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REVIEW ARTICLE

Blood-nerve barrier: Structure and opening

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Abstract

Like the blood-brain barrier and blood-spinal cord barrier, the blood-nerve barrier (BNB) is one of the crucial tissue barriers of the nervous system. It plays a vital role in homeostasis, physiological protection, and pathological reactions. Various factors, such as biological, physical, and chemical factors, can lead to transient or permanent dysfunction of the BNB. With the advancements in biological techniques and the growing peripheral nerve injuries such as trauma and diabetic peripheral neuropathy, the BNB has gained increasing attention. Moreover, the defensive function of the BNB impedes therapeutic deliveries and anesthetic drugs, which compromises the therapeutic experiences and life quality. It should be noted that numerous pathways are involved in the microstructure, function, and opening of the BNB, but the true underlying molecular mechanisms are still under constant exploration and investigation. This review summarizes the microstructure, and signaling pathways of the BNB, and thoroughly discusses the transient or permanent disruptions of the BNB in both physiological and pathological conditions.

Keywords: Blood-nerve barrier, peripheral nerve system, endoneurial blood vessel, structure and function integrity, opening

INTRODUCTION

The normal functions of the physiological system rely on many factors, tissue barrier is one of them. The blood-brain barrier (BBB)[1,2], air-blood barrier[3], gut-blood barrier[4,5], and retinal-blood barrier[6] are the commonly discussed and studied barriers, which play unreplaceable roles in shielding easily vulnerable tissues from physical, chemical, and biological stimulus.[1,7] Blood-nerve barrier (BNB)[7-9], assumes this role in the peripheral nervous system (PNS)[7], is typically considered to consist of the perineurium and endoneurial blood vessels, and is regarded as a critical interface between blood flow and the innermost compartment of nerve[9,0], the TJ sealed basement membrane (BM) and layers of perineurial cells compose the perineurium.[8] Furthermore, the endoneurium and inner part of the perineurium constitute specialized endothelial cell (EC) and myofibroblasts, which are interconnected through tight junctions (TJs).[11,12] Moreover, pericyte is attached to the outer side of the vascular EC, and both types of cells are covered by BM.[9,13] The BNB maintains a safe and stable environment for axons, Schwann cells (SCs), other endoneurial cells, and both functional and non-functional entities.[14] Not only does the BNB play a role in preventing the axons, SCs, and other cells from external injuries to the most, but also restricts the aberrant migration of various cells and soluble substances, for example, potassium (a high level of potassium could disrupt electrical conductance).[14,15] Collectively, the structure and function integrity of the BNB is the precondition of the normal homeostasis of the nerve. Nevertheless, the tight structure and functional junction of BNB may impede the delivery of therapeutic and anesthetic agents.[9,16,17] In such circumstances, the presence of BNB could significantly hinder treatment efficacy and anesthesia effectiveness.

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Due to the similarities in cellular composition, cellular junction, and function, the BNB and BBB are often undeterminedly brought up together for comparison and discussion.[18,19] But the two barriers are extremely different from their structures, as the vessels in the BBB are covered by cells, such as the pericytes and astrocytes, there is a high degree of pericyte coverage, and the rest surface is covered by the astrocyte endfeet.[20,21] However, except for the absence of astrocytes, there is a significant variation in the coverage of pericytes in BNB compared to BBB, with an average of 54% from 2D imaging and 33.86% from 3D imaging.[9] For the insufficient coverage of the EC and the lack of the end foot of the astrocyte, the barrier function of the BNB is weaker than BBB.[22,23] On the contrary, some scholars also believe that the BNB exhibits barrier function despite low cell coverage[9], the shortage in EC coverage might be redressed physiologically by the perineurium.[10] (Figure 1)

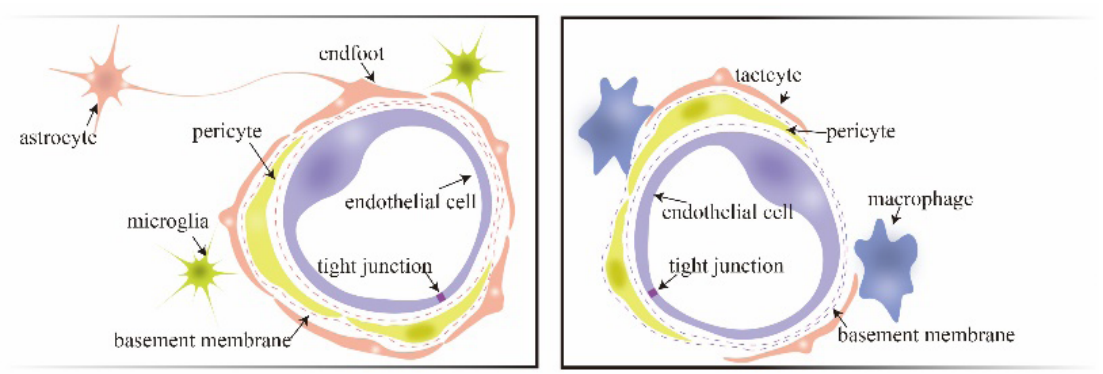
Unlike BBB, which exploration has been put forward to a profound degree[24,25], there is far from research on BNB despite recent years progress. Studies have demonstrated different measures to open the BNB[16,17], and many signaling pathways have been found involved in the structure and function adjustments of it.[26,27] A thorough grasp of the structure, signaling pathway, and physiologically or pathologically transient or permanent permeability disruption of BNB is imminent for further clinical practices and basic research. This review intends to provide a comprehensive summary of the recent advancements in the understanding of the structure, opening, and signaling pathway of BNB, the disruptions of the BNB under physiological and pathological conditions are also deeply discussed to better elucidate their fundamental physiology and pathology, provide an orientation for the exploration of novel instruments, therapeutic drugs, and narcotic drugs toward this barrier.[16,28]

STRUCTURE OF BLOOD-NERVE BARRIER

Despite the BNB is not as extensively researched as BBB, a decent understanding of BNB's overall structure has been gained in recent years. As a functional entity, the BNB relies on the basic unit to carry out its function, its vascular unit (VU) plays a fundamental role in the function.[9] The unit serves not only as the basic building blocks of the BNB function but also as a crucial channel for further in-depth research and exploration of the BNB.[9] Furthermore, some cells and structures enhance the barrier function, and support and protect the barrier, such as SC[9], macrophage[9,29], pericyte[9], tactocyte, and TJ.[7,9] What should be noted is that although transendothelial electrical resistance (TEER)[30] is not a physical structure but a functional parameter, it plays a crucial role in assessing and maintaining the function of the BNB. Therefore, it is important to include the discussion of TEER in this part.

VASCULAR UNIT

BNB, which localizes in an endoneurial blood vessel of the peripheral nerve, plays a crucial role in the maintenance and homeostasis of the nerve and adjacent structures.[9] (Figure 2) VU is



blood-brain barrier blood-nerve barrier

Figure 1. The diagram illustrates the differences between the blood-brain barrier and the blood-nerve barrier. Both barriers consist of endothelial cells, a tight junction that closely connects endothelial cells, pericytes, and the surrounding basement membrane. The difference lies in the presence of the astrocytic end foot and surrounding microcytes in the blood-brain barrier, and the astrocyte and macrophage in the blood-nerve barrier. The endoneurial endothelial cell, closely collaborated pericyte, and slightly connected tactocyte and macrophage consist of the vascular unit of the blood-nerve barrier, the endothelial cell, and the pericyte are firmly enveloped by the basement membrane.

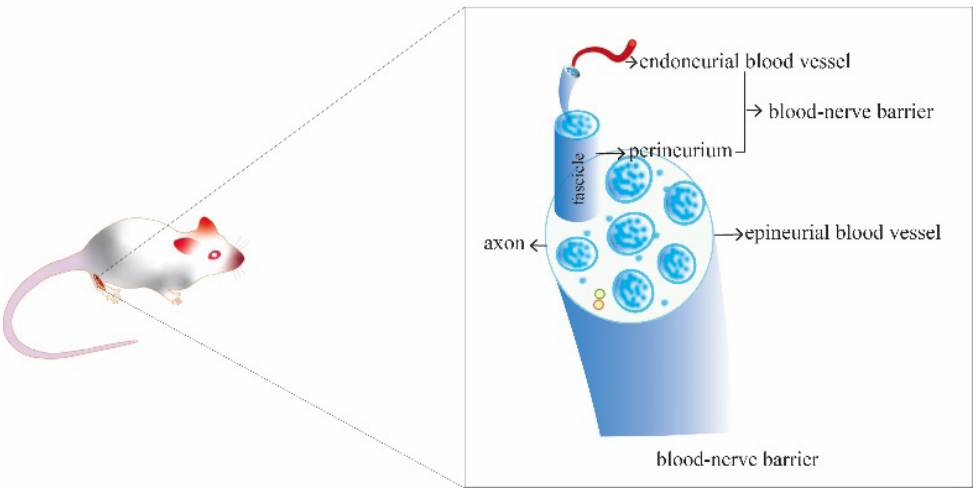


Figure 2. Schematic illustrates the structure of the peripheral nerve and the components of the blood-nerve barrier, which consists of the endoneurial blood vessel and perineurium.

the basic element of BNB, it confers PNS the specializations of transcytosis and parasitosis transport like the central nervous system (CNS).[31,33] Moreover, it is defined as endoneurial EC combined with tightly collaborating pericyte and loosely connected tactocyte and macrophage[9,34], the EC is connected by TJ[11,12] and attaches to pericyte, moreover, the two kinds of cells are covered by BM.[9,13] (Figure 1) It's reported that VU is conserved across organisms, nerve types, and even different mammalian species.[9] Notably, the BNB is not only present in mammals but also in other biological populations. In Drosophila melanogaster, for example, the perineurium and sub perineurium form the BNB, and the cortex glia beneath the subperineurium enclose and provide sheathing for axons.[15]

Even though the endoneurial EC and VU are surrounded by various cells or molecules, it is important to note that the EC in the BNB is not always completely covered by other cells like the BBB. This means that total cellular coverage of the vascular endothelium may not be strictly essential for the normal function of the BNB.[9] This property enables the possibilities of utilizing physical, biological, or chemical forces to functionally open the BNB and facilitate the delivery of therapeutic drugs or anesthetic drugs[16,17,35], techniques such as focused ultrasound and MRI-guided focused ultrasound have been demonstrated to be safe, feasible, and reversible.[16,17,28]

SCHWANN CELL

Schwann cell (SC), which accounts for about 70% of cells in the endoneurium cell population[9], primarily distributes in specific regions, it exhibits path-different but destination-similar functions and mainly encompasses myelinating Schwann cell (mSC) and non-myelinating Schwann cell (nmSC) in the nerve root, trunk and terminal after differentiation and radial sorting.[36,37] MSC predominantly presents in the nerve trunk, surrounding large-diameter axons and forming compact myelin sheath to ensure rapid conduction of potential and excitation[38], but there is no direct interaction between SC and endoneurium in the. VU[9] NmSC provides mechanical support to small-diameter axons to ensure integrity, furthermore, a single nmSC can encompass several axons to form a Remak bundle, but more specific structures and functions of nmSC need to be established.[36] SC itself is widely indicated in adjusting the opening of BNB through modulating the level of transcytosis[9,27,39] despite the cell being the component of VU.[9] Propelled by persistent signals emanating from the ERK signaling pathway after nerve injury, SC could restructure to a functional or even progenitor-like state[40], which enables SC to actively modulate and fine-tune the process of nerve regeneration.[41,42] Furthermore, myelin-associated glycoprotein, as a member of lectins that binds to sialic acid in the SC, is a key molecule in the formation and maintenance of myelinated axons[43,44], it actively roles in the development and progression of BNB-related diseases[43], for example, anti-myelin-associated glycoprotein neuropathy.[45]

MACROPHAGES

Macrophage, which simultaneously expresses F4/80 and Iba1[46,47], (Table 1) belongs to mononuclear phagocyte, predominantly originating from progenitor cells residing in the bone marrow and subsequently migrating to the vasculature.[48,49] Notably, monocyte possesses the remarkable capacity to differentiate, leading to the emergence of diverse macrophage lineages that give rise to specialized populations and functions. In particular, these subtypes mainly include Kupffer cells in the liver, cardiac macrophage in the heart, splenic macrophage in the spleen, alveolar macrophage in the lung, microglia in the CNS, and osteoclast in the bone. Additionally, histiocyte, which is the functionally quiescent state of macrophage, presents within the loose connective tissues.[50,51] (Figure 3) Despite the residual macrophage, which accounts for 2-9% of the endoneurial cell populations[9,52], is the essential element of VU, (Figure 1) the knockout of the cell seems to make no difference to the barrier function, the permeability of the connection and transcytosis transport within BNB remain intact, which means that macrophage might be not responsible for the tightness of the barrier.[9] However, the cell serves as the rescue and backup for the structural and functional integrity of the BNB by efficiently and reliably phagocytosing leaked materials and debris[9,29,53,54], for this, the impairments of the structure and function completeness of BNB could be functional remedied by macrophage. At the time of bearing the role in BNB, macrophage could be adjusted by many factors both in physiological and pathological conditions. For example, prostaglandin D2 synthase could negatively influence the function of macrophages by regulating the expression of COX_2[55], the absence of this synthase is followed by more macrophage recruitment, but these macrophages can't normally exert complete functions. This could result in the accumulation of destroyed myelin and debris, and the impairments to permeability and integrity of the BNB.[54-56]

PERICYTE

The pericyte, which expresses NG2, PDGFRb, a-SMA, and CD146/MCAM[46], (Table 1) is located within the BM of small blood vessel[9,57], (Figure 1) The coverage of pericyte varies from 10% to 100%, and different coverage accompanies with similarly barrier function. The cell plays a pivotal role in facilitating the formation, preservation, and control of the BNB, they also modulate endothelial function within the BNB through the paracrine release of growth factors[58], such as TGF-β[57,59], and VEGF.[57,60] By prompting the pericyte secrets of the VEGF and TGF-β, advanced glycation end-products induce dysfunction of the BNB and hypertrophy of the BM. In addition, pericytes can regulate BM function by secreting fibronectin, collagen type IV, and tissue inhibitor of metalloproteinase.[57] It's also reported that the pericytes function as perivascular macrophages to clear tissue debris and abnormal proteins.[61,62]

TACTOCYTE

Tactocyte, which frees from the envelopment of BM and accounts for about 2% to 12.5% of endoneurial cell[46,63,64] and exhibits an endoplasmic reticulum-riched elongated morphology, is poorly characterized but polymorphic fibroblast-like closely contacts with blood vessel.[65] It possesses protrusions that span the endoneurium, which facilitates direct contact with other cells.[46] (Figure 1) The cell itself is usually described as fibroblast[66], mesenchymal precursor cell[67], pericyte-like cell[68], and talocyte.[69] The cell expresses pericyte markers NG2 and PDGFRb combined with p75 positive but aSMA-negative, research found that the astrocyte expresses a common symbol of progenitor cells: CD34.[65] (Table 1) It could be distinguished from SC and

Table 1: Functional phenotypes of the cells related to the blood-nerve barrier

	F4/80	Iba-1	PDGFR-β	α-SMA	NG2	CD34	CD68	CD31	CD146	p75	caveolin-1	S100b	GLUT1
macrophage	+	+	-	-	-	-	+	-	-	-	-	NA	NA
Schwann cell	-	-	-	-	-	-	NA	-	-	+	-	+	NA
pericyte	-	-	+	+	+	-	NA	-	+	-	-	NA	NA
astrocyte	-	-	+	+/-	+	+	-	-	-	+	-	-	-
endothelial cell	-	-	-	-	-	+	NA	+	-	-	+	NA	+

+: positive; -: negative; NA: not applicable; +/-:variation or heterogeneity

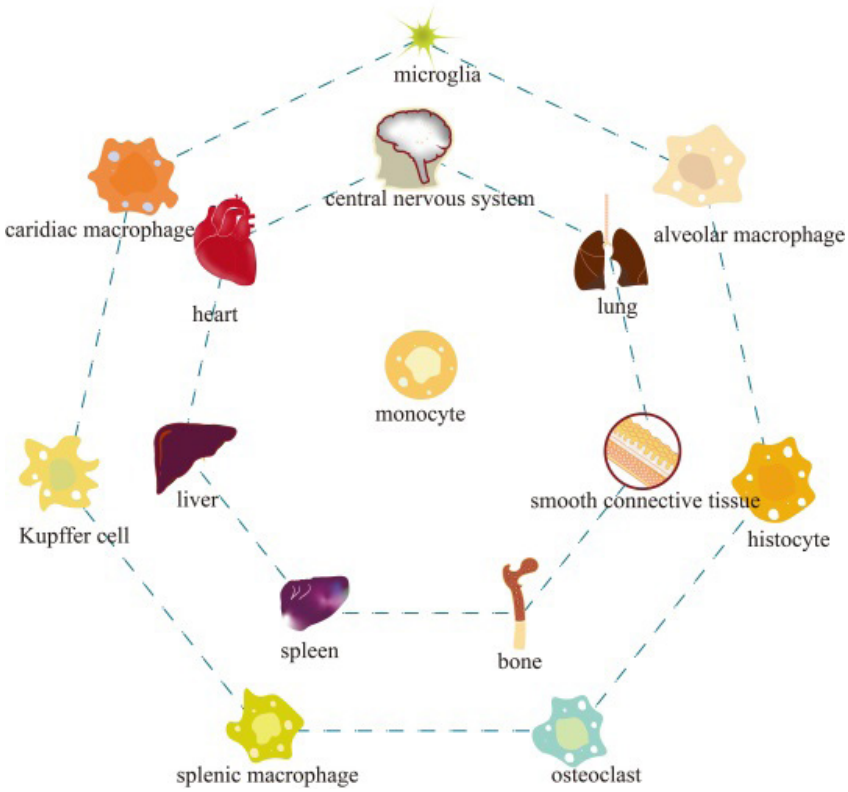


Figure 3. Diagram illustrates the overview of the origin, main tissues, and organ distribution of macrophages. The macrophage is an important defense system, which mainly includes Kupffer cell in the liver, cardiac macrophage in the heart, alveolar macrophage in the lungs, macrophage in the spleen, microglia in the central nervous system, osteoclast in bone tissue, and pericyte in loose connective tissue. They play immune and defensive roles in different tissues, working together to maintain homeostasis.

pericyte for lacking BM envelopment[66], and the negative expression of CD68 differentiates it from macrophage.[66] The distinctive morphological characteristics of tactocyte make it the potential provider of the barrier function of BNB.[9] However, due to limited research, further studies are still needed to explore the characteristics and roles of the cell in BNB.

JUNCTION AND MOLECULE

TJs are formed by protein complexes that tightly join cells together, the barrier function of the BNB heavily relies on TJs. They play a crucial role in biological organisms by restricting the unrestricted diffusion of substances and signals[70], maintaining cell membrane polarity and function, as well as regulating cell differentiation, proliferation, and migration. For this, it has been described to have "gate" and "fence" functions which are keys to maintaining low endothelial permeability.[71]

BNB gene profile consists of numerous TJs, adherent junctions, and cell adaptor molecules, such as endoneurial endothelial microvessel protein expression of α1 catenin, cadherin-5, cadherin-6, claudin-1, claudin-4, claudin-5, ZO-1, platelet endothelial cell adhesion molecule(PECAM), and junctional adhesion molecule(JAM).[10,72-76] The alteration in the perineurium or the integrity of TJ in endoneurial EC makes no difference to the opening of BNB, but the enhanced transcytosis of endoneurial EC significantly modulates it.[7,9] For instance, claudin-1, as a crucial protein in TJ, plays a vital role in maintaining the barrier function, inhibition or knockout of it can significantly impact the function of the perineurium.[8] Among the molecules, claudin-4 is associated with TJ, cadherin-5 is associated with adheren junction, and α1 catenin, as an adhesion molecule, mediates the linking of F-actin cytoskeleton and TJs.[10] Studies found that claudin-5 and ZO-1 are commonly expressed in endoneurial EC[9] and epineurial blood vessels despite the absence of TJs in epineurial vessel[77], what's more, claudin-5 serves as a vital component of TJ rather than epineurium. (Figure 4) The complex molecular abundance of the BNB

indicates the potential presence of significant molecular superfluousness to maintain its normal function, these molecules may possess functions beyond their barrier roles.[10] Both the types and expression levels of these proteins deeply influence their functionality.[78,79] However, current researches primarily focus on the impact of TJ expression on barrier function and have yet to delve into the influence of their interplays in the PNS. Exploring the interactions and their effects on the BNB function is a worthwhile direction.

TRANSENDOTHELIAL ELECTRICAL RESISTANCE

TEER is an indicator of the resistance to electrical current across EC, it's commonly used to assess the integrity and functionality of various barriers.[30] TEER provides valuable insight into barrier permeability and TJ function, which real-time feedback on the integrity of barrier structures, measurement of TEER represents a noninvasive and quantitative technique utilized for the evaluation of the electrical resistance exhibited by cellular layers constituting biological barrier, such as endothelial and epithelial monolayers.[80] The main primary utilization of TEER involves foretelling the physiological integrity of biological membranes in response to diverse chemical and biological entities.[81] It is calculated as the resistance value of the cell layer multiplied by the effective membrane area ($\Omega.cm^2$).[82,83] Unlike the CNS, the TEER in the PNS is little known, but studies showed that the TEER in the CNS valuable about 35 $\Omega \cdot cm^2$ [82,84], moreover, a unanimous consensus regarding the precise value of TEER in the peripheral nerve has yet to be established. What's more, it enables the evaluation of drug efficiency in crossing biological barriers and prediction of the barrier permeability[30], which aids in understanding drug transport characteristics and potential therapeutic effects across different barrier systems.[85] By effectively assessing this process, it guides drug design and optimization, facilitates the selection of suitable administration routes, and enhances drug penetration and treatment efficacy.[30]

OPENING OF BLOOD-NERVE BARRIER

Overviews of the opening

As the barrier between the peripheral nerve and circulating blood, the BNB plays a vital role in the restriction of unexpected substances exchange between blood and nerve, including metabolic waste, nutrients, toxic substances, drugs, and various small molecule substances.[9,10] Sometimes, BNB could be opened physiologically and temporarily, which means that the defense

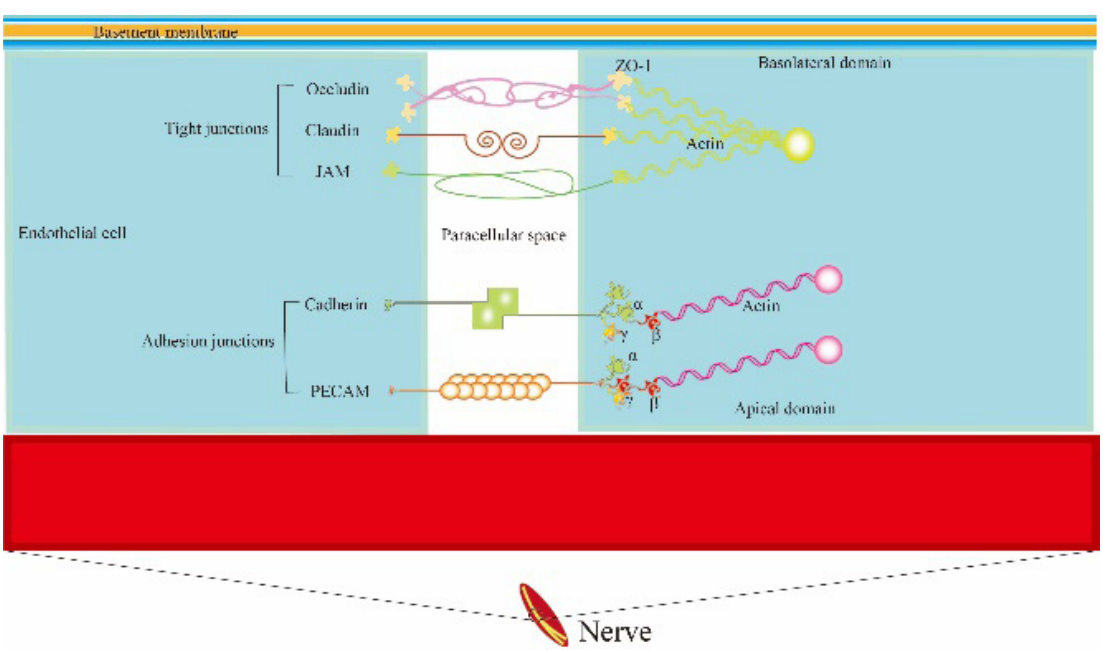


Figure 4. Schematic of the junctional structure of blood-nerve barrier, claudins, occludin, and JAM constitute the core components of tight junctions, cadherin, and platelet endothelial cell adhesion molecules belonging to the adhesion junctions.

function of BNB experiences a temporary reduction for certain substances during specific physiological conditions, such as the delivery of therapeutic drugs and narcotic drugs[17], the BNB subsequently returns to the normal state with the stimulus removed.[16,17] In most cases, however, the opening of the BNB is pathological and usually caused by peripheral nerve injury[79,86], metabolic disease (diabetic peripheral neuropathy)[87], or other pathological conditions (neurodegenerative or neuroimmune disorders).[45,88] This usually combines with the decreased expression of claudin-5, claudin-12, and ZO-1[89], and causes severe negative effects, even irreversible damage to the structure and function integrity of BNB.[7] From the macroscopic perspective, the disruptions of the BNB signify damage to the structure, such as vascular EC, endoneurium, perineurium, and even the nerve.[13] From the microscopic perspective, the disruptions of the BNB further entail the weakening or disappearance of structures, and functions of cellular and molecular, as well as the disruption of interactions among different cells, and molecules, which are often accompanied by alterations in cellular metabolism, changes in the microenvironment, alterations in the concentration of certain RNA and proteins[8,78,79,90], like miRNA[8,78], tumor necrosis factor[43] and Pmp22[7], and the augmentation or attenuation of specific signaling pathways[27,91], such as RET-tyrosine kinase-MAPK signaling pathway[91], Ras/Raf/MEK/ERK signaling pathway[27] and Wnt/β-catenin pathway.[92] By way of illustration, the impairment of SC function leads to an increase in transcytosis level in the peripheral nerve and an increased permeability of BNB.[9] What's more, function or structure damage of BNB dramatically prompts the prothrombin transcription level and relative thrombin inhibitor protease nexin-1 expression, which in turn results in the disturbance of the local coagulation-anticoagulation system. The specific mechanism, however, requires further investigation.[93] In this part, we mainly discuss the functional condition that opens the BNB, and the pathological condition will be discussed in the next section.

Evaluation of the opening of the blood-nerve barrier

The detection of BNB dysfunction serves as a crucial aspect in evaluating nerve functional status, guiding subsequent intervention, and facilitating prognostic evaluation. Undoubtedly, the opening of the barrier is inevitably accompanied by the increase in permeability, which could be indirectly demonstrated by the decreased TEER[94] and enhanced efficacy of therapeutic drugs or anesthetics.[16,17] Of note, the electron microscopy or immunohistochemistry demonstrated increased transcytosis activity on both sides of the BNB is the most direct evidence of the increased permeability.[9,13] A diverse array of chromophores or tracers are employed to evaluate the permeability of the BNB, such as Evans blue[13,95], anti-endothelial barrier antigen (anti-EBA), and anti-rat EC antigen-1 (anti-RECA-1)[13], horseradish peroxidase[9], dextran-FITC[9], retrograde tracer Fluorogold[96] and nanoparticle.[97]

Evans blue is one of the commonly used largest tracers with a molecular weight of 961 Da, it is presently the most widely used tracer, which could tightly and reversibly bind to albumin.[98] Intravenous injection of 1 mL per 100g body weight of Evans blue allows the evaluation of BNB function to be accomplished quickly, the more tracer transferred between the perineurium and ECs, the more severe impairment of the BNB.[13,95] Electron microscopy demonstrated increasing in transcytosis could also be a sign of the impairment of BNB.[9] Furthermore, Dextran-FITC of either 40kDa or 10kDa at a concentration of 1.5 mg/ml, an injection of 10 ml per gram of body weight, and subsequently sacrificed either 30 minutes or 6 hours post-injection for the observation.[9] The horseradish peroxidase is also the agent used for the trance of permeability of BNB, its solution is prepared by dissolving 125mg of horseradish peroxidase type II in 2.5ml of PBS, and the animal is injected with a single dose of 0.5mg per gram of body weight and then harvested at specific time[9], the barrier's permeability could be precisely assessed. Furthermore, anti-RECA-1 is a monoclonal antibody that specifically binds to the surface antigen of both macrovascular and microvascular ECs[99], while anti-EBA is a monoclonal antibody that specifically targets endothelial protein localized in regions of BNB.[100] The two antibodies hold great promise for studying the function and structural integrity of the barriers, a decrease in the ratio of anti-EBA to anti-RECA-1 usually indicates impaired permeability.[13,101] Of note, magnetic resonance imaging and photoacoustic imaging exhibit exceptional spatial resolution, profound tissue penetration, and remarkable contrast. By combining Fe3O4@COOH nanoparticles with biotinylated dextran amine, the forward-directed nanoscale neural tracer can be generated, which

enables protracted release and facilitates real-time tracking of injured nerves using a dual-modal imaging technique.[97] Seitz *et al*. conducted an experiment where they injected biotin-labeled substances intraperitoneally, the tracer quickly reached the blood vessels and tissues of various organs, but could only be detected in the dura mater of the CNS, spinal ganglia, and outer layer of peripheral nerves, later on, IgG was also found in the inner layer of peripheral nerves, while the brain, spinal cord, and spinal nerves remained IgG-free, indicating different permeability of the BNB and partial permeability of the normal BNB to IgG.[102]

Functional opening of the blood-nerve barrier

The presence of BNB protects the nerve against external stimuli and potential damages.[10] However, it poses challenges in efficiently and rapidly delivering local anesthetics and therapeutic drugs to target tissues, which leads to great amounts of inconvenience. Therefore, extensive research in this field focused on ultrasound[16,17], microbubbles[103,104] to address these problems and enhance the treatment efficacies and anesthetic effectiveness. Microbubbles, most of which are metabolized by the Kupffer cells in the liver[105,106], are the contrast agents of diagnostic ultrasound[107], through the shell and gas core are deep diverse, they are usually regarded as the effective candidates of contrast agents.[108] Of note, the microbubbles are also used as theranostic agents for the drug uptake stimulations followed by the dilatation and compression secondary to ultrasound field.[109] Focused ultrasound combined with microbubbles could constitute a noninvasive approach that transiently disrupts the barrier in a specific region, facilitating precise and localized delivery of the therapeutic agent, genetic material, or nanoparticle into targeting tissue.[110] In the neurophysiological assessment following microbubble administration and focused insonation, superharmonic emission and tissue tracer extravasations could be observed.[16] Umansky *et al*. conducted a significant study showing disruption of neural endostructure at 1.2 MPa and 166.7 μl/kg, with notablely anatomical changes at a lower threshold of 0.3 MPa and 40 μl/kg, the compound muscle action potential was partially affected, as the signal alteration in the magnetic resonance imaging supported transient modulation of BNB permeability by focused ultrasound rather than sustained neural impairment.[16] While the combination of microbubbles with low-intensity sound waves significantly prolongs the duration of nerve blockade, it does not exert a significant impact on the duration of blockade when combined with high-intensity sound waves.[16] In the absence of insonation, the sensory nerve block exhibited a diminished rate and shortened duration. However, ultrasound at intensities of 0.1 W/cm^2 or 0.5 W/cm^2 significantly heightened the block rate, while intensity above 0.5 W/cm^2 prolonged the block duration. At 3 W/cm^2 intensity, the block success rate reached 100% with a remarkable extension of duration. Insonation facilitates drug delivery through fluid oscillation and enhances molecule diffusion efficiency and transportation across blood, cell, and extracellular fluid.[16] Furthermore, hydrophobic drugs are less affected by insonation compared to hydrophilic drugs, which could be attributed to the fact that hydrophilic substances encounter hindrances in traversing biological membranes, whereas hydrophobic substances do so effortlessly.[16]

SIGNALING PATHWAYS RELATED TO THE REGULATION OF BLOOD-NERVE BARRIER

The maintenance and regulation of BNB integrity have become a popular research area, as the discovery and study of signaling pathways have revealed the intricate mechanisms involved in BNB regulation.[26,92] It is worth noting that cases of abnormal activation or disruption of these signaling pathways can lead to BNB breakdown and significantly implicate the occurrence and development of various neurological disorders.[111] By gaining a comprehensive understanding of these signaling pathways, we can enhance our knowledge of the biological characteristics of the BNB and provide new insights for future treatment strategies and drug development. This section aims to review the pathological condition of BNB opening through signaling pathways.

Ras/Raf/MEK/ERK signaling pathway

The Ras/Raf/MEK/ERK signaling pathway is a mitogen-activated protein kinase pathway, its activation centers on the conversion of RAS to RAS GTP through the ornithine cycle. This pathway not only deeply acts in cell proliferation but also regulates cellular growth and differentiation.[112] It's well-documented involvement in various diseases, including nerve injuries[113, 114], tumors[115] and wound healing.[116] In the absence of injury, the disruption of the BNB in heterozygous P0-RafTR mice, which is accompanied by elevated

inflammatory cells, can be triggered by signaling activated by Ras in SCs, activation of Ras then leading to upregulation of mRNA, such as the mRNA of MCP-1, IL11, Scye1, and Cxcl10. Of note, the expression of various other factors that may play important roles in the regenerative response, such as MEGF10, neurotrophic factors like GDNF, and angiogenic factors, are also significantly upregulated.[27] (Figure 5) In the context of nerve injury, administering a highly selective MEK1/2 inhibitor effectively blocks the ERK signal, resulting in a remarkable reduction of phosphorylated ERK levels and a remarkable suppression of the inflammatory response caused by the suppression of inflammatory cell recruitment.[27]

Wnt/β-catenin and Sonic Hedgehog signaling pathways

The Wnt/β-catenin pathway holds the ability to adjust the communication between toll-like receptors and Sonic Hedgehog signaling.[92] Its abnormal activation leads to an increased resident macrophages-released VEGF[117], then resulting in the down-regulation of Frizzled-7, VE-cadherin, and the early changes of the vascular endothelial-cadherin/β-catenin/Frizzled-7 complex, which disrupts the adhesive structure of endoneurial ECs, ultimately leading to the disruption of BNB.[92] However, the knockout of Wnt/β-catenin signaling makes no negative influence on the Sonic Hedgehog pathway nor the function of the BNB, which means the interrelation between the Wnt/β-catenin and Sonic Hedgehog pathways is responsible for the disruption of both the function and structure of BNB. Moreover, toll-like receptor 4 or inactive Sonic Hedgehog signaling could regulate the BNB through down-regulate crucial TJ[26], but the activation of Sonic Hedgehog and inactivation of toll-like receptor 4 signalings do not cause any changes in molecular alterations[92], which means that the two pathways could independently modulate the expression of TJ and act in the function of BNB. Inhibition of Hh-Smo signaling resulted in downregulated expression of mRNAs encoding TJs of Occln and claudin-5, and the marks of the Hedgehog system, also the RNA expression of TLR2, CCL2, IL-1b, and CD11b increased.[26] (Figure 6) For that, the Wnt/β-catenin and Sonic Hedgehog signaling pathways regulate

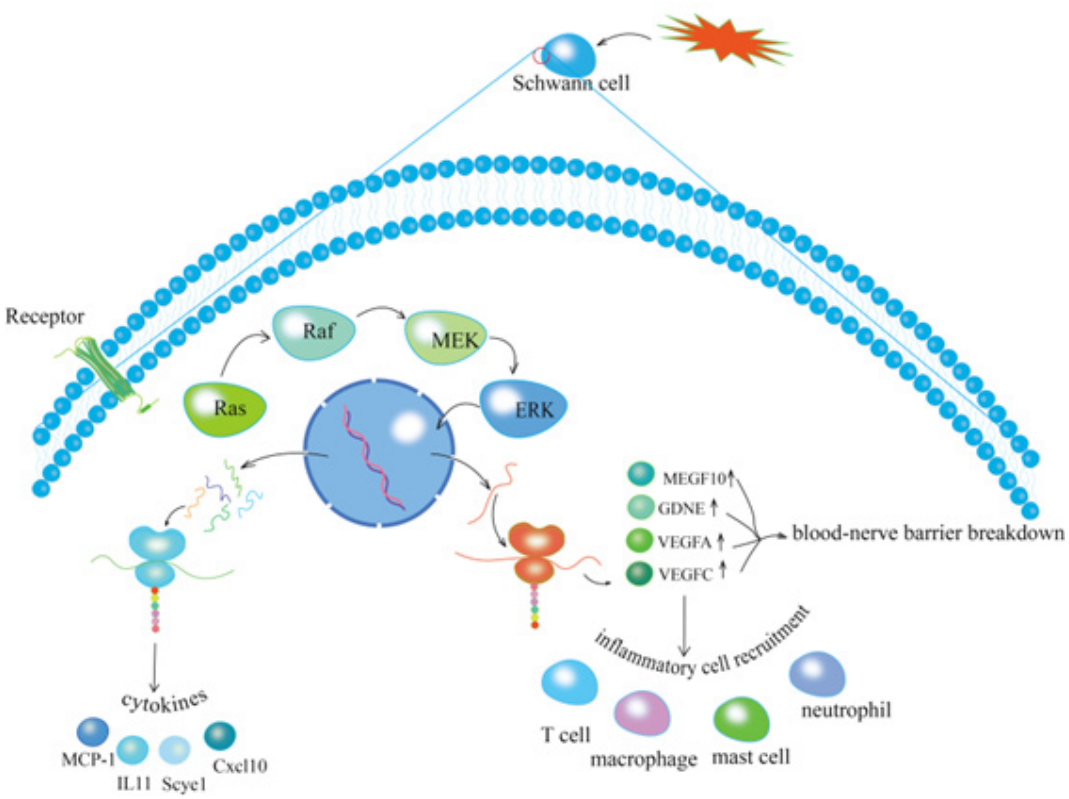


Figure 5. Activation of the Ras/Raf/MEK/ERK signaling pathway leads to the recruitment of inflammatory cells and disruption of the blood-nerve barrier by prompting the expression of many molecules and the infiltration of various inflammatory cells.

the BNB permeability differently, activation of the Wnt/β-catenin signaling pathway disrupts intercellular junctions and increases the barrier's permeability, while the Sonic Hedgehog pathway affects the stability of the barrier by regulating the expression of claudin-5.

RET-tyrosine kinase and MAPK signaling pathway

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor β protein superfamily, it is deeply involved in maintaining the survival of all kinds of neurons.[118-120] It strengthens BNB function by restricting transcytosis, reducing solute permeability, and regulating the expression of cytoskeletal proteins and TJs.[121] After the administration of GDNF, the damaged membrane of human peripheral nerve vascular endothelial cells (pHEndECs) longitudinal F-actin cytoskeletal filaments could relocate to form more continuous adherence and TJs[91], by upregulating the expression of adherence junctions and desmosome components such as ACTN2, RALA, WASL, ARF6, TLN1, and cytoskeletal intercellular junctional complexes such as ACTN3, LLGL1, HCLS1, and SPTAN1, the inhibitor of RET-tyrosine kinase, MAPK2K2 could knock out the increased TEER and permeability[121], which mean GDNF could enhance the properties and TEER of BNB through the RET-tyrosine kinase and MAPK signaling pathway[91,121], (Figure 7) but the specific mechanism remains yet to be determined.

Other pathways

The RECK/MMP9 signaling pathway is a regulatory involved in tissue remodeling, including extracellular matrix degradation and angiogenesis[122], it deeply acts in various physiological and pathological processes, such as wound healing and tissue remodeling.[78,123] Reversion-inducing cysteine-rich protein with Kazal motifs is the inhibitor of Matrix Metalloproteinase 9, which is responsible for the breakdown of extracellular matrix components.[124] As a non-coding RNA, miRNA plays an indirect role in cellular physiological processes by functioning as a regulator for numerous biological processes.[125] Numerous studies verified the functions of miRNA in neurogenesis and nerve regeneration.[126-128] The miRNA-1207-5p/EPB41L5 axis is found to be involved in TGF-β1-mediated exosomal lnc-MMP2-2-induced increase in barrier permeability[129], and the subtle change in the permeability of the blood barrier.[130] The expression of adaptor-associated kinase 1 is restrained by miRNA-384-3p, it has been found to alleviate sevoflurane-induced nerve injury by suppressing the expression of adaptor-associated kinase 1.[131] Also the knockout of the miRNA-155

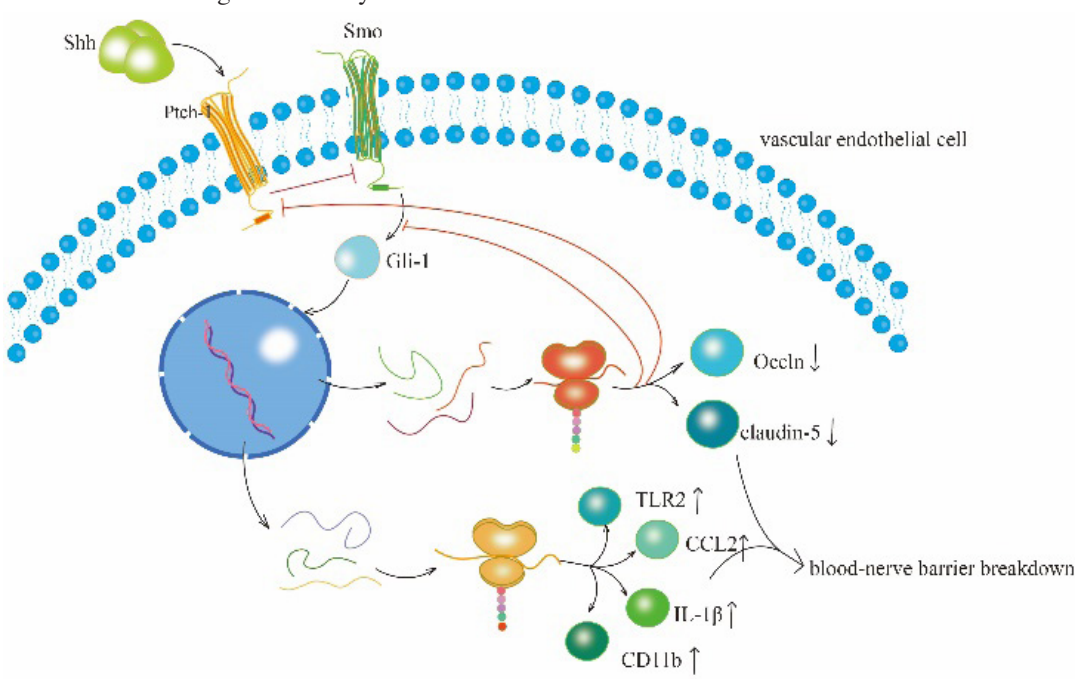


Figure 6. Schematic illustrates the effects of the Sonic Hedgehog signaling pathway on the blood-nerve barrier.

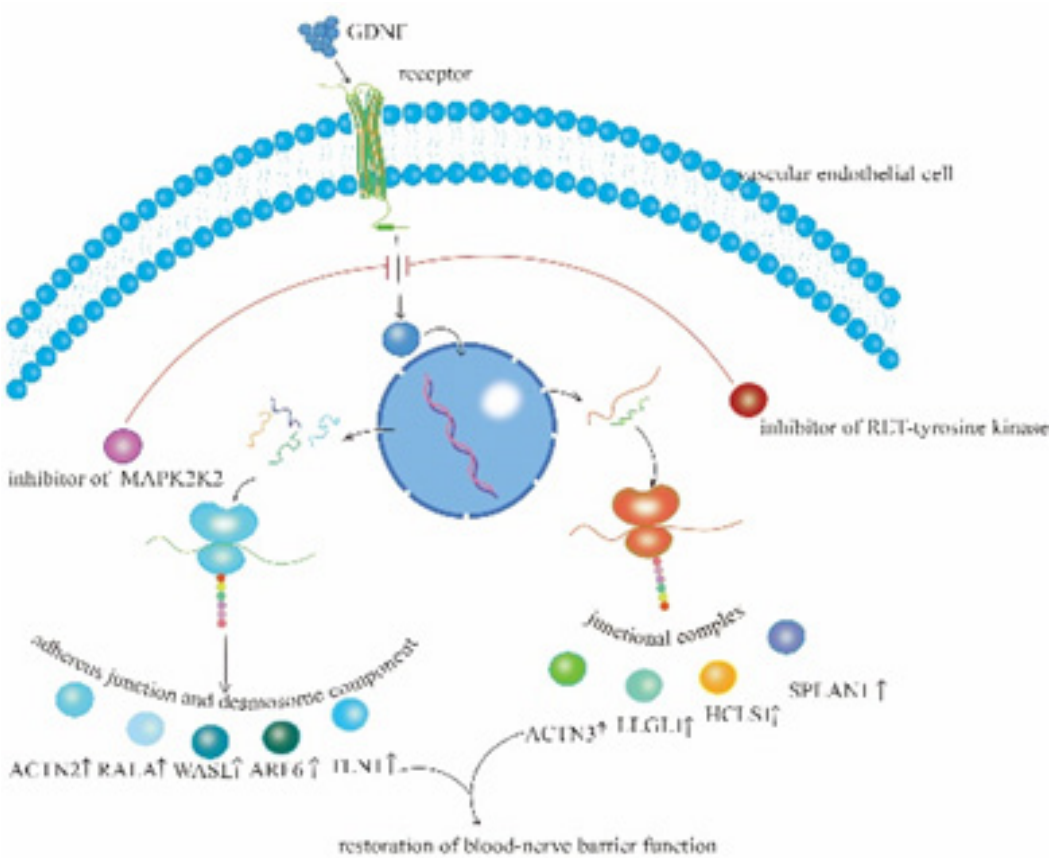


Figure 7. The diagram demonstrates the effect of GNDF on the function blood-nerve barrier. In injured cells, the use of GNDF leads to an increase in the expression of adherens junction and desmosome components such as ACTN2, RALA, WASL, ARF6, TLN1, as well as cytoskeletal intercellular junctional complexes including ACTN3, LLGL1, HCLS1, and SPTAN1. This, in turn, promotes the improvement of BNB barrier function and TEER. Conversely, the use of an inhibitor of RET-tyrosine kinase, MAPK2K2 can completely hinder the aforementioned effects.

could alleviate the diabetic peripheral nerve injury by up-regulating the expression of erythroid 2-like 2.[126] MiRNA also actively participates in the function regulation of BNB, nerve injury is often accompanied by alterations in the activity of miRNA, Reinhold *et al*. reported that miR-21 participated in the regulation of BNB function through the RECK/MMP9 pathway.[78] Stimulus leads to the decrease in miRNA-183 expression and the increase in FoxO1d, which in turn results in a decrease in claudin-5 expression, ultimately leading to impairment of the barrier function.[79]

After nerve injury, miR-21 expression is increased, which consequently downregulates its target RECK, leading to an upregulation of the downstream Mmp9 and decreasing in the expression of claudin-1, and ultimately impairs the function of BNB.[78] On the contrary, the expression of miR-183 decreases continuously following injury, which is in parallel with the decrease of TJ, moreover, its inhibition is associated with activation of forkhead box protein O1, impairment of TEER and BNB function, and use of miR-183 mimic could reverse this situation.[8,79] (Figure 8) These findings indicate that the impact of various miRNAs on the BNB is not consistent, certain miRNAs can strengthen the barrier function of the BNB, while some miRNAs compromise it. The diverse actions of different miRNAs offer potential avenues for future research to leverage their unique characteristics. By harnessing these properties, we may be able to alleviate nerve damage, enhance the recovery of the BNB, and ultimately facilitate neural regeneration and functional rehabilitation.

FUTURE DIRECTIONS OF BLOOD-NERVE BARRIER RESEARCH

Understanding the physiological regulation

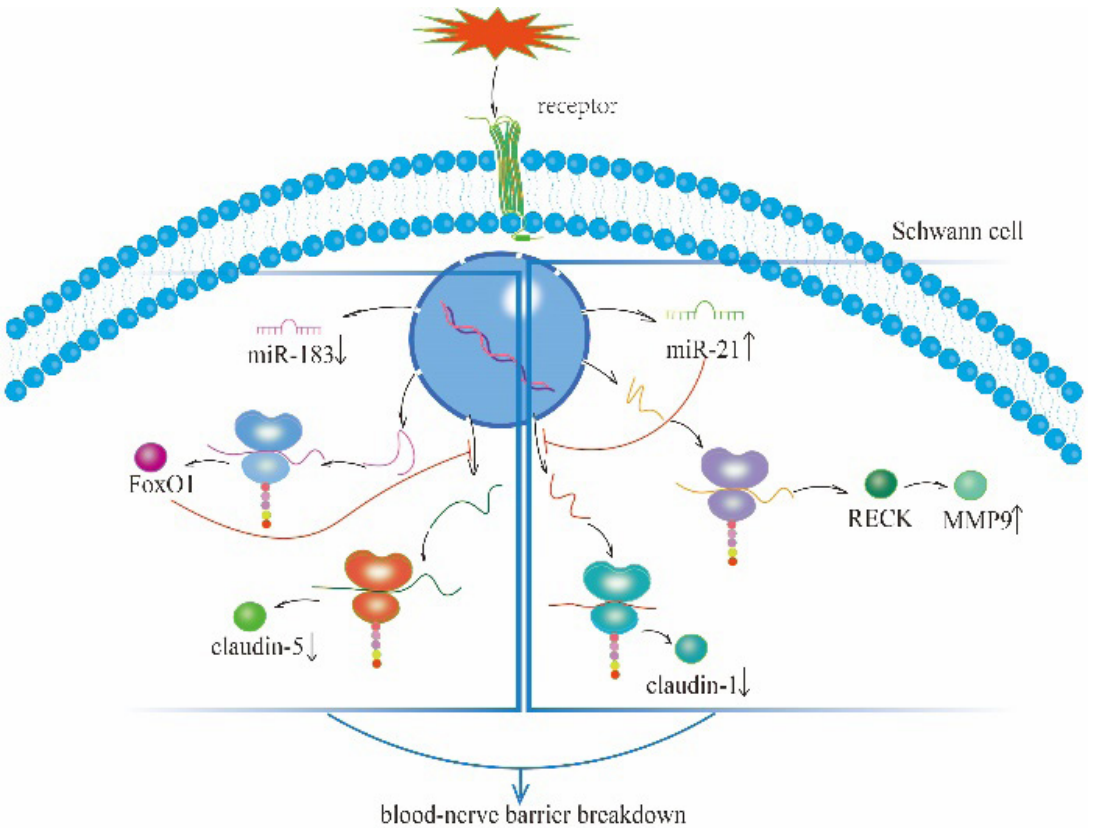


Figure 8. The diagram depicts the alterations in two miRNAs in response to nerve injury, highlighting their involvement in modulating the function of the blood-nerve barrier. Specifically, miRNA-21 exerts a negative effect on the blood-nerve barrier through the RECK/MMP9 signaling pathway.

mechanisms of the BNB is vitally important for their crucial roles in maintaining a stable environment for the peripheral nerve by controlling the permeability of EC in blood vessels. Researchers can investigate the regulatory effects of different cells, hormones, and neurotransmitters on the BNB process. Moreover, in-depth studies are required to unravel the intricate interplays between the BNB and specific diseases such as diabetic peripheral neuropathy and peripheral nerve injury. These investigations should focus on elucidating the structural and functional characteristics of the BNB, as well as the molecular markers and components involved. Additionally, comprehending the complex mechanisms underlying the functional states of BNB components and their impact on barrier function and neural recovery is recommended. Furthermore, it is crucial to explore novel techniques and methodologies that can provide a comprehensive understanding of BNB functionality. For example, advancements in radiographic imaging techniques, such as CT, structural MRI, and even functional MRI, hold promise in capturing the different functional states of the BNB. These imaging modalities offer valuable insights into the dynamic changes occurring within the BNB during disease progression and therapeutic interventions. The development of reliable animal and cell models of BNB is pressing needed, representative models that accurately mimic the complex structure and intricate functions of the BNB will enable researchers to study disease mechanisms, test potential therapeutic interventions, and evaluate drug delivery efficiency. By refining and expanding the experimental models, the understanding of the BNB and its role in various pathological conditions could be achieved. In conclusion, further investigations into the structure, component, molecular marker, and functional state of the BNB are essential for unraveling the pathophysiology of diseases like diabetic peripheral neuropathy and peripheral nerve injury. Such research will not only shed light on the fundamental mechanism underlying these conditions but also pave the way for the development of targeted therapies and improved

drug delivery strategies.

DISCLOSURE

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