# Research developments in the neurovascular unit and the blood-brain barrier (Review)

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Received November 18, 2024; Accepted March 5, 2025

DOI: 10.3892/br.2025.1966

**Abstract.** The neurovascular unit (NVU) is composed of neurons, glial cells, brain microvascular endothelial cells (BMECs), pericytes, and the extracellular matrix. The NVU controls the permeability of the blood-brain barrier (BBB) and protects the brain from harmful blood-borne and endogenous and exogenous substances. Among these, neurons transmit signals, astrocytes provide nutrients, microglia regulate inflammation, and BMECs and pericytes strengthen barrier tightness and coverage. These cells, due to their physical structure, anatomical location, or physiological function, maintain the microenvironment required for normal brain function. In this review, the BBB structure and mechanisms are examined to obtain a better understanding of the factors that influence BBB permeability. The findings may aid in safeguarding the BBB and provide potential therapeutic targets for drugs affecting the central nervous system.

**Contents**



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*Key words:* neurovascular unit, blood-brain barrier, neurons, astrocyte, microglial, brain microvascular endothelial cells, pericytes

## 1. Introduction

The brain is the most complex organ in the body, and it has a highly specialized structure and function. It accounts for ~2% of body weight, but consumes 20% of the metabolic reserves of the body (1). The metabolic needs of the brain are met through blood transport, and a group of cells from blood vessels and nerves known as the neurovascular unit (NVU) complete the blood supply of the brain (2). The NVU is a complex component that is composed of neurons, astrocytes, oligodendrocytes, microglia, pericytes, and brain microvascular endothelial cells (BMECs) (3-5) (Fig. 1). The blood-brain barrier (BBB) is an important NVU component (6). It is impossible to observe the functional activity of the BBB without the NVU.

The BBB controls the movement of nutrients and metabolites into and out of the brain parenchyma, regulates the brain microenvironment by providing transportation of selective substances, controls molecules and cells, and protects the normal structure and function of the brain (7). As early as 1885, Paul Ehrlich marked all tissues except the brain and spinal cord by injecting aniline stains into the blood vessels (8). Subsequently, Edwin Goldmann successfully stained the brain cells by injecting the dye into the spinal fluid, but other tissues and organs remained intact. This researcher then discovered a special barrier between the central nervous system (CNS) and circulating blood (9). Max Lewandowsky further discovered that capillary obstruction prevented some molecules from entering the brain and introduced the term 'Bluthirnschranke' (9). Until the late 19th century, Dr. Lewandowski from Berlin officially named it the BBB (8). It has now been established that the BBB is composed of BMECs, pericytes, astrocytes, and a basement membrane as the barrier matrix (10). The BBB prevents toxic substances, microorganisms, and pathogens in the blood from directly contacting the brain tissue. This protects the brain parenchyma from blood-borne pathogens and prevents drugs and other exogenous compounds from entering the CNS (11,12). Therefore, the BBB function is closely related to stroke (13), traumatic brain injury (TBI) (14), surgical brain injury (SBI) (15), intracerebral hemorrhage (ICH) (16), Alzheimer's disease (17), epilepsy (18), Huntington's disease (17), as well as other neurological diseases.

The present review focuses on the mechanism by which the NVU constructs the BBB. New possibilities for protecting the integrity of the BBB are also provided.

## 2. Neurons and the BBB

*Physiological structure and neuronal functions*. The primary function of the nervous system (NS) is to perceive and analyze changes in the internal and external environment of the body to control it (19). Neurons are the basic structures and functional units of the NS that perform the primary functional activities of the NS (20). Neurons are divided into cell bodies and protrusions, and the protrusions contain dendrites and axons (21). Axons comprise >95% of the total volume of neurons, and they carry electrical impulses and project them to other neurons (22). The perivascular neuron axons include the presynaptic portion that is inserted into the basement membrane through the astrocytic endfeet and forms a synaptic-like transmission with vascular smooth muscle cells to regulate high levels of neuronal activity and brain metabolic requirements (23,24). Higher brain functions depend on the electrophysiological signals that are released by neurons. Hence, the BBB is needed to maintain a precise and balanced ionic environment in the brain (23). Neurons form before the BBB during brain development. Neuronal cells combine with other nerve cells to construct the BBB. The BBB, in turn, protects neurons from harmful toxins, pathogens, and immune cells by controlling substance exchanges. Therefore, it is suggested that BBB formation and neuronal development are interrelated and complement each other. Specialized cells that sense the environment have emerged in the simplest multicellular organisms with epithelial tissue (25). These cells may have evolved into the first sensory neurons. As sensory neurons specialize in function, they require the support and protection of other cells. These supporting cells may then evolve into glial cells that form a tight barrier in the brain. This process would promote the separation of neuroactive substances in the central and peripheral areas, helping to establish a consistent microenvironment in the brain (25,26).

*Pathophysiological mechanisms of neuronal regulation of the BBB*. Neurons are also known as pacemakers of NVUs (27). Neurons convert small changes detected in the surrounding environment into electrical signals and chemical information that are transmitted to neighboring interneurons or astrocytes, thereby affecting vascular tone and regulating BBB permeability (28,29). The necessary regulatory mechanisms are activated to transmit these signals. Neuronal excitability is controlled by calcium and potassium homeostasis, while specific channels and transporters on the BBB provide a favorable environment for synapses and neural activity (30,31). For example, $Na^+$-$K^+$-ATPase plays an important role in cell excitation by regulating the transfer of $Na^+$ and $K^+$ in and out of the brain (32). When ATP production in the brain is insufficient, the $Na^+$-$K^+$-ATPase pump fails, intracellular sodium accumulates, the ion transport gradient collapses, intracellular fluid accumulates, and cells eventually rupture. This causes inflammation and damage to adjacent cells, thus destroying the BBB (33). Voltage-gated $Ca^{2+}$ channels and $K^+$ channels also regulate ion transport across the BBB (34,35). The normal functioning of the $Na^+/HCO3^-$-cotransporter and cation-chloride ion cotransporter in neurons also maintains the BBB integrity and intracranial ion homeostasis (36). These transporters or gated channels are expressed in neurons that can regulate and maintain the normal polarity of nerve cells and regulate cell volume and membrane permeability. This contributes to the selective permeability of the BBB to different substances. The permeability of the BBB is reduced for essential water-soluble nutrients and metabolites required by nerve tissue, and these are allowed to pass through to enter the nutrient nerves of the brain. The BBB acts as a specific filtering system for other toxic substances to prevent them from entering the brain (37).

A result of BBB breakdown is cerebral edema, and this can occur in all types of brain cells and brain parenchyma, such as neurons, astrocytes, and endothelial cells (ECs) (38). Following brain injury, neuronal water disintegrates cell bodies and processes, the neuronal nuclei become sparse, the water-soluble cytoplasm disintegrates, and brain edema becomes significantly aggravated (39). Mature nerve cells cannot proliferate. Therefore, the specific function and viability of nerve cells are impaired, and this can lead to BBB damage and further affect brain function (40). This provides strong support for the role of neurons in the BBB. Although the specific pathways of these regulatory mechanisms require further exploration, some signaling pathways have been shown to affect the BBB by disrupting neurons. It was determined from our previous studies that intraperitoneal injection of closantel allows bumetanide to pass through the BBB and inhibits the phosphorylation of the STE20/SPS1-related proline/alanine-rich protein kinase (SPAK)/$Na^+$-$K^+$-$Cl^-$ cotransporter 1 (NKCC1) signaling pathway in neurons. This reduces nerve cell apoptosis and has a protective BBB effect (41,42). The SPAK/NKCC1 signaling pathway is activated by phosphorylation after brain injury. Ion channels in the cell membrane open, and $Na^+$, $K^+$, $Cl^-$, and water enter the neuronal cells and disrupt the cellular structure. A large amount of neuronal apoptosis, BBB damage, albumin infiltration into the brain parenchyma, and brain edema of the injured side were found to be significantly aggravated in rats (41,42).

Endoplasmic reticulum stress (ERS) was also found to play an important role in neuronal death. $Ca^{2+}$ homeostasis of the neuronal ER is disrupted after brain cell injury. This causes ERS that participates in the pathological process of BBB destruction (43). The protein kinase R-like endoplasmic reticulum kinase (PERK) pathway is part of the unfolded protein response (UPR) during ERS. It is activated by phosphorylation after early ICH, and it further phosphorylates downstream eukaryotic translation initiation factor 2α (eIF2α) and upregulates activating transcription factor 4 (ATF4) to play a pro-apoptotic role. Acyl-CoA synthetase long chain family member 4 (ACSL4) reduces $Fe^{3+}$ to $Fe^{2+}$ through transferrin and transfers it into cells. The reactive oxygen species (ROS) reaction with $Fe^{2+}$ leads to ferroptosis, injury of nerve cells, and destruction of the barrier function, causing secondary cerebral edema (44). Deferoxamine can reduce ERS and protect nerve cells by interfering with $Fe^{3+}$ and inhibiting the PERK signaling pathway (45). However, transmembrane protein 2 (TMEM2) is involved in ERS through non-UPR pathways. TMEM2 decomposes the extracellular high-molecular-weight hyaluronic acid

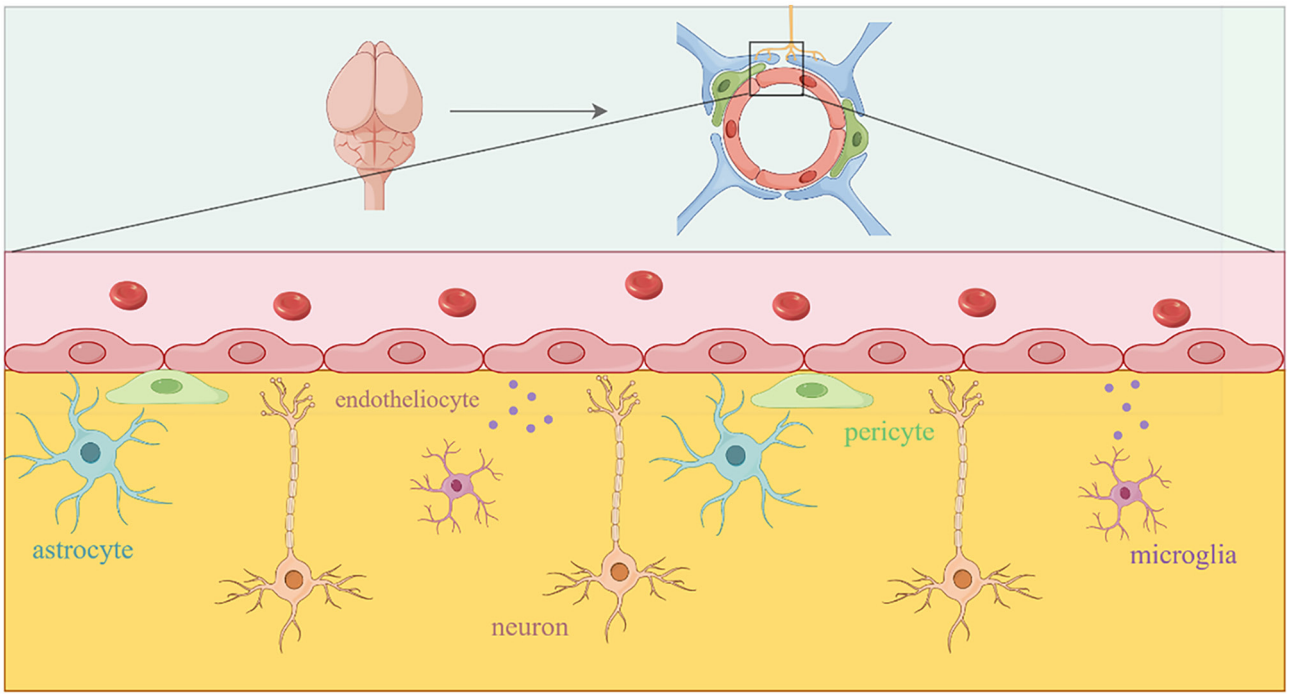


Figure 1. Composition of the neurovascular unit and the blood-brain barrier.

(HMW-HA) into low-molecular-weight (LMW)-HA. The LMW-HA enters cells through the membrane channel, via CD44, inhibiting intracellular p38/ERK signal transduction, and alleviating ERS. Moreover, when TMEM2 is silenced, caspase-3 is highly expressed, and this promotes neuronal apoptosis and cerebral edema (46). In addition, it was determined in our previous study that leucine-rich repeat kinase 2 (LRRK2) inhibition can effectively interfere with Ras-related protein Rab-10 (RAB10) phosphorylation, downregulate lysosomal hydrolase secretion caused by lysosomal overload stress after SBI, and play a protective role in BBB and nerve function (47). Collectively, our previous studies confirmed that the normal structure and function of neurons contribute to BBB integrity maintenance (Fig. 2).

*Summary*. Neurons are considered to be components of the NVU. Neurons can send signals to regulate the neural network when their energy demands change, and this has direct effects on the BBB. Neurons can rapidly transmit chemical information through synapses, and this may be one of the reasons why neurons respond quickly after BBB damage. In addition, ion concentration differences inside and outside the cell will also initiate the release of membrane potential from the neurons, thereby adjusting the brain microenvironment according to the current demand. It is considered that the mechanism by which neurons participate in BBB regulation is related to their signaling function in the brain, and some new therapeutic targets may emerge by studying this cellular communication.

At present, it is generally considered by most, that it is difficult to regenerate neurons in the adult CNS, and the function of the CNS can be restored after damage because of the compensation of other neurons around it. However, whether the BBB can be damaged by regulating the metabolism of peripheral neurons to play a role in repair is still unknown. The position of neurons is crucial. However, the mechanisms that could explain the direct molecular effects on the integrity of the BBB remain to be determined.

## 3. Microglia in the BBB

*Physiological functions of microglia*. Microglia have not yet been determined as direct contributors to BBB maintenance. However, structurally, most para-vascular microglia are located in the perivascular space and are closely related to vascular function (48). Vascular-associated microglia initially maintain the integrity of the BBB by expressing the tight junction (TJ) protein, claudin-5, and make physical contact with ECs (49). Microglia also function through direct contact with neuronal synapses. Microglia monitor their surroundings and continuously extend and retract their cellular protuberance, making direct contact with neuronal synapses at an hourly rate. This continuous and rapid monitoring is a unique function of microglia in the brain (50). In addition, microglia can secrete a variety of cytokines and growth factors, such as brain-derived neurotrophic factor (BDNF), IL-10, and transforming growth factor-β (TGF-β), to help repair local nerve tissue (51,52). These functions of microglia play important roles in restoring brain balance (53). Normally, the CNS is an immunologically privileged region, and the BBB separates the CNS from the inflammatory state to maintain brain homeostasis (54). However, microglia release high levels of inflammatory factors as they are CNS immune cells. Following brain injury, they are the first to be activated among all immune cells to destroy the BBB and participate in the pathological process of neuroinflammation (55,56). The inflammatory environment in the brain is an important factor in BBB destruction. Immune cells cross the BBB and infiltrate

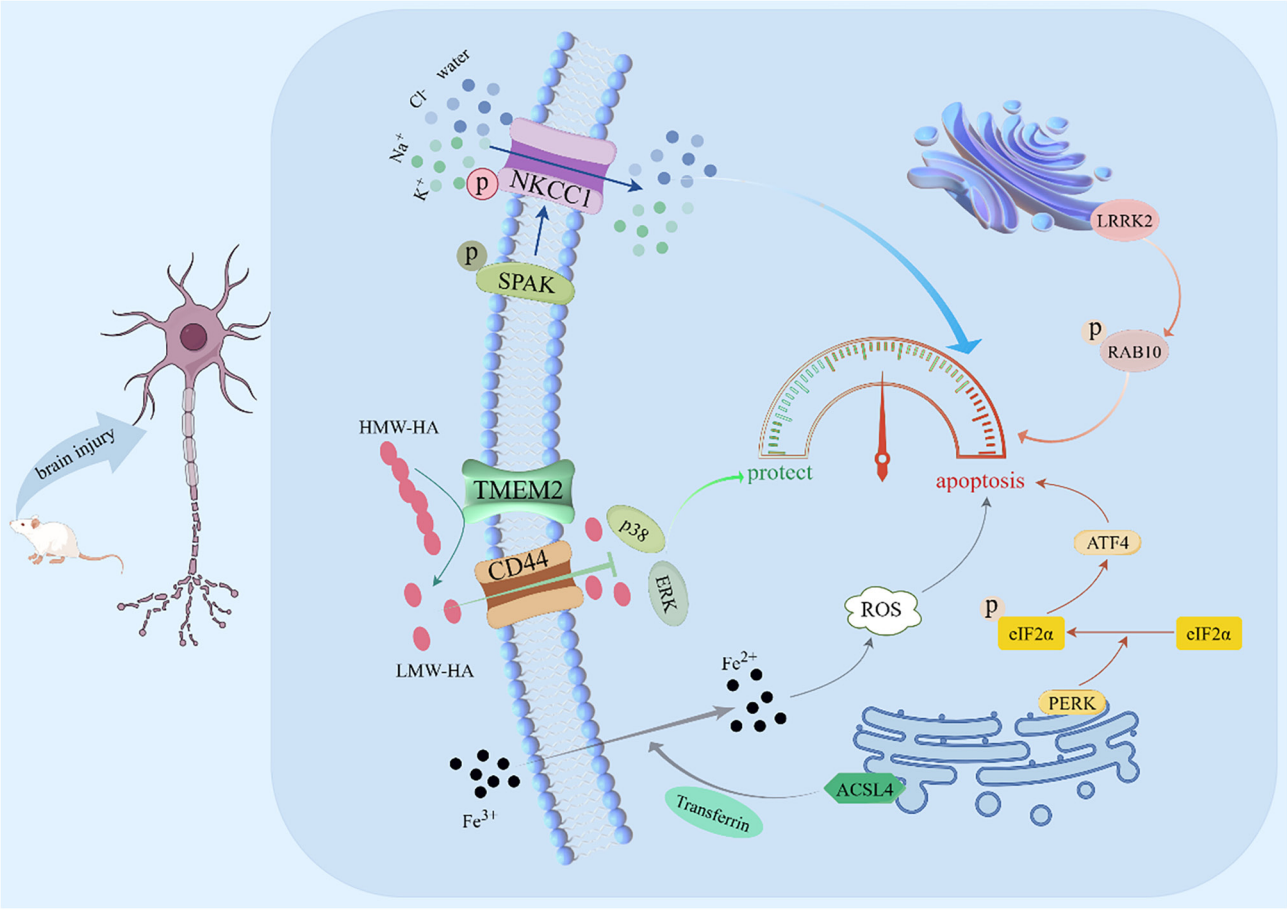


Figure 2. Molecular mechanism of neuronal apoptosis after brain injury. For example, after brain injury, SPAK is activated in neurons and phosphorylates downstream NKCC1. Sodium potassium chloride plasma and water enter neuronal cells, and the cells swell and rupture. This causes a pro-apoptotic effect. The TMEM2 membrane protein breaks down extracellular high-molecular-weight hyaluronic acid to low-molecular-weight hyaluronic acid and inhibits p38 and ERK signaling through CD44 entry into cells to exert brain protective effects. ACSL4 in the endoplasmic reticulum can convert 3-valent iron to 2-valent iron and activate the ROS system to accelerate cell apoptosis. PERK upregulates ATF4 to promote apoptosis by phosphorylating eIF2α. In addition, lysosomes promote neuronal apoptosis by secreting LRRK2 to phosphorylate RAB10. The arrows represent activation or upregulation, and the transverse lines represent inhibition. SPAK, STE20/SPS1-related proline/alanine-rich protein kinase; NKCC1, $Na^+$-$K^+$-$Cl^-$ cotransporter 1; TMEM2, transmembrane protein 2; HMW-HA, high-molecular-weight hyaluronic acid; LMW-HA, low-molecular-weight hyaluronic acid; ACSL4, acyl-CoA synthetase long chain family member 4; ROS, reactive oxygen species; PERK, R-like endoplasmic reticulum kinase; ATF4, activating transcription factor 4; eIF2α, eukaryotic translation initiation factor 2α; LRRK2, leucine-rich repeat kinase 2; RAB10, Ras-related protein Rab-10; p, phosphorylated.

the brain parenchyma through paracellular and transcellular pathways, and this leads to extensive neuroinflammation (57). It is known that neuroinflammation is an important driver of numerous diseases and is closely related to the BBB. Proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-18, tumor necrosis factor (TNF), chemokine ligand (CCL)1 and CCL5, small molecule messengers (prostaglandins, NO, and reactive oxygen species) are produced in large quantities during neuroinflammation. Microglia are one of the main cells involved in this process. As the key cell of brain inflammation, when its immune function is activated, inflammatory factors are released in large quantities. This induces aquaporin 4 (AQP4) upregulation. TJ expression disruption in the ECs affects their functions, leading to vascular disintegration and severe barrier dysfunction (58,59). The damaged barrier displays more leakage, and some of the serum proteins enter the brain to recruit microglia into the blood vessels to phagocytize ECs and destroy the BBB (60). Moreover, serum proteins that leak into brain tissue can bind to local cells and induce secondary brain injury.

*Pathophysiological mechanisms of microglia regulation of the BBB*. The aforementioned inflammatory effects of microglia are closely related to their morphology. Microglia are activated and polarized into different phenotypes that include M1 (pro-inflammatory effect) and M2 (anti-inflammatory effect) (61). Cerebral edema is one of the most common BBB injury manifestations and can be caused by neuronal cytotoxic edema. Neuronal axons are damaged, and myelin degradation produces a large amount of cellular debris (62). M1 microglia are capable of engulfing these cellular debris, apoptotic neurons, and harmful substances such as inflammatory cytokines and chemokines that are produced during injury (63,64). However, with an increase in M1 microglia, the phagocytic function gradually decreases, inflammatory cells are recruited, and neurotoxic substances accumulate in the brain. This leads to neuronal death and further destruction of the BBB (65,66). NF-κB regulates the expression of most microglia characteristic genes of the M1 phenotype. Peroxisome proliferator activated receptor (PPAR)-γ in activated microglia can inhibit NF-κB signaling to downregulate the microglia 'M1' and

reduce the expression levels of inducible nitric oxide synthase (iNOS), TNF, IL-1, IL-6, and IL-23 (67-69). In addition, NF-κB has been revealed to promote IL-4 secretion and microglia M2 polarization, thereby improving the tube formation function and cell connection ability of BMECs (70,71). Research has shown that small nucleolar RNA host gene 8 activates microglia to release inflammatory factors to damage BMECs by targeting the sirtuin1 (SIRT1)-mediated NF-κB pathway (72). High mobility group protein B1 interacts with Toll-like receptor 4 to activate the NF-κB/NLRP3 signaling pathway through myeloid differential protein-88 and induce the transcription of proinflammatory cytokines (66). Inhibition of this signaling pathway induces the upregulation of M2 markers, CD206 and arginase 1, and the downregulation of M1 marker proteins, CD86 and iNOS, resulting in the conversion of microglia (73,74). The Notch1/Hes1/Stat3 signaling pathway plays an important role in microglia activation, reprogramming M1 to M2 by inhibiting NF-κB p65 translocation (75). Microglia-mediated inflammation is an important cause of BBB disruption. As the most predominant proinflammatory cell in the NS, microglia also disrupt the BBB by engulfing astrocyte endfeet during persistent inflammation (49).

Recent studies have found that the expression of triggering receptor expressed on myeloid cells 2 (TREM2) in microglia is closely related to BBB destruction after brain injury (76,77). These findings suggest that TREM2 is closely related to the BBB, which is consistent with the results of our study (78). Following brain injury, TREM2 was revealed to be highly expressed in the microglia of the injured side of the brain and negatively correlated with the diffusion of inflammatory factors. TREM2 downregulation resulted in upregulation of NF-κB-mediated inflammatory signals such as IL-6, IL-1β, and TNF-α. With a large amount of nerve cell apoptosis, the BBB was disrupted, albumin exudated into the brain parenchyma surrounding the lesion, and the brain water content on the affected side increased. These results indicated that TREM2 can protect nerve cells and maintain CNS homeostasis (78).

*Summary*. It was determined that under pathological conditions, microglia morphologies rapidly change to larger cell bodies and shorter processes. This change may be due to a shift in the role of microglia from central monitors to phagocytes. This may have bidirectional effects after BBB disruption. Microglia help remove cellular debris, but they also release inflammatory factors and aggravate neuroinflammation. The benefits and harms of this bidirectional effect of microglia on the BBB cannot be assessed. As important nerve cells, microglia cannot be removed to conduct a holistic study. Thus, the mechanism by which microglia restore the function of the BBB has yet to be determined.

## 4. Astrocytes in the BBB

*Anatomical location and physiological function of astrocytes*. As the key point of brain metabolism regulation, astrocytic endfeet are attached to blood vessels and establish a direct interface with the vascular chamber of the NVU. They are closely attached to the basal membrane of blood vessels and form a boundary along the perivascular space, an important part of the BBB (79). Astrocytes are widely distributed in the CNS, and they fill the space outside neurons and blood vessels and secrete neurotrophic factors that support and nourish neurons (80). Astrocytes have isolation and barrier functions. They can prevent neighboring neurons from interfering with each other and have a certain selective effect on the transport of substances, and they participate in BBB formation (81,82). As satellite cells of the NS, astrocytes divide the cortex and gray matter into functional areas. In addition, a single glial cell regulates the internal and external output, guiding nerve axon regeneration (79,83). A single astrocyte forms higher-order glial vascular units along capillaries that match local neural activity and blood flow, coordinate neuronal firing thresholds, and provide metabolic support for neurons and their synapses (84). Astrocytic endfeet extend to the periphery of brain microvessels, control the barrier function of BMECs through derived factors and physical contact, and regulate the BBB bidirectionally (85).

Astrocytes can divide and proliferate, unlike neurons. A damaged or degenerated brain primarily relies on astrocyte proliferation to fill the tissue defect. It has been confirmed that astrocytes can be induced to differentiate into neurons *in vitro*, and this provides hope for the treatment of a variety of neurodegenerative diseases (86). It has been observed that altered astrocyte communication can induce neuronal apoptosis and phagocytosis of broken neurons, and this has direct consequences for neuropathology (87,88). However, astrocytes detect glutamatergic and gabaergic neuronal signals and translate them into commands to expand/contract blood vessels (89). They promote the transmission of ions, amino acids, neurotransmitters, and other substances in the brain by absorbing and regulating nerve molecules, and they also establish water homeostasis in the brain (90,91). Thus, they regulate the local microenvironment and affect the extent of BBB damage and subsequent repair. Astrocytes can stabilize $K^+$ and $H^+$ concentrations in the extracellular fluid of the CNS during normal brain activity (92). Water is drained from the brain and out of the body, while neurotransmitters and ions are recycled. When proliferating astrocytes produce scarring, the absorption capacity for $K^+$ and $H^+$ is correspondingly weakened. This results in high $K^+$ in the local extracellular fluid. These boundary and scar functions play important roles in maintaining the physiological homeostasis of the CNS, supporting nerve function and signal transduction (93,94). Following brain injury, the morphology of astrocytes changes from static to reactive. This morphological change affects the BBB and induces brain pathology (95,96). In summary, astrocytes support neuronal cells by regulating neurofactors and preventing hemodynamic and metabolic disorders. They create conditions for the survival and recovery of neurons, protect the permeability integrity of the BBB, and alleviate secondary brain injury.

There is increasing evidence that astrocytes play a key role in BBB breakdown after brain injury. There are abundant organic anion transporters (OAPs) in the contact interface between the astrocytic endfeet and a blood vessel. When this contact is broken, the density of OAPs, such as Kir4.1 and AQP4, that are carried by the astrocytic endfeet decreases. The ion and water imbalances cannot maintain the BBB function, cell edema increases, the cytoskeleton is destroyed, and the brain is damaged further (97,98). Astrocytes secrete vascular

permeability factors that enhance the BBB permeability, including vascular endothelial growth factor (VEGF) (99), NO (100), glutamic acid 69, matrix metalloproteinases (MMPs) (101), and endothelin (102).

*Pathophysiological mechanisms of astrocyte regulation of the BBB*. Astrocytes can generate retinoic acid (103), sonic Hedgehog (Shh) (104), angiopoietin-1 (Ang-1) (105), glia-derived neurotrophic factor (106), insulin-like growth factor (107), and apolipoprotein E (108) to help restore the BBB. Numerous factors released by astrocytes have matching receptors on the brain endothelium that can transmit neuronal activity signals to the ECs and improve the efficiency of nutrition and metabolism of the brain endothelium (109). Genes involved in BBB regulation have also been found in perivascular astrocytes, including integrin subunit α7 (Itga7) (110). Itga7 promotes EC junction integrity by adhering to laminin. Knockdown of Itga7 downregulates fibronectin expression, a major component of the brain barrier. This results in a significant reduction in astrocyte coverage around brain microvessels (111,112). Therefore, how does Itga7 work? Studies have shown that Itga7 activates STAT3 via TNF and increases the expression of serpin family A member 3 encoding the α1-anticoagulant trypsin protein. This prompts astrocytes to enter an inflammatory state (110,113). However, triglyceride-rich lipoprotein (TGRL) liposomes activate the ER protein, CHOP, and NF-κB pathways through the JNK/cJUN/ATF3 pathway to further aggravate inflammatory factor secretions (114). Growth differentiation factor 15 (GDF15) is an important factor in astrocyte remodeling, and it is related to the enhancement of tight BBB connections. TGRL lipolysis products activate GDF15 in astrocytes. Increased GDF15 expression promotes astrocyte remodeling and enhances barrier properties between ECs via claudin-5 (115). Astrocyte-derived TGF-β may maintain BBB function by regulating Hedgehog signaling in BMECs (116). TGF-β signal overactivation is a necessary and sufficient condition to cause neurological dysfunction and BBB-related pathology (117). With further research, cellular metabolism was found to be involved in these coordination processes. For example, high blood glucose inhibits astrocyte activation and destroys endfeet. This increases neutrophil infiltration in the brain, leading to BBB leakage (118). Hypertonic saline alleviates BBB permeability by inhibiting the downregulation of VEGF expression by NF-κB p65 (119). Intracellular high iron levels regulate BMECs by increasing hepcidin and reducing the uptake of iron from the circulation by the BBB (120).

Several receptors have been found on brain ECs and astrocytes to cause an increase in intracellular $Ca^{2+}$ upon activation. Upregulation of $Ca^{2+}$ regulates the activities of neurons, astrocytes, and ECs (121,122). When $Ca^{2+}$ ions are activated, they send signals from the CNS nerve cells to neighboring cells through the astrocytes. This releases a signal to dilate the vascular space, and a TJ is opened (123,124). Perivascular astrocyte processes regulate the TJs around brain ECs. This pattern of signal transduction between neuroglia and ECs is of great significance for the physiological and pathological regulation of the BBB by brain ECs. Several astrocyte-derived factors have been found to disrupt vascular permeability by decreasing the endothelial TJ protein expression or inducing EC apoptosis. VEGFA expression is a key driver of BBB permeability, and astrocytes drive BBB opening by VEGFA (125). Upregulation of Apelin-13 promotes the phosphorylation of ERK, Akt, and AQP4 in astrocytes (126). Following the upregulation of Apelin-13/APOE4 and NF-κB/HIF-1α signals, MMP9 and VEGF secretions also increase. The reduction of astrocytes endfeet that cover blood vessels and TJ damage are evidence of BBB damage (127,128). Lee *et al* (129) further found that src-suppressed C-kinase substrate reduced VEGF by decreasing Apelin-1 while stimulating the upregulation of Ang-1. This regulates cerebral angiogenesis and TJs between cells, thereby affecting BBB differentiation (129). Proteins such as astrocytic N-myc downstream regulatory gene 2 and thrombospondin-2 also act as regulators of BBB permeability. They play roles in BBB permeability and immune cell infiltration regulation by affecting MMP expression (130,131).

Astrocytes are often one of the major cell types that initiate the inflammatory cascade. Astrocyte swelling is considered to be an early sign of cytotoxic edema. The inflammatory environment caused by brain injury mediates astrocyte signaling, and this is an important cause of cellular swelling (132). The degradation of the extracellular matrix (ECM) and TJ proteins activates astrocytes, stimulates glial cells to release inflammatory factors that aggravate neuroinflammation, disrupts the TJs of vascular ECs, and accelerates BBB permeability (42,133). Collagen-IV is a major BBB basement membrane component, and its degradation can lead to basement membrane dysfunction. Our previous study showed that MMP-9 was secreted by astrocytes after a TBI (133). This degraded endothelial TJ proteins and the ECM, including occludin and collagen-IV. It was also determined that in addition to directly destroying basement membrane and TJ proteins, MMP-9 also affects TJs by inhibiting the Hedgehog pathway. MMP-9 is upregulated after brain injury, and it partially blocks the Hedgehog pathway and reduces Scube2 and Shh expression levels in astrocytes. This causes further damage to the BBB. Inhibition of this signaling pathway has a protective effect on the BBB mechanism (133,134).

*Summary*. It is considered that the contribution of astrocytes to the BBB is primarily due to their supporting, nutritional, and other auxiliary effects on nerve cells. Astrocytes can secrete a variety of neurotrophic factors to promote the repair or activation of ECs, BMECs, and pericytes so that they provide better barrier characteristics. Furthermore, astrocytes adhere to the wall of microvessels, encase parts of the microvessels, and strengthen the basement membrane. All of these functions play important roles in maintaining BBB homeostasis. However, the role of astrocytes is much more than this. Astrocytes are also able to release glutamate and thus participate in electrical signaling in the NS. They have an important physiological role in the NS, and this also provides new insights for the treatment of complex neurological diseases.

Astrocytes have attracted notable attention due to their pivotal role in the NVU and have been studied as aforementioned. BBB homeostasis imbalance has been implicated in numerous CNS diseases. The role and mechanism of astrocytes in maintaining BBB homeostasis, especially in the transition from normal BBB homeostasis to imbalances of BBB homeostasis in disease states, require further investigations.

## 5. BMECs in the BBB

*Physiological structure and BMEC functions*. The BBB is a selective barrier formed by ECs that line the brain microvessels. As a key NVU regulator, BMECs are key cells for BBB integrity (135). BMECs are a single layer of flat ECs that line the inner surface of cerebral blood vessels. BMECs have a more obese appearance than ECs from other non-neural tissues (136). BMECs have some unique features, such as the presence of numerous intercellular TJs that generate high transendothelial impedance and delay paracellular flux. They lack the fenestra structure of ECs and have low levels of pinocytosis of the liquid phase material (137). In addition, BMECs possess asymmetrically localized enzymes and vector-mediated transport systems.

These characteristics make the connection between two cells tighter, forming a liquid impenetrable barrier that prevents molecules and ions from passing through the intercellular space (37,138). The basement membrane of brain microvessels is intact, and a few phagocytic vesicles are distributed in the vascular lumen. In addition, the number of mitochondria is high (139). BMEC mitochondria account for approximately one-tenth of the cytoplasmic volume, a proportion much higher than that of ECs in other non-BBB tissues (140,141). The high mitochondria proportion helps to improve the efficiency of metabolism and transport, enhance the signal transmitted by sensing neurons, and promote the propagation of vasodilator signals to the tiny arteries in the brain to regulate the vasodilation function (142). BMECs can also transport oxygen and glucose from the blood to the brain to increase its energy supply while emitting carbon dioxide and other waste products in the opposite direction (143,144). This is essential to maintain the physiological function of the NVU and regulate the pH of normal brain metabolism, and is one of the reasons for the sensitivity of the BBB to oxidative stress (145). BMECs can form a tighter capillary endothelium than peripheral ECs, and this aspect renders the BBB essentially impermeable to macromolecules (135). The TJs fill the gaps between BMECs and limit paracellular permeability by expressing TJ proteins such as occludin, claudins, and zonula occludens. These suggest that the TJs of BMECs are indispensable for a normal structure and function of the BBB. The TJs connect adjacent cells and seal intercellular spaces, and this prevents top and bottom membrane proteins from spreading laterally (146,147). The purpose is to block the paracellular fusion pathway of ECs to form a physical barrier. Hydrophobic molecules are allowed to diffuse internally, whereas hydrophilic molecules are hindered by barriers, thus maintaining CNS homeostasis (148). Therefore, drugs that target nerve regions or nerve cells must have the ability to cross the BBB to achieve the desired therapeutic effect (149). BMECs contain transporters, such as ATP synthase-binding cassette (ABC), ABC subfamily member 1, and solute carrier organic anion transporter family member 1C1, as well as receptors, such as endothelial protein C receptor, insulin receptors, transferrin receptors (TfRs), and LDL receptor related protein 1, that can help CNS drugs open the BBB (150). Multiple receptors on BMECs enable cells to respond to inflammatory mediators (151). One of the hallmarks of vascular inflammation is the increased expression of adhesion molecules on the BMEC membrane. Vascular inflammation can affect the infiltration of peripheral immune cells, activate microglia, change the shape of blood vessels, affect angiogenesis, and ultimately destroy blood vessels (152,153).

*Pathophysiological mechanisms of BMEC regulation of the BBB*. The barrier function of BMECs is maintained through close association with other cell types within the NVU. Direct contact between ECs and astrocytes is necessary for BBB formation and functioning (154). When co-cultured with astrocytes, endothelial soluble factors showed higher transendothelial resistance (155). Changes in ECs and astrocytes were also found in cerebral cortex biopsy specimens from patients with brain injuries (156). This may have been related to EC morphological changes. The injured EC surfaces showed longitudinal folds and invagination, the activity was enhanced, and granulation and some multivesicular bodies were observed in the cytoplasm. The space between ECs and astrocytes was significantly enlarged (157). The interaction between astrocytes and ECs involves intercellular and intracellular communication, which is key to BBB induction and maintenance (84,158). Research has found that autophagy related 7 (Atg7) regulates the function of astrocyte adhesion to the BMECs to maintain BBB homeostasis through endothelial fibronectin (159). Atg7 affects the phosphorylation of the cyclic adenosine monophosphate-responsive element-binding protein by regulating protein kinase A activity, thereby triggering endothelial fibronectin expression. Loss of endothelial Atg7 downregulates fibronectin expression, a component of the BBB, and reduces the extent of astrocyte coverage of cerebral microvessels (159). Other signaling mechanisms between ECs and the BBB have also been gradually discovered. Among them, the VEGF/VEGF receptor (VEGFR) pathway, Notch, and Wnt pathway are considered to be the key signaling pathways that drive the phenotype of BMECs during BBB formation (160). The VEGF/VEGFR signaling pathway is related to the proliferation, invasion, migration, and survival of ECs and is involved in the activation of most mechanisms of angiogenesis (161). The endothelial nitric oxide synthase (eNOS)/NO pathway regulates vascular remodeling, and inhibition of this signaling pathway destroys VEGF. This leads to impaired angiogenesis (162). The eNOS/NO pathway in ECs is related to BDNF. After the impairment of this pathway, BDNF is downregulated, and this affects neurotransmitter release, thereby impairing neuronal regeneration and leading to BBB leakage (163). It is worth noting that the eNOS/NO pathway in BMECs is closely aligned with mitochondria-associated oxidative stress, and as a downstream effector of VEGF, the reduction of NO impairs the VEGF/VEGFR pathway (164). Wnt signaling is also essential for CNS angiogenesis and BBB formation (165). In addition, Notch signaling is involved in EC proliferation and survival processes (166). SIRT1 promotes angiogenesis by promoting VEGFR-2 expression through Notch signaling (167). Taken together, BBB integrity depends to a large extent on the interaction of BEMCs with other cells in the NVU.

VEGF is a growth factor that induces angiogenesis and increases vascular permeability, and it has a key role in BBB integrity loss (168). The blocking of VEGF was demonstrated to alleviate the degradation of the TJ protein occludin (169).

Collagen-IV is a major BBB basement membrane component, and its degradation can lead to dysfunction of the basement membrane; this may be one of the causes of BBB disruption after a brain injury (170). Our results, in a previous study showed that in the brain tissues surrounding the TBI in rats, VEGF expression increased, and occludin and collagen-IV expression levels decreased. The basement membrane and TJs of the BBB were disrupted. The TUNEL and Fluoro Jade C-positive cell levels increased, nerve cells were apoptotic, and the BBB was impaired. VEGF inhibition protected the BBB and reduced TBI-induced brain edema. This effect may be achieved through MMP9 regulation of occludin and collagen-IV (133).

*Summary*. As one of the major cell types constituting the BBB, the contribution of BMECs to the BBB originates from their structural location. BMECs form the BBB through TJs and gap junctions that prevent the passage of macromolecular substances. Of course, in addition to forming barriers, BMECs also participate in a variety of neural metabolism activities and release neuroactive substances that affect neural function. These mechanisms all serve to further regulate the brain microenvironment and protect the BBB.

## 6. Pericytes in the BBB

*Physiological structure and functions of pericytes*. Pericytes were discovered as early as 1873 by French scientist Charles Benjamin Rouget, and they were originally called 'Rouget's' cells. They were later officially named 'pericytes' by Karl Wilhelm Zimmermann (171). Pericytes are an important component of the NVU, and they attach to the lateral wall of the lumen of ECs in capillaries, embed into ECs, and cover approximately one-third of the capillary wall. Together with vascular ECs, they constitute the cellular component of the blood vessel wall and play an important role in the BBB (172). Within the NVU, pericytes are located in a unique location between neurons, astrocytes, and ECs, and their coverage is inversely proportional to the permeability of the blood vessels (173). The cytoplasm of pericyte stellate processes is wrapped outside the vascular basement membrane and is primarily distributed at the TJs of ECs. ECs are surrounded by pericytes and communicate directly with the microvascular endothelium through physical contact and paracrine signals. The structural substances constituting the basement membrane and the ECM, including integrins, N-cadherin, fibronectin, and connexin, regulate capillary permeability and participate in the perfusion and infiltration of microcirculation (171). Pericytes have contractility similar to smooth muscle cells and can regulate cerebral vessel formation and maturation (174). The cerebral angiogenesis signaling pathway is an important regulator that maintains the normal structure of the BBB, and its signaling pathway abnormality can lead to an aberrant BBB structure. Studies have shown that pericytes express receptors for vascular mediators, such as catecholamines, β-adrenoceptors, thromboxane $A_2$, vasoactive intestinal peptide, endothelin-1, and vasopressin, that play crucial roles in maintaining vascular wall stability (175,176). Peripheral cells also participate in angiogenesis by expressing TGF-β, VEGF, Ang-1/2, angiotensin I, and other vascular-related factors (177). Type IV collagen, polysaccharides, and laminin synthesized by pericytes are also important for basement membrane formation. TfR1 is one of the known markers of the BBB, and TfR1 is downregulated in ECs lacking pericellular contact (178). It has been shown that capillary permeability increases after pericyte depletion. This phenomenon supports that pericytes have an important role in maintaining endothelial barrier function (179).

*Pathophysiological mechanisms of pericyte regulation of the BBB*. Pericytes share a basement membrane with ECs as they can regulate microcirculatory blood flow. Direct synapse-like contacts are formed with ECs via N-cadherin and connexin (180). The interaction between pericytes and BMECs is essential for basement membranes and TJs and directly affects BBB permeability. Gap junctions, focal adhesion, and nail hole junctions are three ways in which pericytes interact with ECs (181). Gap junctions transmit ionic currents and small molecules between neighboring cells (182). Focal adhesion kinase was revealed to immobilize capillary pericytes on ECs (183). Pericytes play a key role in BBB homeostasis by communicating with ECs through these specialized roles (184). Daneman *et al* (185) found that ECs co-cultured with pericytes had reduced intercellular spaces and increased transepithelial electrical resistance. Abundant TJ proteins that included occludin and claudin 5 were present at the cell boundary, suggesting that the pericytes enhanced the TJ function (185). ECs associated with pericytes are more resistant to apoptosis than ECs alone and have a protective effect on ECs. This provides new evidence that pericytes together with ECs support BBB structural integrity. The phagocytic function of ECs is enhanced after pericyte depletion. Vascular permeability genes, such as Angpt2, Plvap, and LAM, were increased, and molecular transporters were overexpressed. Some of the macromolecules are allowed to cross the BBB to affect the brain parenchyma (186). In addition, the receptor, PDGFR, expressed by pericytes can respond rapidly after brain injury, mediate pericyte migration to ECs, release fibrinolytic enzymes, and increase BBB permeability (187). It is worth exploring that in the absence of pericytes, embryonic brain ECs can still express some BBB-specific molecules. This may be induced by neural progenitor cells that then regulate the functional integrity of the BBB during development through pericytes and adult astrocytes (188).

Pericytes are an important part of brain capillaries that have a phagocytic function and can remove cell debris and regulate capillary blood flow and BBB permeability. Pericytes in different vascular beds have different frequencies and can transform into multipotent vascular stem cells that generate microglia in the late stage of brain injury and participate in microglia proliferation to acquire a microglial phenotype with a phagocytic capacity (189,190). Pericytes can also direct astrocytic endfeet into the endothelium (191). The interaction between pericytes, ECs, and astrocytes can regulate angiogenesis and hemodynamics and maintain BBB integrity. This is essential for normal brain function.

Currently, pericyte loss is considered to be a hallmark of BBB dysfunction, and it causes a variety of secondary brain injuries such as brain edema and brain parenchymal lesions (192). Oxidative stress, a high lipid and high glucose

internal environment, and toxic substances in the blood can damage pericytes. This then destroys the BBB. Aβ is the most widely studied of the toxic substances that cause BBB damage. Aβ binds to RAGE to increase oxidative stress, promoting Aβ accumulation in the brain. This process causes BMEC cytotoxicity and induces pericyte ferroptosis (193-195).

*Summary*. The role of pericytes in the BBB has been further revealed with increasing research. Pericytes are located lateral to the BMECs and control the endothelial function through direct contact to maintain BBB stability. In addition, pericytes are involved in physiological processes such as substance transport and signal transmission to ensure normal brain function. Understanding pericyte functions helps us to better understand BBB processes and their importance in protecting the brain from harmful agents.

## 7. Conclusion

The normal functioning of the brain depends on the interactions between the components of the NVU. The positive role of these cells in CNS homeostasis is encouraging. Neurons, glial cells, pericytes and vascular ECs constitute the BBB, which provides a stable internal environment for the realization of nerve function. Among them, astrocytes are closely related to neuronutrition and repair, and it is speculated that astrocytes may also have some benign effects on nerve cells and brain microvascular diseases, such as the promotion of microvascular and nerve injury regeneration to help BBB function repair. However, further studies are required to confirm this. The focus on the relationship between neuroinflammation and the BBB has uncovered a notable phenomenon. Activated microglia and reactive astrocytes communicate and cooperate with each other in the NVU to achieve immune 'optimization'. Brain inflammatory signals are amplified not only by microglial activation but also by the unique anatomy of astrocytes in the immune network (196,197). Microglia and astrocyte interactions may become an effective and accurate therapeutic target in the future with further technological advances. These NVU cell-to-cell interactions are critical to regulate cerebrovascular functions. BBB permeability alterations after acute CNS injury are due to a loss or alteration of these cellular structures, and understanding the structure and mechanisms of these cells is important to control injury progression.

Various NVU components and their interactions are essential to maintain BBB stability. The damage of the BBB is the pathological basis of vascular cerebral edema. The destruction of the BBB can lead to brain edema and increased intracranial pressure, which in turn can aggravate brain edema and cause functional and structural damage to brain tissue. If the brain edema progresses from localized to diffuse, irreversible secondary pathological changes are formed, and even brain death is developed. Brian edema is often secondary to neurological diseases such as TBI, intracranial space occupying lesions, cerebrovascular diseases, cerebral parasitosis and some cerebral congenital diseases. The severity of cerebral edema is related to the course and prognosis of intracranial diseases and has important significance. In the field of neurology, if we can better understand the mechanism of the BBB and effectively prevent and treat the secondary brain edema, we can achieve better results in the treatment of intracranial diseases. The therapeutic potential of the BBB in a variety of neurological diseases has great potential. Hence, it is necessary to investigate the effect of the NVU on the BBB as a whole to further reveal possible therapeutic mechanisms.

The recovery of the barrier function is considered to be the key to the treatment of NS diseases. An exploration of the NVU composition and its cell-cell interactions will help to further unlock the mechanism of the BBB and provide new insights for the clinical treatment of NS diseases. Drug therapy should protect the whole NVU from different angles and in various ways. The exploration of multi-target protective agents has become one of the important research and development directions for the treatment of NS diseases in the future.

In conclusion, it is considered that the NVU should be regarded as a functional whole, and the dynamic interaction between all cells affects brain function. However, most of the current BBB studies have only focused on a single nerve cell or target, and few have studied a variety of nerve cells and brain microvascular endothelium as a whole. In the future, focus will be on the cellular structure and function of the NVU, and exploration on how a variety of cells combine to repair the BBB after disruption.

## Acknowledgements

Not applicable.

## Funding

The present review was supported by the National Natural Science Foundation of China (grant no. 82205254), the 11th Batch of Science and Technology Development Projects of Suzhou in 2023 (grant no. SKY2023014), the University-local Collaborative Innovation Research Project (grant no. 20239606), the Jiangsu Traditional Chinese Medicine Science and Technology Development Program project (grant no. MS2023101) and the Wumen Medical Project of Suzhou (grant no. SKYXD2022034).

## Availability of data and materials

The literature analyzed during the current study are available in the PubMed (https://pubmed.ncbi.nlm.nih.gov/), Embase (https://www.embase.com/), Ovid (https://ovidsp.ovid.com/), CNKI (https://www.cnki.net/) and Cochrane Library (https://www.cochranelibrary.com/library) repositories.

## Authors' contributions

BD and YG conceived and designed the manuscript. MW, XH and YH searched the literature and wrote the manuscript. JY created the figures. BD reviewed and revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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