Mini Review

Blood-neural barrier: its diversity and coordinated cell-to-cell communication

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The cerebral microvessels possess barrier characteristics which are tightly sealed excluding many toxic substances and protecting neural tissues. The specialized blood-neural barriers as well as the cerebral microvascular barrier are recognized in the retina, inner ear, spinal cord, and cerebrospinal fluid. Microvascular endothelial cells in the brain closely interact with other components such as astrocytes, pericytes, perivascular microglia and neurons to form functional 'neurovascular unit'. Communication between endothelial cells and other surrounding cells enhances the barrier functions, consequently resulting in maintenance and elaboration of proper brain homeostasis. Furthermore, the disruption of the neurovascular unit is closely involved in cerebrovascular disorders. In this review, we focus on the location and function of these various blood-neural barriers, and the importance of the cell-to-cell communication for development and maintenance of the barrier integrity at the neurovascular unit. We also demonstrate the close relation between the alteration of the blood-neural barriers and cerebrovascular disorders. [BMB reports 2008; 41(5): 345-352]

Blood-neural barriers

Blood barriers between the blood and neural tissues are collectively referred to the blood-neural barriers (1). The blood-neural barriers include the blood-brain barrier, blood-cerebrospinal fluid barrier, blood-retinal barrier, blood-spinal cord barrier, blood-labyrinth barrier and blood-nerve barrier (2). So far, the regulatory mechanisms of each blood-neural barrier are not fully understood. Considering that a number of neurological disorders are accompanied by blood-neural barriers dysfunction, elucidating the nature of blood-neural barrier system that contributes to brain homeostasis will provide insight into such disorders.

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The blood-brain barrier

Brain microvessels connected by tight and adherence junctions form the blood-brain barrier (BBB). The BBB is a selective barrier that limits the movement of blood-borne substances into the extracellular fluid of the brain. The specialized endothelial cells are embedded in a basement membrane and surrounded by astrocyte end-feet and pericytes. Neurons and microglia are also observed at the perivascular region (Fig. 1A). The BBB formation and function depend on close cell-to-cell connection at the neurovascular unit; in particular, astrocytes possess efficient mechanisms to modulate barrier properties by acting as a mediator of cell-cell signaling (3). Pericytes together with astrocytes function as a key player of BBB differentiation (4). The factors influencing the BBB integrity also stem from neurons and microglia.

The blood-cerebrospinal fluid barrier

Cerebrospinal cord fluid (CSF) is a circulating liquid through the ventricles. CSF functions as a cushion in the brain and spinal cord and helps to nourish them. CSF is secreted in the lateral ventricles mainly by the choroid plexus. The choroid plexus are capillaries with fairly leaky and fenestrated properties. However, the choroid plexus ependymal cells are linked with tight junctions forming the blood-CSF barrier (Fig. 1B). These ependymal cells also contain ion pumps that transports only certain ions across their membranes inducing ionic gradients (5). Brain tumors, hypertension and trauma are involved in the breakdown of the blood-CSF barrier.

The blood-retinal barrier

The visual image is focused on the retina in the eye. Blood vessels supply the oxygen and nutrients to the retina. The blood-retinal barrier (BRB) in the retina can be divided into two distinct regions: the inner BRB and outer BRB (Fig. 1C). The choroid vessels are fenestrated and the barrier is formed by the retinal pigment epithelium layer, which is the outer BRB (Fig. 1C). The inner BRB is formed by the specialized microvessels of the retina and their surrounding pericytes and astrocyte end-feet. The inner BRB plays essential roles in protecting neural tissues from toxic materials and in maintaining neural functions of retina. Astrocytes recognize retinal hypoxia
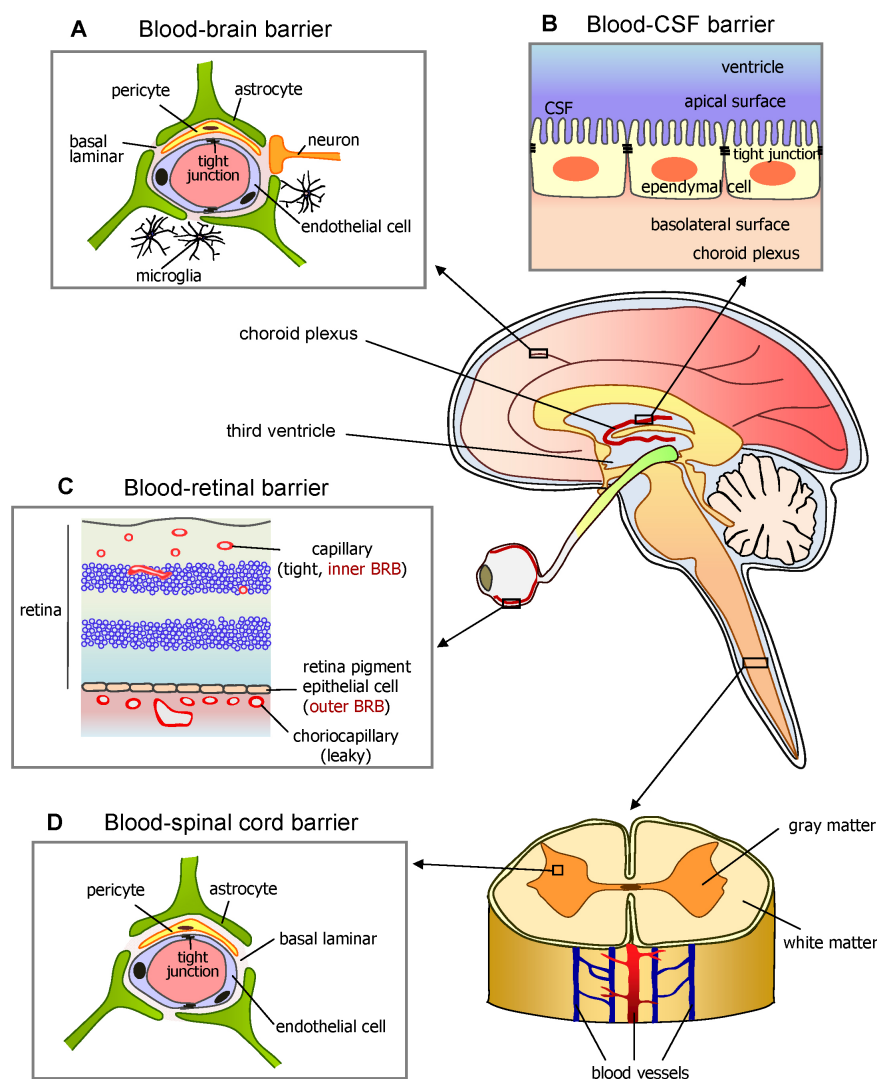
Fig. 1. The BBB, blood-CSF barrier and BRB are present at different sites in the brain. (A) The specialized endothelial cells are embedded in a basement membrane and surrounded by astrocyte end-feet and pericytes. Neurons and microglia are also observed at the perivascular region. The physical barrier of the BBB is formed by tight junctions. (B) The choroid plexus ependymal cells are joined with tight junctions, forming the blood-CSF barrier. (C) The schematic figure shows the localization of the inner BRB and outer BRB in the retina. (D) The cross image of the spinal cord is seen, which contains the gray matter and white matter. The blood-spinal cord barrier is well-differentiated microvessels surrounded by pericytes and astrocyte endfeet.

and guide for the developing retinal vasculature by increasing the expression of hypoxia-inducible angiogenic factors, such as hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) (6, 7). Oxygen delivered by the new forming retinal vasculature is an important factor in the cessation of retinal angiogenesis and in the induction of retinal barriergenesis (8, 9).

The blood-spinal cord barrier

The spinal cord is part of the central nervous system and is attached to the brain stem. The blood-spinal cord barrier (BSCB) is well-differentiated microvessels surrounded by pericytes and astrocytic endfeet (Fig. 1D), which are similar to those of BBB (10). Minor differences between BSCB and BBB are observed; for example, the spinal cord vessels contain glycogen deposits, not normally seen in the brain microvessels (11), and the spi-

nal cord shows greater permeability than the brain (12). Traumatic spinal cord injury results in the intraparenchymal hemorrhage, inflammation, disruption of the BSCB and angiogenesis (10, 11).

The blood-labyrinth barrier

The inner ear is referred to the labyrinth. The labyrinth constantly maintains its remarkably stable homeostasis by the regulatory mechanisms such as an ion transport system, a blood-labyrinth barrier (BLB), and a constant blood supply (13). Perilymph and endolymph in the inner ear contain distinct ionic composition and electrical charge (14), which are completely separated by the specialized BLB such as blood-endolymph barrier and the endolymph-perilymph barrier (2). The expression of tight junction proteins in the Reissner's membrane, endothelial cells, basal cells and marginal cells contrib-

utes to the compartmentalization in the inner ear, and also limits ion movements by actively transporting only certain ions across their membranes (15, 16) (Fig. 2A). The BLB disturbance is closely related with imbalance of ion, osmosis, or metabolism between the compartments.

The blood-nerve barrier

The blood-nerve barrier (BNB) consists of endoneurial capillaries and the perineurial sheath, a connective tissue (Fig. 2B). Endoneurial capillaries in the endoneurial space are derived from blood vessels of epineurium and perineurium (17). These endoneurial capillaries are joined with a network of intercellular tight junctions. In addition, the perineurial sheath restricts the fluid movements between the extracellular spaces

surrounding nerve fascicles and the endoneurium (18). Dysfunction of blood-nerve barrier integrity is closely associated with many peripheral nerve disorders.

Effects of cell-cell interactions on blood-neural barriers

At different sites, a variety of blood-neural barriers is functionally and dynamically regulated by the factors recruited or released from adjacent cells. Factors derived from differentiated neuroglia influence the barrier formation (barriergenesis) at the neurovascular unit. Thus, we describe the role of perivascular cells and their molecular factors in the formation of blood-neural barriers.
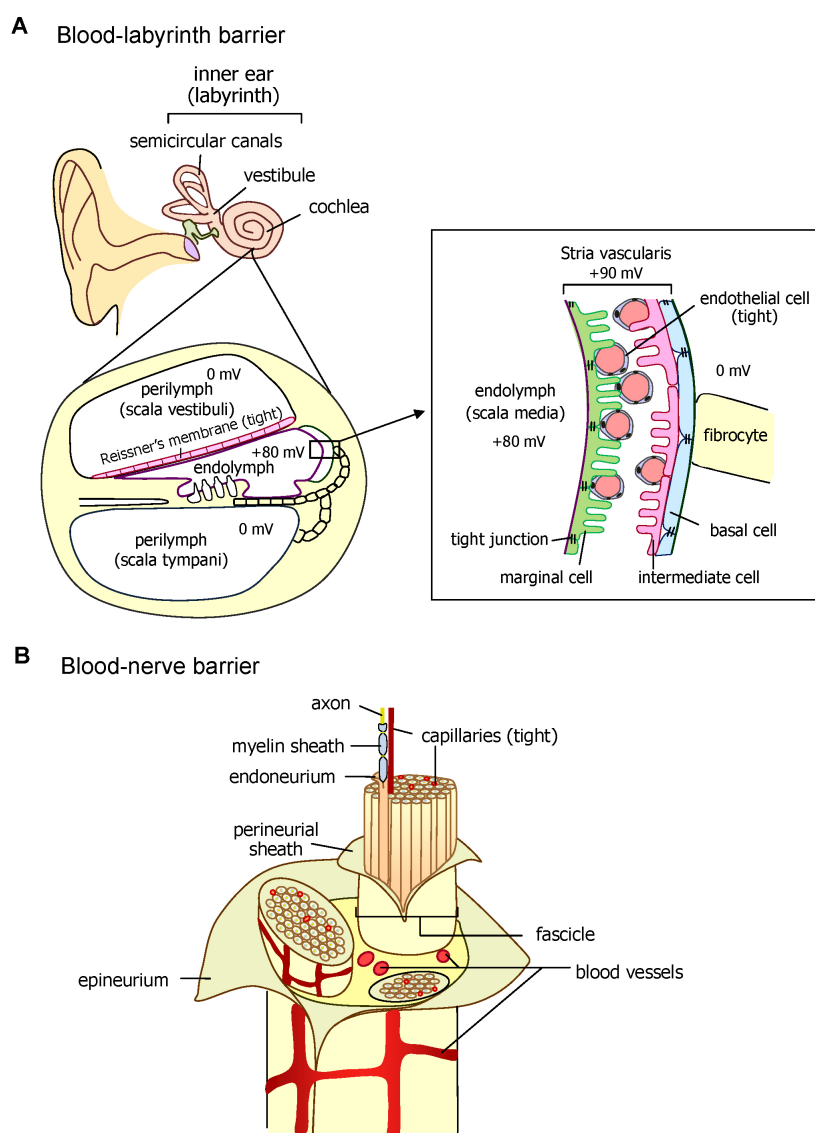

Fig. 2. The schematic drawing shows the blood-labyrinth barrier in the inner ear and the blood-nerve barrier in the peripheral nervous system. (A) The inner ear contains three regions, semicircular canals, vestibule and cochlea. The cochlear (originated from the Greek 'snail') consists of three fluid-filled compartments, scala vestibuli, scala tympani and scala media. Tight junctions are expressed in the Reissner's membrane, marginal cells, basal cells, and endothelial cells. Endolymph is secreted by the vascularized mucosae of marginal cells, intermediate cells and basal cells in the stria vascularis. (B) The blood-nerve barrier consists of the endoneurial capillaries and the perineurial sheath. Each axon is sheathed by an endoneurium, the fascicles of nerve fibers are surrounded by the perineurial sheath, and all the fascicles are sheathed by the epineurium. Endoneurial capillaries are originated from the epineurial and perineurial blood vessels.

Pericytes-endothelial cells interactions

Reciprocal interactions between pericytes and endothelial cells function as essential regulators for vascular development, stabilization, maturation and remodeling. The release of secretion factors by endothelial cells can induce the migration of pericytes towards the endothelial cell wall and subsequent maturation of the vessels. Platelet-derived growth factor (PDGF)-B produced by endothelial cells binds to its cognate receptor PDGFR-β on pericytes, consequently recruiting pericytes near the endothelial cells and leading to vascular maturation (19). Other signaling in the reverse way can occur. Angiopoietin-1 (Ang1) is expressed by pericytes and binds to the endothelial receptor tyrosine kinase Tie-2 (20). Ang1 induces the remodeling and stabilization of the vasculature and prevents the vascular leakage acting as an anti-permeability factor (21). Interestingly, TGF-β1 is likely to be expressed by both endothelial cells and pericytes. TGF-β1 induces barrier functions by inhibiting migration and proliferation of endothelial cells and pericytes (22) and by enhancing endothelial-mesenchymal interactions (23). Vascular stabilization is also mediated by N-cadherin induced endothelium/pericyte contacts (24) (Fig. 3A).

Astrocytes-endothelial cells interactions

The recent research related to the interaction between endothelial cells and astrocytes in blood-neural barriers is of great interest. A secreted factor derived from endothelial cells, leukemia inhibitory factor (LIF), induces the astrocytic differentiation (25). Blood-borne elevated oxygen tension itself also induces retinal astrocytes differentiation (9). As the oxygen level increases because of the presence of vessels, the expression of A-kinase anchor protein12 (AKAP12) is upregulated in astrocytes (3). AKAP12 may reciprocally regulate the secretion of Ang1 and VEGF through HIF-1α in astrocytes during a critical
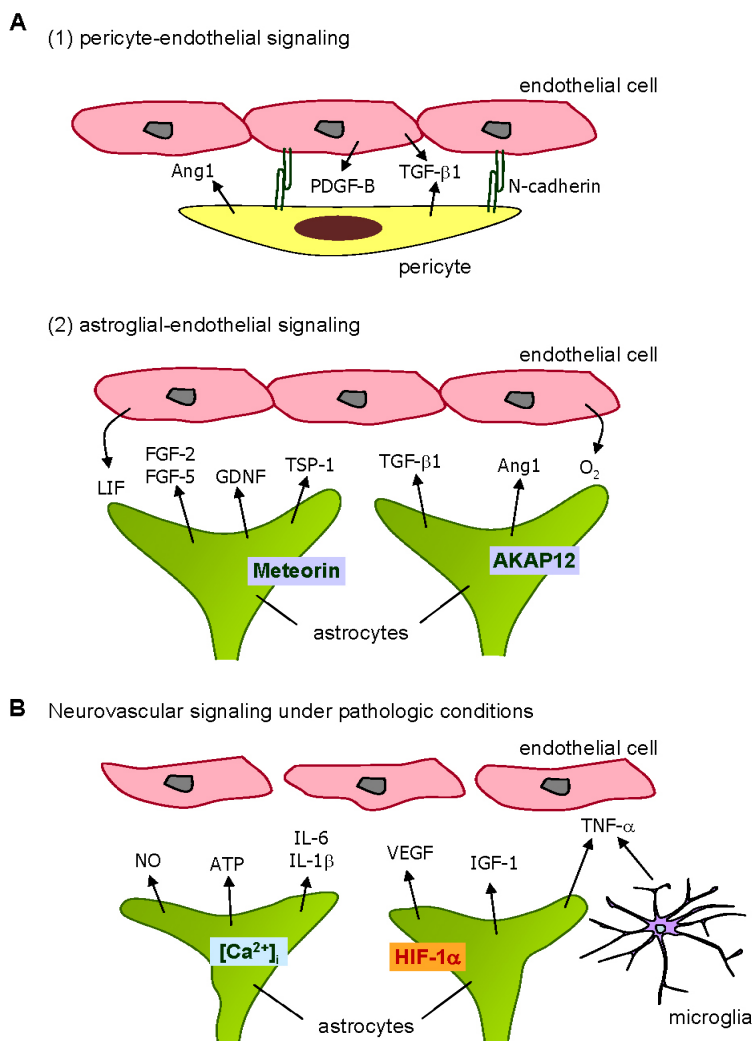

Fig. 3. Cell-cell signaling under physiologic and pathologic conditions. (A) Cellular components in blood-neural barriers and cell-cell signaling between these cells in normal conditions. (1) Bi-directional pericyte-endothelial signals induce the barrier properties. Endothelial cells releasing PDGF-B and TGF-β1 affect the pericytes differentiation. Pericytes-derived Ang1 and TGF-β1 play a role in the endothelial barrier properties. N-cadherin is localized in the endothelium/pericyte contacts. (2) The expression of AKAP12 and Meteorin is upregulated in the presence of oxygen in astrocytes. Astrocyte-derived factors such as Ang1, TGF-β1, TSP-1, FGF-2, FGF-5 and GDNF bind to their cognate receptors on endothelium. In addition, endothelium-derived factors, LIF and O₂, possess a function to induce astrocytes differentiation. (B) Under the neuronal disorders, activated microglia and astrocytes secrete the TNF-α, disrupting the endothelial barrier properties. In astrocytes, deprivation of oxygen and nutrients induces the HIF-1α, IGF-1 and VEGF. In addition, astrocytic calcium ion elevation can play a role for the production of the astrocyte-derived neurotrophic factors, such as NO, interleukins and ATP.

period in brain development, and enhance barrier properties *in vitro* by upregulating the tight junction proteins (3, 8) (Fig. 3A). In concert with AKAP12, Meteorin is upregulated by an elevated oxygen level in astrocytes and is secreted from undifferentiated neural progenitors, astrocyte lineage, and astrocyte endfeet that surround the blood vessels (26, 27). Meteorin may regulate glial cell differentiation and attenuate brain endothelial cell angiogenesis via thrombospondin-1 (TSP-1) (Fig. 3A). TSP-1 has a function to inhibit angiogenesis and to promote the astrocytes differentiation (28). Growth factors such as fibroblast growth factor-2 (FGF-2) and FGF-5 contribute to the formation, maintenance, and stabilization of the BBB (29). The other astrocyte-derived factors such as TGF-β1 and glia-derived neurotrophic factor (GDNF) induce barrier features of brain endothelial cells (1, 30, 31) (Fig. 3A).

Neurons-endothelial cells interactions

Blood vessels and neurons use similar guidance cues. The reciprocal interactions between blood vessels and neurons play essential roles for the neurovascular network and normal brain function. Neuron may contribute to building up the blood-neural barriers by direct interaction or by neuron-glial interactions. Astrocytes are able to respond to neuronal activity and transmit signals to the blood and vice versa. An astrocyte-derived secretion protein, TSP-1, promotes synaptogenesis and anti-angiogenesis (28, 32), influencing both on neurons and endothelial cells. Purinergic signaling such as adenosine triphosphate (ATP) induces TSP-1 level in astrocytes, and ATP-dependent calcium signaling among endothelial cells, astrocytes and neurons mediates adequate cerebrovascular response to fine neuronal activity (33, 34). In addition, astrocytic calcium ion elevation can produce the astrocyte-derived neurotrophic factors, such as epoxyeicosatrienoic acid, nitric oxide (NO), and cyclo-oxygenase-2 metabolites, leading to increased cerebrovascular blood flow (35, 36). The mature endothelium has a reciprocal function to induce a stable brain microenvironment that enables proper neuronal activity (37). Thus, cell-to-cell communication in blood-neural barriers is complicatedly regulated by a reciprocal feedback mechanism.

Microglia-endothelial cells interactions

Microglia are the central nervous system intrinsic macrophages and can release a large number of immunoregulatory, inflammatory, and cytotoxic mediators. Microglia are found in the perivascular space (38), indicating that interactions between microglia and endothelial cells can contribute to the blood-neural barrier properties. Blood-derived macrophages enhance the *in vitro* BBB properties (39). On the other hand, microglia are a potent source of tumor necrosis factor (TNF)-α, a cytotoxic factor for the disruption of tight junctions (40) (Fig. 3B). Thus, the exact mechanisms how microglia influence the BBB integrity remain to be investigated.

Transporters in blood-neural barriers

Although blood-neural barriers block entry of substances into the brain tissues, the essential molecules such as glucose and amino acids should be transported across the endothelial cells. Some compounds move against a concentration gradient, requiring ATP for active transport. The ATP-binding cassette transporters include P-glycoprotein (Pgp) and multidrug resistance-related proteins. Pgp in the endothelium prevents entry of blood-borne substances into the brain and facilitates their transport out of the brain parenchyma (41). Other transporters including glucose transporter-1 and L-type amino acid transporter-1 have a function for glucose and amino acids, respectively, to move down the concentration gradient, allowing the bi-directional movements of nutrients in and out of the brain (42). The Na⁺-dependent glutamate transporters move glutamate from brain to blood against the large opposing concentration gradient (43). Astrocytes support the selective transport barrier by inducing the expression and localization of transporters (44).

Blood-neural barriers in neurologic disorders

The disruption of blood-neural barriers is closely related with cerebrovascular diseases such as Alzheimer's disease, Parkinson's disease, ischemia, tumors, and diabetic retinopathy. Proinflammatory and proangiogenic factors can mediate blood-neural barrier dysfunction characterized by leaky vessels, astrocyte degeneration, recruitment of activated immune cells and neuronal cell death. In several disorders, decreasing nutrients and oxygen levels can induce NO and free radicals. Astrocyte-derived NO can interact with free radicals, leading to the neurodegenerative process via impairment of mitochondrial function (45, 46). Deprivation of oxygen and nutrients also induces hypoxia, which then upregulates the expression of growth factors either directly or through HIF-1 (Fig. 3B). HIF-1α-induced VEGF is a most potent angiogenic factor that increases blood-neural barriers permeability (47), and is a survival factor that promotes endothelial and neuronal survival (48). Numerous astrocyte-derived angiogenic factors including insulin-like growth factor-1 (IGF-1), TNF-α, and interleukins (ILs) are involved in the neurodegenerative disorders (49, 50) (Fig. 3B). Disrupted endothelial cell tight junctions, glial reactivity and neuronal apoptosis are often associated with excessive glutamate levels in the central nervous system (51). In diabetic retinopathy, insulin deficiency may cause alteration of glial cells which disrupts glutamate metabolism and transport, leading to accumulation of extracellular glutamate, and degeneration of neuronal cells (52). In addition, abnormal astrocytic activity is observed, accompanied by neuroretinal damage, capillary basement membrane thickening, loss of pericytes, and the BRB disruption (53).

Conclusion and future perspective

We cannot explain brain function only by analyzing a single cell type. Rather, study of blood-neural barriers as a neuro-vascular unit can provide an essential clue for better understanding of physiologic and pathologic properties of the brain, because the coordinated cell-to-cell interactions between perivascular cells establish and maintain the blood-neural barriers. In particular, astrocytes possess significantly efficient mechanisms by acting as a principal intermediary sensor between neurons, microglia and brain capillaries. Moreover, specialized blood-neural barriers are connected with each other. However, the integrated research focusing on the connection between specialized blood-neural barriers is not well-established yet. Considering that neurologic disorders damage the structure and function of several blood-neural barriers at the same time, the integrated study on blood-neural barriers will provide an important clue for a number of strategies to the successful neurotherapy.

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