Emerging molecular mechanism for cerebral small vessel disease: Lessons from hereditary small vessel disease

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Abstract
Cerebral small vessel disease is a common disorder in the elderly. The findings of hereditary small vessel disease studies clearly show that small vessel diseases have a distinct molecular pathway that is different from that in large vessels. However, the anatomical and functional heterogeneity of the cerebral small vessel system makes it difficult to understand the concept and molecular mechanism for small vessel disease. The purpose of this review is to explain the heterogeneity of small vessels and the importance of the components of the capillary system in the pathogenesis of cerebral small vessel disease. Although traditional investigations have focused more attention on the arteriole, the most functional part of small arteries is the capillary. Therefore, the capillary might play an important role in the pathogenesis of small vessel disease. In the capillary, pericytes and astrocytes are unique components with marked diversity. However, the molecular signature and function of pericytes remain unknown. Furthermore, the morphology and molecular signature of astrocytes in the cortex and white matter are quite different. Therefore, the mechanism of small vessel disease is not simple, and must be investigated considering the diversities of small vessels. In the capillary, cross-talk between cell components exists. Among these cell signaling pathways, recent findings on the gene responsible for hereditary small vessel disease show that transforming growth factor-β and platelet-derived growth factor-β could contribute to the molecular pathogenesis of small vessel disease. These findings provide useful information for the development of a new therapeutic strategy for small vessel disease.

Introduction
The vessel system in the brain is fundamental for maintaining brain function. Among the components of the vessel system, the small vessels play an important role in maintaining the function. Diseases that mainly involve small vessels are known as cerebral small vessel disease (SVD). SVD is a common disorder in elderly populations, and contributes to dementia, gait disturbance and stroke.1 The concept of SVD changes our understanding of cerebral vascular disease in that the molecular pathogenesis of SVD is different from that of large vessel disease, which is mainly caused by arteriosclerosis. With advances in molecular genetics, the genes that cause cerebral SVD have been identified. Study findings have clearly shown that SVD has a distinct molecular pathway that does not involve large vessels. However, little is known about the molecular basis of SVD.2

The concept of cerebral SVD is still obscure. Although small vessels are anatomically and functionally different, we are still not sure which type of small vessel contributes to the pathogenesis of cerebral SVD. Furthermore, although a blood–brain barrier and perivascular drainage of interstitial fluid from the brain parenchyma have been proposed as a function in the small vessels in the brain, the precise functions and involved components of small vessels are not fully understood.3,4 To understand cerebral SVD, the function and precise structure of the small vessels in the brain must be clarified. Capillaries and surrounding astrocytes are unique, and play an important role in the function of small vessels. However, previous studies on cerebral small vessels have mainly focused on the arterioles, which are larger than capillaries.1 The purpose of the present review is to explain the heterogeneity of the cerebral small vessel system, and the importance of mural cells in the pathogenesis of cerebral SVD, based on the
findings from studies about the molecular pathogenesis of hereditary SVD.

Heterogeneity of small vessels

A characteristic feature of the brain arteries is that they mainly lose their external elastic membrane. This feature of the cerebral arteries might contribute to the high frequency of brain vessel aneurysms. Among the small vessels in the brain, there are at least three different types of vessels: pial artery, arteriole and capillary. The pial artery is composed of the following cells and connective tissue layers (starting from the luminal side): endothelial cells, basement membrane, internal elastic membrane, smooth muscle cells, basement membrane, leptomeningeal cells and connective tissue. These small arteries have anastomoses. The anatomical differences among these three vessels include the absence or presence of the internal elastic membrane, smooth muscle cells and perivascular space. The elastic membrane is absent in the arterioles, and smooth muscle cells and the perivascular space are both absent in the capillaries.

There are two types of arterioles, according to their anatomical position in the cerebrum. One is the superficial perforating artery arising from the pial artery (smaller than large arteries), and the other is the deep perforating artery arising from the anterior, middle and posterior cerebral arteries (large arteries). Superficial perforating arteries are further divided into four types by the depth of the vessels from the cerebral surface. Two types of superficial perforating arteries irrigate the different layers in the cortex, and the others irrigate the corticomedullary junction and white matter. In the cortex, small arteries are more abundant than in white matter, and make anastomoses.

The superficial perforating artery that branches into the periventricular area and irrigates the white matter is known as the medullary artery. The medullary artery is divided into two types by its shape after penetrating the cortex. After penetrating the cortex, one artery extends straightly through the white matter and the other bends at a right angle at the subcortical area to access the deep white matter. The arteries make anastomoses around the ventricular wall. The superficial perforating arteries coil, loop and spiral within wide adventitial spaces at the corticomedullary junction; the function of these structures is unknown. These arteries have thick adventitial sheaths and large perivascular spaces in the white matter not in the cortex. Meanwhile, the deep perforating artery branches out directly from the large artery, and most of the arteries reach the basal ganglia and thalamus. In addition, some of these arteries have a dual leptomeningeal cell layer, resulting in a relatively large perivascular space. This unique structure might appear as a relatively large perivascular space on T2-weighted magnetic resonance imaging (MRI).

Another important characteristic of the small vessels is their regulation by the neuron. The pial artery is densely innervated by the peripheral nervous system. In contrast, small cortical arteries (arteriole or capillary) are innervated by interneurons in the cortex or subcortical pathway neurons. It is not known if these neuronal regulations also exist in small arteries in the subcortical area; however, the small vessels in the white matter might be less tightly regulated by the nervous system.

Diversities of capillaries

The most important function of capillaries in the small vessel system is as a blood–brain barrier. The non-fenestrated endothelial cells and tight junction compose this system. The endothelial cells are enveloped with pericytes and astrocyte end-feet. This structure is sometimes called a neurovascular unit; however, the contribution of the nervous system to capillaries in the subcortical area is not clear. In the present review, these components will be described as a capillary unit, including capillaries, astrocytes and pericytes. In a capillary unit, the endothelial cell plays several important roles for barrier function: forming a tight junction, selective transport system and endocytosis. To maintain these characteristic features, the endothelial cells in capillary express several unique molecules, which are not observed in the endothelial cells in arterioles; for example, claudin and occludin in tight junctions, glucose transporter 1 for selective transportation and caveolin 1 for selective transcytosis. In addition, a recent study showed extravasation of clots in the capillary to the brain parenchyma by the endothelial cells, suggesting that the function and characteristics of endothelial cells in the capillary might be different from those in the arteriole. Furthermore, it is not known if all the endothelial cells in the central nervous system are identical regardless of their location. Endothelial cells are tightly associated with pericytes, which are mural cells in the capillary, by autocrine and paracrine signaling. If the characteristics and function of pericytes vary according to their location, the same might also be true for endothelial cells.

In capillaries, smooth muscle cells are absent, and pericytes cover some extent of the abluminal side of endothelial cells. Compared with other species, the small vessels in the human cortex are covered by a larger number of pericytes. Recent findings show that pericytes play an important role in maintaining the blood–brain barrier function. In addition, a decrease in the number of pericytes causes neurodegeneration through a non-ischemic or hypo-oxygenic pathway. Pericytes are cells attached to the abluminal side of endothelial cells in the capillary and covering the basement membrane along with endothelial cells. There are several molecular signatures that can distinguish most of the pericytes from other cells; however, none of the single molecular markers can distinguish all of the pericytes from other brain cells. The lineage of the pericytes in the central nervous system is different in each part of brain. The embryonic sources of pericytes include neuroectoderm-derived neural crest cells, which give rise to pericytes in the forebrain, and mesoderm-derived mesenchymal stem cells, which give rise to pericytes in the midbrain, brain stem and spinal cord.

This complex is enveloped by astrocytes. Although the contribution of the astrocytes to maintain the barrier function is not fully understood, the astrocytes might contribute to the direction of the selective transportation between the
luminal and the brain parenchyma.\textsuperscript{26,27} The astrocyte also has marked heterogeneities, including protoplasmic and fibrous astrocytes.\textsuperscript{26,28–31} The protoplasmic astrocyte is predominantly found in the cortex, and has many branching processes with end-feet that envelop the synapse and capillaries. Furthermore, some of the branches extend to the surface of the cerebrum. In contrast, the fibrous astrocyte is found in white matter, and has a few unbranched processes with end-feet that envelop Ranvier nodes and capillaries. Although the lineage differences of each astrocyte in the cerebrum is still obscure, the astrocytes in the spinal cord have a distinct lineage depending on the anatomical position in the spinal cord.\textsuperscript{32}

Small arteries have marked anatomical and functional diversities. Most prominently, small vessels in the cortex and white matter are different in many aspects. The difference is not simply explained by the difference of the circulation dynamics or number of capillaries. The regulations by the nervous system and the type of cells that compose the capillaries are fundamentally different between small vessels in the cortex and white matter. These results indicate that small vessels do not have a single architecture. We should to pay more attention to the heterogeneities of cerebral small vessels when we study the molecular pathogenesis of SVD.

**Which type of small vessel is responsible for the clinical features of SVD?**

MRI has shown several aspects of SVD, white matter hyperintensity, lacunar infarction, microbleeds, cortical subarachnoid hemorrhage, cortical microinfarction and cortical thinning.\textsuperscript{2,33} Among these features, which feature is mostly responsible for the clinical symptoms of SVD? The most prominent feature of SVD on MRI is white matter hyperintensity (WMHI). Indeed, several hereditary SVD show diffuse WMHI; thus, there is no doubt that WMHI is a result of small vessel alterations. The lower density of capillaries in white matter might explain the vulnerability of the white matter in SVD.\textsuperscript{14} As the medullary artery is severely affected in sporadic SVD, Okeda et al. proposed the earthen pipe hypothesis for the molecular pathogenesis of SVD.\textsuperscript{34,35} They speculated that the hypoperfusion resulting from a loss of autoregulation of small vessels contributes to the white matter pathology in SVD.

Although the autoregulation disturbance hypothesis might explain a part of the molecular pathogenesis of white matter injury, the loss of the smooth muscle cell layer cannot simply explain the entire feature of SVD. The pathological findings of idiopathic basal ganglia calcification do not support this hypothesis. Patients with idiopathic basal ganglia calcification present massive calcifications in the perforating arteriole, specifically, in a portion of the media intima.\textsuperscript{36–38} In the small vessels of patients with this disease, the contracting property of the arteriole should be completely diminished. However, WMHI is not an early finding in these patients.\textsuperscript{39–41} The difference between this small vessel pathology and the other SVD is that the affected area is calcified and protected from the bloodstream. Therefore, an additional mechanism should exist to explain the molecular pathogenesis of WMHI. In addition, accumulating evidence shows that WMHI is not strongly correlated with the clinical symptoms in hereditary SVD.\textsuperscript{42–46} This feature markedly precedes the onset of the neurological symptoms. Thus, the significance of WMHI on development of clinical manifestations in SVD should be carefully assessed.\textsuperscript{47,48}

The capillary, which plays the most important role in the small vessel system, could contribute to the clinical manifestations in SVD.\textsuperscript{49} Capillary alterations can cause cognitive impairment and movement dysfunction through different mechanisms: (i) dysfunction of the barrier function; (ii) dysregulation of microcirculation dependent on neuronal activity; and (iii) failure of interstitial fluid draining.\textsuperscript{17,50,51} Previous studies have attempted to explain the selective vulnerability for white matter, as changes in the white matter are mostly prominent in SVD. However, if WMHI is just a consequence of the dysfunction of the small vessel system, we might lose track of the true pathogenesis that contributes to the neurological manifestations of SVD. Further studies should focus on the alteration of the microcirculation system, including the capillary and surrounding cells, to understand the pathogenesis of SVD.

**Which component of small vessels is important for the pathogenesis of SVD?**

The degeneration of the smooth muscle cells and the splitting of the internal elastic membrane are characteristic features in sporadic and some hereditary small vessels. The splitting of the internal elastic fiber might cause the transition of smooth muscle cells and their migration and proliferation to the media intima, not apoptosis.\textsuperscript{52} Furthermore, the disturbance of elastic fiber by reducing the amounts of component protein, elastin, does not cause SVD.\textsuperscript{52–55} In contrast, patients with the mutation in actin, which is mainly expressed in the smooth muscle cells, resemble those of sporadic SVD.\textsuperscript{56} Therefore, the degeneration of smooth muscle cells might contribute to the pathogenesis of SVD.

However, the capillary, a functional small vessel, does not have smooth muscle cells. The capillary has several unique structures that distinguish it from other vessels and small vessels in other organs. These unique structures might explain why these disorders specifically affect the brain. Thus, whether the components of the capillary unit are important for pathogenesis of SVD will be addressed in the present review.

One of the components of the cells in the capillary unit is the pericyte. Pericytes are cells that share the basement membrane with endothelial cells. However, the lack of markers to identify pericytes makes it difficult to investigate the involvement of these cells in the human brain.\textsuperscript{15,17,20} The involvement of pericytes in SVD is well characterized in diabetic retinopathy. In this disorder, pericyte apoptosis is an early manifestation. The absence of pericytes is recognized as a “pericyte ghost”, which represents the trace of the pericyte as a space between the basement membrane.\textsuperscript{57} In contrast, in brain parenchyma, it would be difficult to recognize these traces. Therefore, there is a limitation to recognizing
pericyte alterations in the human brain. However, the importance of pericytes for maintaining the neuron and the blood–brain barrier has recently been recognized.15,17,20 The involvement of pericytes has been observed in patients and a mouse model of cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a hereditary SVD,58,59 or idiopathic basal ganglia calcification.22,23,36,39,60 Thus, it would be interesting to investigate the contribution of pericytes in cerebral SVD.

The other unique component in the capillary unit is the astrocyte. The morphology and molecular signature of astrocytes is different between those in the cortex and white matter.26,28,31 Fibrous astrocytes are plentiful in the white matter, and have cylindrical processes with dense glial filaments stained with glial fibrillary acidic protein. Protoplasmic astrocytes are popular in the gray matter, and have more irregular processes and few glial filaments. Protoplasmic astrocytes contact and sheathe synapses and blood vessels. Therefore, there is a possibility that an alteration on a specific type of astrocyte results in the vulnerability of specific areas in the brain. For example, the mutation of the glial fibrillary acidic protein, which is a fundamental skeletal protein in the astrocyte and more popular in the fibrous astrocytes than protoplasmic astrocytes, causes demyelination in the white matter. Although in the patients with a mutation in the GFA gene, the astrocytes in white matter are predominantly affected, the clinical manifestations of the patients are quite different from the SVD. Thus, it might be difficult to consider that the astrocyte takes a primary role in the pathogenesis of SVD.

Finally, the perivascular space, known as the Virchow–Robin space, is a unique structure in the small vessel systems in the brain. Several hypotheses for the significance of the space between the adventia and parenchyma (glia limitans) in the brain have been provided.61 One of the hypotheses is that the space functions as a pathway for drainage of fluid or proteins from the brain parenchyma.3,62 Weller et al. use the term, “protein elimination-failure angiopathy,” for the disorder caused by the impairment of drainage pathway by small vessels.3 Cerebral amyloid angiopathy, which predominantly involves the cortical and pial arteries, has been considered part of the elimination failure disorders.3 The disease has been classified into two types depending on the presence or absence of amyloid accumulation in capillaries.7,63,64 In cerebral amyloid angiopathy, amyloid deposit in the internal space of the mural cells results in the disappearance of smooth muscle cells. In addition, WMHI is well observed in patients with Alzheimer’s disease. Although the elimination failure hypothesis is promising, more evidence should be accumulated to prove that the perivascular space functions as a drainage system in the brain.

Alteration of the signaling pathway between the cell components of the microcirculation system causes SVD

The identification of the gene responsible for hereditary SVD provides the molecular pathway for SVD. Several molecular mechanisms have been identified in SVD: (i) the alteration of structural proteins in the small vessel system,65 (ii) accumulation of the abnormal proteins or dysfunctional metabolism in the small vessel system,66,67 and (iii) alteration of the cell signaling pathway in the small vessel system. The present review will focus on the contribution of the cell signaling pathway on the pathogenesis of SVD.

We recently identified the causative genes for hereditary SVD, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL).68 Mutations in the high-temperature requirement A (HTRA) serine peptidase 1 (HTRA1) gene cause the disease. Disorganization of the internal elastic membrane and loss of vascular smooth muscle cells were observed in small cerebral arteries in CARASIL.69 These pathological findings resemble those observed in patients with non-hereditary cerebral SVD.34,35 HTRA1 has a serine protease activity, and decreases transforming growth factor-β (TGF-β) family signaling.70 CARASIL-associated mutant HTRA1 show decreased protease activity and fail to decrease TGF-β family signaling.68,69 Furthermore, the fibronectin containing extra type III domain A and versican, which are induced by increased TGF-β signaling, accumulate and TGF-β1 is increased in the media intima of small cerebral arteries of patients with CARASIL.68,71 These findings show that increased TGF-β signaling plays a pivotal role in the pathogenesis of SVD in CARASIL. HTRA1 decreases TGF-β1 signaling by interfering with the maturation of proTGF-β1 in the intracellular space. HTRA1 cleaves the pro-domain of proTGF-β1, and cleaved proTGF-β1 is degraded.71 Consequently, the amount of mature TGF-β1 is reduced. The intracellular cleavage of proTGF-β1 is a novel mechanism to regulate the amount of TGF-β1.71,72 The relationship between the dysregulation of TGF-β signaling and the loss of smooth muscle cells in small cerebral vessels might show an emerging molecular mechanism for cerebral SVD. TGF-β is a well-known cytokine that is secreted from endothelial cells, pericytes and astrocytes.15,17 The receptors for TGF-β are also expressed in these cells. Therefore, TGF-β signaling could affect autocrine or paracrine signaling. Although it is still not clear in which cell HTRA1 is expressed, the endothelial cell is a possible candidate and regulates TGF-β signaling.73,74

Another component of the cell signaling pathway, which functions between endothelial cells and pericytes, is the platelet-derived growth factor-β (PDGFβ).15,17 The decreased PDGFβ or receptor for PDGFβ decreases the number of pericytes, and results in the dysfunction of the blood–brain barrier accompanied with neurodegeneration.22–25 Mutations in PDGFβ or receptor for PRGFβ cause idiopathic basal ganglia calcification.39,60 Although the neuropathological findings with these mutations have not been reported, neuropathological findings in patients with idiopathic basal ganglia calcification showed calcium deposition in pericytes.38 The mural cells have the capacity to transition into several characteristic states. For example, smooth muscle cells transition from the contracting type to the non-contracting type as well as the osteogenic type, depending on the balance of the signaling pathway.74,75 It would be interesting to investigate the transition of pericytes to osteogenic pericyte as a result of decreased PDGF-β signaling.
Conclusion

Small cerebral vessels are a lost world in the brain architecture. In the pharmacological field, the role of the small vessels in the brain in relation to the function of the blood-brain barrier and the molecules in the tight junction has been investigated. Indeed, the tight junction plays an important role in maintaining the barrier function; however, recent advances in small vessel research show that selective endocytosis in the capillary also plays an important role in the barrier function.13,14 Furthermore, the capillary unit also functions in the draining of interstitial fluid.3 The fine regulation of microcirculation in the cortex might also be important to maintain brain function.13,14 The pericytes, astrocytes and neuronal regulation could take an important role for these functions. To clarify the pathogenesis of SVD, further research on the anatomical and functional heterogeneity in the small vessels and surrounding cells is required. Furthermore, additional insight on how the cell signaling pathway maintains the small vessel units will provide useful information for the development of a new therapeutic strategy to prevent the progression of SVD.

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