natl Acad Sci U S A. 2000;97(13):7585–90.
48. Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, et al. LINGO-1 negatively regulates myelination by oligodendrocytes. Nat Neurosci. 2005;8(6):745–51.
49. Etxeberria A, Mangin JM, Aguirre A, Gallo V. Adult-born SVZ progenitors receive transient synapses during remyelination in corpus callosum. Nat Neurosci. 2010;13(3):287–9.
50. Pham LD, Hayakawa K, Seo JH, Nguyen MN, Som AT, Lee BJ, et al. Crosstalk between oligodendrocytes and cerebral endothelium contributes to vascular remodeling after white matter injury. Glia. 2012;60(6):875–81.
51. Franklin RJ, Ffrench-Constant C. Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci. 2008;9(11):839–55.
52. Joutel A, Haddad I, Ratelade J, Nelson MT. Perturbations of the cerebrovascular matrisome: a convergent mechanism in small vessel disease of the brain? J Cereb Blood Flow Metab. 2016;36(1):143–57.
53. Lo EH, Moskowitz MA, Jacobs TP. Exciting, radical, suicidal: how brain cells die after stroke. Stroke. 2005;36(2):189–92.
54. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol. 2010;10(12):826–37.
55. Macrez R, Ali C, Toutirais O, Le Mauff B, Defer G, Dirnagl U, et al. Stroke and the immune system: from pathophysiology to new therapeutic strategies. Lancet Neurol. 2011;10(5):471–80.
56. Asahi M, Asahi K, Jung JC, del Zoppo GJ, Fini ME, Lo EH. Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. J Cereb Blood Flow Metab. 2000;20(12):1681–9.
57. Gasche Y, Fujimura M, Morita-Fujimura Y, Copin JC, Kawase M, Massengale J, et al. Early appearance of activated matrix metalloproteinase-9 after focal cerebral ischemia in mice: a possible role in blood-brain barrier dysfunction. J Cereb Blood Flow Metab. 1999;19(9):1020–8.
58. Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab. 1999;19(6):624–33.
59. Montaner J, Alvarez-Sabin J, Molina C, Angles A, Abilleira S, Arenillas J, et al. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke. 2001;32(8):1759–66.
60. Kelly PJ, Morrow JD, Ning M, Koroshetz W, Lo EH, Terry E, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the biomarker evaluation for antioxidant therapies in stroke (BEAT-stroke) study. Stroke. 2008;39(1):100–4.
61. Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke. 1998;29(10):2189–95.
62. Lo EH. Experimental models, neurovascular mechanisms and translational issues in stroke research. Br J Pharmacol. 2008;153(Suppl 1):S396–405.
63. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci. 2009;10(12):861–72.
64. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004;114(3):330–8.
65. Ding G, Jiang Q, Li L, Zhang L, Zhang ZG, Ledbetter KA, et al. Angiogenesis detected after embolic stroke in rat brain using magnetic resonance T2∗WI. Stroke. 2008;39(5):1563–8.
66. Krupinski J, Kumar P, Kumar S, Kaluza J. Increased expression of TGF-beta 1 in brain tissue after ischemic stroke in humans. Stroke. 1996;27(5):852–7.
67. Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med. 2006;12(4):441–5.
68. Lee SR, Kim HY, Rogowska J, Zhao BQ, Bhide P, Parent JM, et al. Involvement of matrix metalloproteinase in neuroblast cell migration from the subventricular zone after stroke. J Neurosci. 2006;26(13):3491–5.

69. Hayakawa K, Pham LD, Som AT, Lee BJ, Guo S, Lo EH, et al. Vascular endothelial growth factor regulates the migration of oligodendrocyte precursor cells. J Neurosci. 2011;31(29):10666–70.
70. Butt AM, Ibrahim M, Ruge FM, Berry M. Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody rip. Glia. 1995;14(3):185–97.
71. Orthmann-Murphy JL, Abrams CK, Scherer SS. Gap junctions couple astrocytes and oligodendrocytes. J Mol Neurosci. 2008;35(1):101–16.
72. Arai K, Lo EH. Astrocytes protect oligodendrocyte precursor cells via MEK/ERK and PI3K/Akt signaling. J Neurosci Res. 2010;88(4):758–63.
73. Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci. 2009;10(1):9–22.