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MicroRNAs in central nervous system diseases: a prospective role in regulating blood-brain barrier integrity

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Abstract

Given the essential role of the blood-brain barrier (BBB) in the central nervous system (CNS), cumulative investigations have been performed to elucidate how modulation of BBB structural and functional integrity affects the pathogenesis of CNS diseases such as stroke, traumatic brain injuries, dementia, and cerebral infection. Recent studies have demonstrated that microRNAs (miRNAs) contribute to the maintenance of the BBB and thereby mediate CNS homeostasis. This review summarizes emerging studies that demonstrate cerebral miRNAs regulate BBB function in CNS disorders, emphasizing the direct role of miRNAs in BBB molecular composition. Evidence presented in this review will encourage a deeper understanding of the mechanisms by which miRNAs regulate BBB function, and facilitate the development of new miRNAs-based therapies in patients with CNS diseases.

Keywords

MicroRNAs; Central nervous system diseases; Blood-brain barrier; Vascular integrity

1. Introduction

1.1 Blood-brain barrier

The central nervous system (CNS) is one of the most complex and highly organized systems in the human body and the blood-brain barrier (BBB) is a highly sealed cell-to-cell contacted barrier that regulates the transportation of proteins, nutrients, and molecules from blood to brain, and metabolic waste products from the brain into circulation (Ballabh et al., 2004; Maiuolo et al., 2018; Zhao et al., 2015d). The BBB is centrally positioned within the neurovascular unit and several factors contribute to the physical and functional BBB. Cerebral endothelial cells (ECs) are the major component of the brain microvasculature, playing an essential role in the maintenance of BBB integrity and cerebral homeostasis (Daneman, 2012; Sweeney et al., 2019). Junction proteins mainly include tight junctions

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(TJs) and adherens junctions (AJs) (Hartsock and Nelson, 2008). TJs are responsible for connecting neighboring endothelial cells to reduce intercellular distance, thus, preventing free exchange between the CNS and vasculature. Many transmembrane proteins are required to form a functional BBB such as claudins, occludin, zonula occludens (ZO), and junctional adhesion molecules (JAMs). Claudins, the major transmembrane molecules, have been well demonstrated to play critical barrier functions in both human cell lines and rodent knockout models as summarized in a previous publication (Daneman, 2012). AJs initiate and stabilize cell-cell adhesion, thereby regulating intracellular signaling and the transcription of important transmembrane components. The core of the AJs includes certain cadherin and catenin family members. Vascular endothelial (VE)-cadherin is exclusively responsible for endothelial AJs' assembly and barrier architecture. E-cadherin is the core transmembrane protein and N-cadherin forms the pericyte-endothelial junction (Wolburg and Lippoldt, 2002). Other components such as pericytes, astrocytes, and the basement membrane (BM) may also contribute to BBB function and structure. Pericytes maintain BBB integrity by covering the capillaries, and astrocytes participate in BBB maintenance by interacting with pericytes and ECs through their endfeet (Wong et al., 2013). The BM is a unique form of the extracellular matrix. Although the molecular and cellular mechanisms are not clear, an increasing number of studies suggest that the BM also actively participates in vascular integrity (Xu et al., 2019).

The complicated structural and functional integrity of the BBB separates the circulating blood from the brain and controls the entry of most blood-derived molecules into the brain (Keaney and Campbell, 2015). Therefore, maintaining BBB integrity and function is critical to regulating the microenvironment of the CNS.

1.2 BBB disruption in CNS disorders

As revealed in numerous studies, BBB breakdown is significantly enhanced under pathophysiological conditions and disruption of the BBB has been observed in many pathologies of CNS diseases, ranging from stroke to inflammatory responses (Daneman, 2012; Jiang et al., 2018; Kamphuis et al., 2015; Liebner et al., 2018; Yang and Rosenberg, 2011). Although it has been well accepted that BBB leakage is a secondary event that occurs in ischemic stroke and traumatic brain injury (TBI), it remains unclear whether BBB leakage occurs in other CNS disorders such as Alzheimer's disease (AD). BBB breakdown may play an important role in facilitating the entry of immune components to remove existing debris. It may also be harmful by causing brain edema, myelin sheath damage, and further neuronal dysfunction. Both ischemic and hemorrhagic stroke cause BBB breakdown, which could have resulted from increased MMP activity and free reactive oxygen species (ROS) as illustrated in previous reviews (Haley and Lawrence, 2017; Keep et al., 2018). As shown in Figure 1, several molecular events lead to brain endothelial barrier disruption, including alterations in junctions, endothelial cell death, and increased endothelial cell transcytosis (Maiuolo et al., 2018). Structural and functional abnormality of endothelial cell-to-cell junctions, mainly the TJs, is the most notable feature during stroke-induced BBB opening. In addition, increased vascular permeability and loss of TJ-related proteins in brain tumors have been reported (Groothuis et al., 1991; Liebner et al., 2000). Other neuroinflammation-related diseases such as TBI, vascular cognitive impairment and the human

immunodeficiency virus (HIV) infection can also cause inflammatory responses in many cell types in the periphery and within the CNS, all leading to an altered BBB integrity (McRae, 2016; Price et al., 2016; Toyama et al., 2019). AD, the most common type of dementia, is considered to be the accumulation of blood-derived products including the amyloid beta peptide (A β) and tau protein in brain parenchyma (Sweeney et al., 2018). BBB disruption may allow influx of these products into the brain, which consequently initiates multiple pathways of neurodegeneration. In the course of HIV infection, HIV-1 Tat, a viral protein produced from the HIV-affected cell, has been observed to decrease the expression of TJs and involves multiple signaling pathways (McRae, 2016).

1.3 MicroRNAs

MicroRNAs (miRNAs) are small endogenous non-coding RNAs that modulate gene expression by directly targeting the 3'-untranslated regions (3'-UTR) of messenger RNAs (mRNAs). In humans, at least 98% of the total genome does not contain protein-coding DNA sequences but can be transcribed into non-coding RNAs (International Human Genome Sequencing, 2004). The binding of miRNAs leads to either destabilization or degradation of targeted mRNAs, thereby inhibiting mRNA translation (Inui et al., 2010). Although functional miRNAs (named functional non-coding RNAs at that time) were first reported in the early 1990s, which were studied as functional regulators of RNA-RNA interaction, the involvement of miRNAs in diseases was not reported until 2005 (Lee et al., 1993; Lu et al., 2005; Wightman et al., 1993). Since then, miRNAs have been shown to be important post-transcriptional regulators of gene expression and play vital roles in the cellular process of numerous diseases including cerebrovascular diseases (Blandford et al., 2018; Yin et al., 2014). Vascular endothelial cells are enriched with various miRNAs that are known to be involved in endothelial biology and physiological function.

2. MiRNAs in CNS disorders

To date, studies clearly depict a crucial role of miRNA in brain development and function (Im and Kenny, 2012; Kamphuis et al., 2015; Reijerkerk et al., 2013; Saba and Schratt, 2010; Sun and Shi, 2015; Sun et al., 2018). As important post-transcriptional regulators of gene expression, miRNAs constitute an essential regulatory control of immune cell development and function by turning on several processes triggered by immune cells delicately, and regulating apoptosis in various cell types (Baltimore et al., 2008). In addition, some miRNAs can also modulate BBB integrity by binding to the 3'-UTR of mRNAs encoding several essential junctional proteins. MiR-21, miR-150 and miR-181 are the most studied miRNAs in the regulation of cell development and pathology during CNS diseases. However, most studies were conducted in males only and sex differences were not taken into account. Developmental changes in the expression of miRNAs has been found in rat cortex, and sex differences in ischemic sensitivity may cause different regulation of miRNA responses (Lusardi et al., 2014; Murphy et al., 2014). Sohranji et al. summarized this difference in miRNA expression recently, finding that sex-specific effects exist in the expression of at least two miRNAs: Let7f and miR363-3p (Sohrabji and Selvamani, 2019). In addition, a small cohort of miRNAs were found to be highly expressed in adult females

compared with middle-aged females (Selvamani et al., 2014). In this regard, a variety of models that account for sex differences and age are still needed.

2.1 MiRNAs in Stroke

Stroke is a severe CNS disorder that results in vascular and neuronal damage, severe brain tissue injury, and neurological deficits. The most common type of stroke is ischemic stroke, which occurs when the blood supply to oxygen- and glucose-enriched vessels is rapidly interrupted in specific brain regions. Accumulating evidence has shown that ischemic stroke induces rapid alterations of endothelial integrity and function (Haley and Lawrence, 2017; Yang and Rosenberg, 2011). Several mechanisms, including the production of ROS and the activation of MMPs or inflammatory cytokines contribute to the vascular disruption, which ultimately leads to BBB dysfunction (Yin et al., 2014). Some miRNAs have been reported to play a role in post-stroke endothelial pathophysiology and BBB regulation (Table 1).

2.1.1 MiRNAs modulate BBB function in ischemic stroke—Early studies have suggested that miRNAs manipulate BBB function in rodent models of ischemic stroke. As shown in Table 1, most miRNAs negatively regulate BBB permeability, such as miR-155, miR-150, miR-210, miR-143, miR-130a, and miR-15a/16-1 (Bai et al., 2018; Caballero-Garrido et al., 2015; Fang et al., 2016; Ma et al., 2017; Yao et al., 2018; Zuo et al., 2019). These miRNAs can enhance ischemia-induced BBB leakage by directly or indirectly targeting TJs including ZO-1, claudin-5, occludin, cadherin, and β -catenin. For example, in a model of distal middle cerebral artery occlusion (dMCAo), improved microvascular integrity in the peri-infarct area was found in miR-155 inhibitor-treated mice (Caballero-Garrido et al., 2015). Further studies revealed that improved TJ integrity in the inhibitor-treated mice could be associated with stabilization of ZO-1, and it was mediated by the miR-155-targeted protein, Ras homolog enriched in brain. In line with the *in vivo* finding, miR-155 inhibition improved monolayer integrity in human primary cerebral microvascular endothelial cells (hCMEC/D3) after oxygen-glucose deprivation (OGD) exposure and significantly increased the expression of claudin-1 and ZO-1 (Pena-Philippides et al., 2018). Luciferase reporter assay revealed that claudin-1 is directly targeted by miR-155. Inhibition of miR-155 also enhanced the interaction between claudin-1 and ZO-1 and indirectly upregulated the expression of ZO-1. Claudin-5 is considered as the most abundantly expressed claudin in endothelial cell-to-cell junctions, and claudin-5-deficient mice die at birth due to BBB leakage of small molecules (Nitta et al., 2003). MiR-15a/16-1 has been recently reported to impair BBB integrity by directly binding to the 3'-UTR of claudin-5, and endothelial cell-specific deletion of the miR-15a/16-1 cluster in mice presented only minor BBB leakage compared with wild-type controls (Zuo et al., 2019). Moreover, miR-210 is identified as being complementary to the 3'-UTR of mRNA transcripts of occludin and β -catenin, which constitute the TJs and AJs respectively (Ma et al., 2017). When miR-143 activity was inhibited by circular RNA DLGAP4, BBB integrity was maintained in a mouse stroke model (Bai et al., 2018). In this study, miR-143, as a positive modulator of endothelial-mesenchymal transition, inhibited the expression of TJ proteins by targeting homologous to the E6-AP C-terminal domain E3 ubiquitin protein ligase 1. Next, overexpression of miR-34a increased BBB permeability in murine cerebrovascular endothelial cells and inhibited the expression of ZO-1 (Bukeirat et al., 2016). Bioinformatics

analysis indicated that miR-34a targeted several mitochondria-associated genes but did not directly target any TJ-related genes. Together with other findings, miR-34a is suggested to enhance BBB permeability through impairing mitochondrial function by binding to cytochrome c gene (CYCS).

On the contrary, miR-149–5p, miR-539, miR-21, and miR-132 are proven to preserve BBB integrity after ischemic stroke via various mechanisms including enhancing TJ expression, regulating MMP-9, and blocking the mitogen-activated protein kinase (MAPK) signaling pathway (Fan et al., 2018; Song et al., 2018; Yao et al., 2018; Zuo et al., 2019). MiR-21, originally identified as an oncogene involved in the pathological process of many diseases, is the most well-studied miRNA during CNS diseases-induced alteration of BBB permeability (Ge et al., 2015; Ji et al., 2018; Yao et al., 2018). The upregulated miR-21 decreases BBB permeability in a rat model of cerebral ischemia-reperfusion (IR) injury and represses the expression of MAPK signaling pathway-related proteins by directly targeting the 3'-UTR of mitogen-activated protein kinase kinase 3 (MAP2K3) (Yao et al., 2018). Besides, MMP-9 has been shown to be a critical effector of BBB breakdown in many CNS disorders, including ischemic stroke. And MMP-9 can be upregulated by a variety of signals such as vascular endothelial growth factor (VEGF), ROS and inflammatory cytokines (Lakhan et al., 2013). MiR-132 and miR-539 were recently reported to preserve post-stroke BBB integrity by directly binding to MMP-9 (Fan et al., 2018; Zuo et al., 2019). MiR-132 is a neuron-enriched miRNA that plays important roles in the developmental plasticity of the neural system and maintains brain vascular integrity (Xu et al., 2017b). Evidence in intact zebrafish suggested that miR-132 regulates the expression of VE-cadherin by directly targeting eukaryotic elongation factor 2 kinase (Xu et al., 2017a). Mice treated with exogenous miR-132 present reduced brain edema compared with the control group; moreover, agomir-132 significantly suppresses the expression of MMP-9 and inhibits the degradation of VE-cadherin and β -catenin in ischemic stroke mice (Zuo et al., 2019).

2.1.2 MiRNAs regulate BBB function in hemorrhagic stroke—Although hemorrhage-induced BBB disruption was less studied than ischemic-induced BBB dysfunction, cerebral hemorrhage can also cause increased BBB permeability, brain edema, and inflammation. There is growing interest in assessing miRNA levels and function in hemorrhage-induced BBB disability. MiR-126, as the only identified miRNA to show an EC-specific expression pattern, has been well studied in vascular development and inflammation (Fish et al., 2008; Harris et al., 2008; Wang et al., 2008). Endothelial miR-126 inhibits the expression of vascular adhesion molecule-1 (VCAM-1), which mediates leukocyte adherence to endothelial cells and limits immune cell infiltration into the brain parenchyma (Harris et al., 2008). In a rat model of collagenase-induced intracerebral hemorrhage (ICH), miR-126–3p was found to attenuate ICH-induced BBB disruption at 24h after ICH, and silencing of miR-126–3p resulted in impaired barrier integrity in rat brain microvascular endothelial cells (BMECs) (Xi et al., 2017). MiR-132 was initially studied for its anti-inflammatory role in several diseases, such as Kaposi's sarcoma-associated herpesvirus infection and human inflammatory bowel disease (Lagos et al., 2010; Maharshak et al., 2013; Shaked et al., 2009). In the lentiviral miR-132-treated mouse brain, both edema and Evans Blue (EB) leakage were decreased at day 3 after ICH compared with

the controls, and the expression levels of classic TJs, such as claudin-5 and ZO-1, were significantly increased (Zhang et al., 2017). These data suggested that miR-132 provided a protective effect on the BBB. Furthermore, in line with the reduced serum levels of miR-27a-3p in ICH patients, rats administrated the miR-27a-3p mimic had attenuated brain edema, EB leakage, and leukocyte infiltration to the perihematomal zone at 24 h after ICH (Xi et al., 2018; Zhu et al., 2015).

The mechanisms involved in miRNA-modulated BBB permeability after ICH can be variable. MiR-126-3p reduces the expression of the [phosphoinositide-3-kinase](#) regulatory subunit 2 in the perihematomal region and regulates the PI3K/Akt pathway that is responsible for BBB leakage (Kilic et al., 2006; Xi et al., 2017). Whereas, miR-27a-3p regulates endothelial water channel proteins in rat BMECs by directly binding to the 3'-UTR of aquaporin (AQP)-11, which is highly expressed in the endothelium of brain capillaries and is positively associated with increased BBB permeability (Koike et al., 2016).

2.2 MiRNAs manipulate BBB function in other CNS disorders

A summary of miRNAs involved in BBB function in other CNS disorders was presented in Table 2. Studies from both animal and *in vitro* models were included.

2.2 Traumatic brain injury and spinal cord injury

TBI is a complex process that occurs when an external force traumatically injures the brain. The role of miR-21 on BBB integrity in CNS diseases was first studied in TBI. MiR-21 was upregulated in the rat cerebral cortex after moderate TBI and also observed in vessel-like structures in the brain (Redell et al., 2011). Ge et al. demonstrated that up-regulation of miR-21 in rat brains alleviated brain edema and BBB leakage of EB after TBI. The protective effects of miR-21 are achieved by inhibiting apoptosis and promoting the expression of angiopoietin-1 and Tie-2 (Ang-1/Tie-2 axis) in BMECs (Ge et al., 2015; Navi et al., 2014). In addition, miR-21-3p and miR-21-5p were also found to be involved in BBB damage after TBI. MiR-21-5p alleviates scratch injury-induced BBB leakage in rat BMECs through suppressing inflammation and apoptosis, which could be related to its down-regulation of nuclear factor of kappa light polypeptide gene enhancer in B cells (NF- κ B) and up-regulation of Akt and Ang-1/Tie-2 activities (Ge et al., 2016; Ge et al., 2019). In contrast, miR-21-3p aggravates TBI-induced BBB damage in mice and regulates OGD-induced cellular apoptosis and inflammation in the bEnd.3 endothelial cell line by targeting methionine adenosyltransferase 2B (MAT2B) (Ge et al., 2019). Moreover, miR-212 and miR-132 are highly expressed in the CNS and participate in synaptic plasticity, neurite outgrowth and endothelial vasodilatory function (Ucar et al., 2012; Wanet et al., 2012). Burek et al. revealed that the miR-212/132 cluster helps maintain BBB integrity by directly targeting the 3'-UTR of claudin-1, JAM 3, occluding, and tight junction-associated protein 1 (Burek et al., 2019).

In a rat model of spinal cord edema, IR resulted in the dysfunction of the blood-spinal cord barrier (BSCB) at 48 h after surgery, and microarray analysis showed that ten miRNAs were upregulated, and seven were downregulated (Li et al., 2016). Further genomic screening and luciferase assays identified that miR-320a protects BSCB integrity by binding to the 3'-UTR

of the water-channel protein AQP1, a small integral membrane protein of epithelial and/or glial cells. Whereas, miR-129-5p was found to prevent BSCB leakage by directly targeting the 3'-UTR of high-mobility group box-1 (HMGB1) in a mouse model of spinal cord IR injury (Li et al., 2017).

2.3 MiRNAs in dementia

Dementia is a class of brain vascular diseases contributing to any degree of cognitive impairment or dementia, and it is one of the severest public health concerns for the modern aging world. The two most common forms are AD and vascular dementia (VD) (Goodman et al., 2017). Increasing reports indicated that BBB damage is an important feature in the pathogenesis of dementia and abnormal BBB permeability correlated with enlarged regions of cerebral damage in the white matter of patients with dementia (Toyama et al., 2019; Wong et al., 2019). A meta-analysis revealed that patients with dementia present increased BBB permeability and that BBB impairment mainly occurs in the hippocampus (Farrall and Wardlaw, 2009; Montagne et al., 2015).

As described above, BBB integrity is strictly controlled under physiological condition, whereas AD patients present increased cerebrospinal fluid/serum or cerebrospinal fluid/plasma ratio of albumin and reduced expression of TJ-related proteins (Algotsson and Winblad, 2007; Yamazaki and Kanekiyo, 2017). Moreover, the accumulation of A β initiates BBB dysregulation by affecting multiple components of the neurovascular unit. MiR-33 regulates the A β level in mouse brains by regulating the ATP-binding cassette transporter A1 (ABCA1), a key regulator of apolipoprotein E lipidation (Kim et al., 2015). Overexpression of miR-33 impairs cellular cholesterol efflux and dramatically increases the extracellular A β level by suppressing ABCA1. MiR-107 was found to be significantly downregulated in endothelial cells pre-incubated with A β and overexpression of miR-107 largely abrogated A β -induced BBB disruption and endothelial cell dysfunction (Liu et al., 2016). This protective role of miR-107 is achieved by miR-107-induced down-regulation of endophilin-1, which is involved in the regulation of BBB permeability and expression of TJ-related proteins (Chen et al., 2015). Moreover, in the human cerebral microvascular endothelial cell line hCMEC/D3, overexpression of miR-224-5p or miR-497-5p decreased BBB permeability in the AD microenvironment (Zhu et al., 2019). Dual-luciferase assay revealed that miR-224-5p or miR-497-5p directly binds to the 3'-UTR of endophilin-1 mRNA, and increases the expression of ZO-1, occluding, and claudin-5 via a miR-224-5p/Endophilin-1 or miR-497-5p/Endophilin-1 signaling cascade.

There are several lines of mechanisms involved in VD, including oxidative stress, neuroinflammation, endothelial cell damage, and astrocyte/pericyte alteration (Parfenov et al., 2019). BBB leakiness is likely due to hypoxia and neuroinflammation-triggered vascular deterioration and apoptosis, resulting in the redistribution of TJs (Biron et al., 2011). Changes in miRNA levels contribute to altered expression of dementia-related proteins, consequently impacting the development of this disease (Viegas et al., 2017). Toyama et al. observed that miR-501-3p was upregulated after bilateral common carotid artery stenosis surgery using a mouse model of vascular cognitive impairment (Toyama et al., 2018). What's more, miR-501-3p directly binds to the 3'-UTR of ZO-1 mRNA and downregulated

transendothelial electric resistance. Manipulating target genes that regulate BBB or TJ integrity could be effective therapy for dementia.

2.4 MiRNAs in brain tumors

Glioma is one of the most common types of primary brain tumors, including astrocytoma, ependymoma, glioblastoma, and oligodendroglioma. Oncogenic or tumor-suppressive miRNAs have been discovered in malignant glioma in recent years (Nie et al., 2019; Petrescu et al., 2019). As shown in Table 2, numerous miRNAs can modulate the permeability and integrity of the BBB in an *in vivo* or *in vitro* model of glioma. In a mouse model of brain metastasis, several miRNAs, including miR-181c, were found in cancer-derived extracellular vesicles (EVs). MiR-181c triggers the breakdown of the BBB and promotes the extravasation of cancer cells through the BBB (Tominaga et al., 2015). Although the contribution of miR-181c in this process is not clear, it was found that breast cancer patients presented an increased level of circulating miR-181c, suggesting a major role of miR-181c in the destruction of the BBB. MiR-509 was found to be significantly decreased in brain metastatic lesions, and miR-509 contributes to decreased BBB permeability through promoting the secretion of TNF- α and inhibiting MMP-9 expression by binding to RhoC (Xing et al., 2015). In a mouse model of neuroblastoma, it was reported that miR-497 reduces the proliferation of chemoresistant neuroblastoma cells and inhibited vascular permeability by targeting key genes such as vascular endothelial growth factor A (VEGF-A), which causes a decrease in transendothelial electric resistance of cultured endothelial cells (Argaw et al., 2009; Soriano et al., 2016).

Growing evidence has revealed that other miRNAs are also involved in the manipulation of blood-tumor barrier (BTB) in human endothelial cells as summarized in Table 2. Overexpression of miR-181a, miR-18a, miR-34c, miR-34a, miR-144, miR-140, miR-181d-5d, miR-137, miR-148b-3p, piR-DQ590027/miR17HG, and miR-429 significantly increases the permeability of BTB and disrupts or downregulates the expression of junction-related proteins (Cai et al., 2015; Dai et al., 2018; Guo et al., 2017; Leng et al., 2018; Ma et al., 2016; Ma et al., 2014; Sa et al., 2017; Yu et al., 2017; Zhao et al., 2015a; Zhao et al., 2015b; Zhao et al., 2015c). Knockdown of miR-200b and miR-330-3p restores BTB integrity *in vitro* (Liu et al., 2017; Ma and Xue, 2016).

2.5 MiRNAs in autoimmune disorders

Multiple Sclerosis (MS) is a chronic immune-mediated disease that attacks the CNS and affects the brain, spinal cord, and optic nerves. In active MS lesions, large numbers of leukocytes infiltrate into brain parenchyma through the immune-activated BBB, which is tightly regulated by various cell adhesion molecules, integrins, cytokines, and chemokines (Aube et al., 2014). Traditional treatments are not available to halt the disease progression and approaches aiming to relieve symptoms and reduce disease progression have been limited to date (Kamphuis et al., 2015). MiR-155 is one of the most highly expressed miRNAs in acute MS lesions and was found to be rapidly upregulated under inflammatory conditions (Lopez-Ramirez et al., 2014). MiR-155 impairs BBB integrity and reorganizes the structure of vinculin and ZO-1 by binding to two focal adhesion components: dedicator of cytokinesis 1 (DOCK-1) and syntenin-1, two interendothelial junctional complex

molecules: claudin-1 and annexin-2. Evidence from the *in vitro* studies suggested that more miRNAs, such as miR-125a-5p and miR-126, are involved. MiR-125a-5p, known as a tumor suppressor miRNA, is reduced in ECs derived from MS lesions and was reported to regulate barrier function-related proteins features such as claudin-5 and ZO-1 under a currently unexplored mechanism (Reijerkerk et al., 2013). MiR-126 and its complement miR-126* originating from the same precursor are amongst the most abundant miRNAs expressed in resting endothelium (Harris et al., 2008). In a human BMEC based *in vitro* BBB model, down-regulation of endothelial miR-126 and miR-126* enhances monocyte and T cell adhesion to human brain ECs, and increases the expression of E-selectin and VCAM1 respectively, while overexpression of miR-126 reduced VCAM1 and CCL2 levels (Cerutti et al., 2017).

In addition, miR-98 and let-7g* were also identified under neuroinflammatory conditions (Rom et al., 2015). Both miR-98 and let-7g* belong to highly conserved miRNAs, the let-7 group. They are capable of decreasing leukocyte adhesion and migration across the BBB by targeting the inflammatory chemokine ligands, CCL2 and CCL5.

2.6 MiRNAs in cerebral infections

The HIV-1 Tat protein has been shown to affect BBB integrity and permeability in HIV neuropathogenesis (Hui et al., 2012). It was found that miR-101 overexpression effectively suppresses the expression of VE-cadherin and claudin-5 in human BMECs (Mishra and Singh, 2013). MiR-101 has been previously summarized as an important mediator of tumorigenesis in a subset of cancer cells, glioblastoma multiforme, acute lymphocytic leukemia and plays a significant role in cerebral infection (Gui and Shen, 2012). In human BMECs, miR-101 directly binds to mutated VE-cadherin 3'-UTR and consequently influences the expression level of claudin-5. Brain endothelial miR-155 also negatively regulates BBB function during neuroinflammation (Caballero-Garrido et al., 2015; Pena-Philippides et al., 2018). It was demonstrated in a mouse model of severe cerebral malaria that miR-155 enhances BBB permeability through interaction with the Ang/Tie2 axis (Barker et al., 2017). In addition, coxsackievirus A16 is another highly neurotropic virus that is commonly seen in children's brain enterovirus infection disease. Recently, Song et al. found that miR-1303 increases BBB permeability in human umbilical vein ECs and this effect is associated with degradation of claudin-4, claudin-5, VE-cadherin, and ZO-1 (Song et al., 2018).

2.7 MiRNAs in other CNS disorders

MiRNAs are also found to be involved in other CNS disorders such as hyperhomocysteinemia, lead poisoning, and methamphetamine abuse. Hyperhomocysteinemia causes the BBB damage, and miR-29 mimics significantly increases the BBB permeability in hyperhomocysteinemic mouse brain endothelial cells by elevating the expression of MMP-9. Further work has demonstrated that miR-29b regulated BBB dysfunction through DNA methyltransferase 3 beta (DNMT3β) and MMP-9. Lead exposure significantly increases the leakage of the blood-cerebrospinal fluid barrier (BCB) in immortalized murine choroidal epithelial cells, and treatment of a miR-203 inhibitor attenuates lead-induced BCB leakage by binding to the 3'-UTR of tricellulin (Su et al.,

2015). Methamphetamine exposure disrupts the BBB structure and Silencing miR-143 ameliorates the increased permeability induced by methamphetamine by targeting p53 up-regulated modulator of apoptosis (PUMA), consequently regulating the expression of TJ related proteins (Bai et al., 2016).

3. MiRNAs as the target of BBB-based therapies

The BBB is clearly very important for both physiological and pathological conditions of CNS function and miRNAs are important regulators of protein expression that are involved in many cellular processes (Sweeney et al., 2019). Some miRNAs, such as miR-30c, miR-143/miR-145, miR-15a/16-1, and miRNA let-7b, have been identified as candidate biomarkers to aid diagnosis in clinical trials (Baraniskin et al., 2018; Derkow et al., 2018; Sun et al., 2018; Wei et al., 2016). The approval of patisiran, the first RNA interference-based drug, heralds a new era for in this field. Several RNA-based therapies are moving through more mature clinical research and has been summarized in a recent publication (Setten et al., 2019). However, most studies focusing on the therapeutic usage of miRNAs are still at the phase of animal experiments. MiR-494 has been shown to alleviate I/R injury in mouse models via various signal pathways (Lan et al., 2012; Wang et al., 2010; Xiong et al., 2014) and is the only ongoing clinical trial designed to identify its role in the recovery of ischemic stroke ([Clinicaltrial.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT03577093). Identifier: [NCT03577093](https://clinicaltrials.gov/ct2/show/study?term=NCT03577093)). In this regard, more clinical studies will be needed to bridge the gap between preclinical and clinical studies.

In animal studies, intravenous injection of miRNA agomir/mimic or miRNA hairpin inhibitor/antagomir is the most common way of miRNA delivery (Sun et al., 2018). However, the injured brain is not the only target of these special drugs, because miRNA inhibitors or antagomirs may affect other organs or systems as well. The use of exosomes is a possible way to overcome this side effect. Exosomes are 30–100 nm extracellular lipid membrane vesicles secreted by most cell types. Exosomes that contain miRNAs and other small or large molecules can travel to distant tissues to directly influence various aspects of cell behavior (Zhao and Zlokovic, 2017). In some neurological diseases including neurodegenerative disorders, exosome miRNAs can pass through the BBB or transport across the synapse, thereby affecting communication between the blood and brain or neural activities (Blandford et al., 2018). Results from recent studies suggested that exosomes can be efficient delivery tools and their usage in the application of miRNA therapeutic strategies for CNS disorders is under active investigation (Tominaga et al., 2015). However, several issues should be further addressed before using exosomes in clinical trials: the optimization of methods to obtain the most proficient and pure exosomes is critical to ensure therapeutic safety (Lener et al., 2015). In addition, for an effective and particularly safe translation of exosome-based therapies into clinical practice, a higher level of cooperation between researchers, clinicians, and other medical professionals is essential. In addition, miRNA sponges can be another gene therapy strategy that employ artificial non-coding RNA with multiple binding sites for a specific miRNA. Some circle RNAs may act as endogenous sponges that interact with miRNAs and regulate the expression of miRNA-targeted genes or serve as protein decoys to directly control cellular function (Bai et al., 2018; Yu and Kuo, 2019). However, whether the accumulation of circle RNAs is deleterious for nervous system function is still unexplored.

Although these above-described therapeutic molecules are designed to only bind to their targets, blocking or enhancing a single miRNA could probably produce multifaceted effects due to its ability to facilitate multiple compensatory pathways. Moreover, miRNAs at non-physiological concentrations likely have unknown targets that lead to adverse effects by targeting normal cell homeostasis (Rupaimoole and Slack, 2017). It is important to carefully and comprehensively investigate the mRNA “targetome” before proceeding to clinical therapeutics.

Furthermore, the timing of miRNA regulation must also be considered according to their cellular development due to possible developmental changes in miRNA expression.

4. Summary and perspective

MiRNAs are versatile regulatory molecules that influence almost every aspect of BBB structure and function. First, the translational dysfunction of individual proteins that constitute the BBB structure can impair BBB physiological function. Second, miRNAs are involved in regulating biological processes such as apoptosis and inflammation, which can exacerbate BBB dysfunction. Although much progress has been made, the role of miRNAs in BBB function remains poorly characterized. Understanding the mechanisms of miRNAs in regulating BBB structure and function may lead us to discover novel pharmaceutical targets for the development of effective microRNA-based therapies against CNS disorders.

Other miRNAs are also found to regulate endothelial cell barrier permeability in many different peripheral organs or tissues. For instance, decreased expression of miR-30 family members was found to be associated with the loss of cell adhesion by targeting snail-1, which is shown to repress the expression of claudins, occludin, and E-cadherin in pancreatic cells (Cano et al., 2000; Ikenouchi et al., 2003; Joglekar et al., 2009). Rho-activated protein kinase (ROCK1) was found to reduce vascular integrity by preceding cell-cell junction delocalization and miR-146 suppressed the expression of ROCK1 in prostate carcinoma cells (Huang et al., 2008; Lu et al., 2014). MiR-874 promoted intestinal barrier permeability and regulated the expression of occludin and claudin-1 by binding to aquaporins, whereas let-7b preserves the intestinal barrier and promotes the expression of occludin, ZO-1, and protein phosphatase 2A by regulating p38 MAPK molecular pathways (Liu et al., 2018; Zhi et al., 2014). Besides, miR-142-3p/5p, miR-26a, and miR-98 were also widely reported to regulate endothelial barrier integrity in testes, heart, thyroid, lung, and skin (Good et al., 2018; Hu et al., 2015; Hu et al., 2018; Xu et al., 2018; Zhu et al., 2016). Several of these miRNAs may play an essential role in the BBB modulation in CNS disorders as well, and further studies are warranted.

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Abbreviations:

ABCA1

ATP-binding cassette transporter A1

AD	Alzheimer's disease
Aβ	Amyloid beta peptide
AJs	Adherens junction proteins
Ang	Angiopoietin
AQP	Aquaporin
BBB	Blood-brain barrier
BCB	Blood cerebrospinal fluid barrier
BMEC	Brain microvascular endothelial cell
BSCB	blood-spinal cord barrier
BTB	Blood tumor barrier
CNS	Central nervous system
CYCS	Cytochrome c
dMCAo	Distal middle cerebral artery occlusion
DNMT3β	DNA methyltransferase 3 beta
DOCK-1	Dedicator of cytokinesis 1
EB	Evans Blue
ECs	Endothelial cells
EVs	Extracellular vesicles
FOXC1	Forkhead box C1
HIV	Human immunodeficiency viruses
HMGB1	High-mobility group box-1
HSF2	Heat shock transcription factor 2
ICH	Intracerebral hemorrhage
IR	Ischemia-reperfusion
JAM	Junctional adhesion molecule
KLF6	Krüppel-like factor 6
MAT2B	Methionine adenosyltransferase 2B
MAPK	Mitogen-activated protein kinase
MEF2D	Myocyte enhancer factor 2D

MiRNAs	MicroRNAs
MMPs	Matrix metalloproteases
mRNAs	Messenger RNAs
MS	Multiple sclerosis
MAZ	Myc-associated zinc-finger protein
NFYA	Nuclear factor YA
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B cells
OGD	Oxygen-glucose deprivation
PDPK-1	Phosphoinositide-dependent protein kinase-1
PKC	Protein kinase C
PUMA	P53 unregulated modulator of apoptosis
ROCK1	Rho-activated protein kinase 1
ROS	Reactive oxygen species
S1PR2	Sphingosine-1-phosphate receptor 2
SOX5	Sex determining region Y-box protein 5
TBI	Traumatic brain injury
TJs	Tight junction proteins
TNF-α	Tumor necrosis factor alpha
USF1	Upstream stimulatory factor 1
VCAM1	Vascular adhesion molecule 1
VD	Vascular dementia
VE-cadherin	Vascular endothelial-cadherin
VEGF-A	Vascular endothelial growth factor A
ZO	Zonula occludens
3'-UTR	3'- untranslated regions

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Highlights:

MiRNAs play a crucial role in the regulation of BBB structure and function.

MiRNAs directly regulate the expression of junctional proteins in CNS diseases.

MiRNAs mediate BBB structure and function via multiple mechanisms in CNS diseases.

Figure 1. BBB disruption in CNS diseases

Simplified pathologies of common CNS diseases and the most relevant alterations in the BBB. CNS disorders cause a series of molecular events including apoptosis and activation of ROS, proinflammatory/anti-inflammatory factors, MMPs and immune cells. All of these pathological events contribute to BBB damage by altering BBB composition.

Table 1

MiRNAs modulate BBB function in stroke

Disorders	miRNAs	Study subject	Proposed contribution to BBB	Target gene/Mechanism	Refs
Ischemic stroke	Negative				
	miR-150	Rat	Increases BBB permeability	Reducing the claudin-5 expression by binding to angiopoietin receptor Tie-2	(Fang et al., 2016)
	miR-210	Rat	MiR-210 disrupts BBB integrity	Suppressing the expression of junction proteins by directly binding to occludin and β -catenin	(Ma et al., 2017)
	miR-143	Mouse	The inhibited miR-143 activity maintains BBB integrity	Regulating TJs expression by binding to Hectd 1	(Bai et al., 2018)
	miR-130a	Rat	MiR-130a increases BBB permeability	Down-regulating the expression of occludin by binding to homeobox A5	(Wang et al., 2018)
	miR-15a/16-1	mouse	MiR-15a/16-1 impairs BBB integrity	Directly binding to claudin-5	(Zuo et al., 2019)
	miR-34a	bEnd.3 cell line	MiR-34a enhances BBB permeability	Impairing mitochondrial function in ECs by binding to CYCS	(Bukeirat et al., 2016)
	miR-155	HBMECs	MiR-155 impairs endothelial monolayer integrity	Reducing the expression of ZO-1 by directly binding to claudin-1	(Pena-Philippides et al., 2018)
	Positive				
	miR-149-5p	Rat	MiR-149-5p reduces BBB permeability	Enhancing N-cadherin expression by binding to S1PR2	(Song et al., 2018)
	miR-539	Rat	MiR-539 preserves BBB integrity	Mediating BBB structure by binding to MMP-9	(Fan et al., 2018)
	miR-21	Rat	MiR-21 alleviates I/R-induced BBB disruption	Blocking the MAPK signaling pathway by binding to MAPK2K3	(Yao et al., 2018)
	miR-132	Mouse	MiR-132 preserves BBB integrity	Regulating the degradation of VE-cadherin and β -catenin by directly binding to MMP-9	(Zuo et al., 2019)
Hemorrhagic stroke	Positive				
	miR-126-3p	Rat	MiR-126-3p preserves BBB integrity and function	Inhibiting neural inflammation by targeting PIK3/Akt pathway	(Xi et al., 2017)
	miR-132	Mouse	MiR-132 mitigates BBB permeability	Inhibiting the expression of ZO-1 and claudin-5	(Zhang et al., 2017)
	miR-27a-3p	Rat	MiR-27a-3p attenuates brain edema and BBB leakage	Protecting against post-ICH complications by directly binding to AQP11	(Xi et al., 2018)

Table 2

MiRNAs modulate BBB function in non-stroke CNS diseases

Diseases	miRNAs	Study subject	Proposed contribution to BBB	Target gene/Mechanism	Refs
TBI	Negative				
	miR-21-3p	Mouse	MiR-21-3p aggravates BBB damage	Regulating TJ protein expression by binding to MAT2B	(Ge et al., 2019)
	Positive				
	miR-21	Rat	MiR-21 alleviates brain edema and BBB leakage	Reducing the loss of TJ proteins by promoting the expression of Ang-1/Tie-2 axis in BMECs	(Ge et al., 2015; Navi et al., 2014)
	miR-212/132	Mouse	MiR212/132 maintains the BBB integrity	Directly targeting claudin-1, JAM3, and Tjap1	(Burek et al., 2019)
Spinal cord injury	miR-21-5p	Rat BMVECs	MiR-21-5p alleviates BBB leakage <i>in vitro</i>	Suppressing inflammation and apoptosis	(Ge et al., 2016)
	Positive				
	miR-320a	Rat	mir-320a protects BSCB integrity	Binding to AQP1	(Li et al., 2016)
	miR-129-5p	Mouse	miR-129-5p prevents BSCB leakage	Targeting HMGB1	(Li et al., 2017)
	AD				
	Negative				
	miR-33	Mouse	MiR-33 impairs cellular cholesterol efflux and increases the accumulation of A β in neural cells	Regulating A β metabolism by directly targeting ABCA1, promoting A β secretion and impairing A β clearance	(Kim et al., 2015)
VD	Positive				
	miR-107	hCMEC/D3	MiR-107 prevents A β -induced BBB disruption	Targeting endophilin-1	(Liu et al., 2016)
	miR-224-5p(miR497-5p)	hCMEC/D3	MiR-224-5p decreases the BBB permeability	Increasing the expression of ZO-1, occludin, and claudin-5 by binding to endophilin-1	(Zhu et al., 2019)
	miR-501-3p	Mouse	MiR-501-3p decreases the BBB permeability	Directly binding to ZO-1	(Toyama et al., 2018)
	Brain tumor				
	Negative				
	miR-181c	Mouse	Breast cancer cell-derived EVs contained miR-181 triggers the BBB disruption	Controlling the reorganization of actin filament by suppressing PDPK-1	(Tominaga et al., 2015)
Brain tumor	miR-181a	hCMEC/D3	MiR-181a impairs BTB integrity	Regulating the expression of ZO-1, occludin, and claudin-5 by binding to KLF6	(Ma et al., 2014)
	miR-18a	hCMEC/D3	MiR-18a increases the permeability of BTB	Down-regulating the expressions of ZO-1, claudin-5, and occludin by binding to MEF2D	(Zhao et al., 2015c)
	miR-34c	hBMEC/D3	MiR-34c impairs BTB integrity	Regulating the BBB integrity by directly binding to MAZ gene	(Zhao et al., 2015a)
	miR-34a	Glioma ECs	MiR-34a impairs BTB integrity	Reducing the expression of TJ-related proteins by directly binding to PKC ϵ gene	(Zhao et al., 2015b)

Diseases	miRNAs	Study subject	Proposed contribution to BBB	Target gene/Mechanism	Refs
	miR-144	hCMEC/D3	MiR-144 increases BTB permeability	Regulating the expression of ZO-1, occludin, and claudin-5 by binding to HSF2	(Cai et al., 2015)
	miR-140	hCMEC/D3	MiR-140 increases BTB permeability	Regulating the expression of ZO-1, occludin, and claudin-5 by targeting NFYA	(Ma et al., 2016)
	miR-181d-5d	hCMEC/D3	MiR-181d-5d increases the BTB permeability	Regulating the expression of ZO-1, occludin, and claudin-5 by binding to SOX5	(Guo et al., 2017)
	miR-137	hCMEC/D3	MiR-137 increases BTB permeability	Regulating the expression of ZO-1, occludin, and claudin-5 by binding to USF1	(Yu et al., 2017)
	miR-148b-3p	hCMEC/D3	MiR-148b-3p increases BTB permeability	Regulating the expression of ZO-1, occludin, and CXCR7 by binding to ZO-2 and FOXC1	(Sa et al., 2017)
	piR-DQ590027/ miR-17HG	hCMEC/D3	piR-DQ590027/ miR-17HG increases the BBB permeability	Decreasing the expression of ZO-1, occludin, and claudin-5 by binding to miR-153 (miR-377) which binds to FOXR2	(Leng et al., 2018)
	miR-429	hCMEC/D3	MiR-429 increases the BTB permeability	Regulating the expression of tight junction proteins by binding to ZO-1 and occludin genes	(Dai et al., 2018)
	Positive miR-509	Mouse	MiR-509 decreases the BBB permeability	Promoting the secretion of TNF- α and inhibiting the expression of MMP-9 by binding to RhoC	(Xing et al., 2015)
	miR-497	Mouse	MiR-497 reduces the permeability of blood vessels	Targeting CHEK1, AKT3, and VEGF-A	(Soriano et al., 2016)
	miR-200b	hCMEC/D3	MiR-200b restores the BTB integrity	Regulating the expression of occludin and claudin-5 by binding to RhoA and ROCKII genes	(Ma and Xue, 2016)
	miR-330-3p	hCMEC/D3	MiR-330-3p decreases the BTB permeability	Increasing the expression of TJs such as ZO-1, occludin, and claudin-5 by binding to PKC α	(Liu et al., 2017)
	MS Negative miR-155	Mouse	MiR-155 impairs BBB integrity	Reorganizing the structures of vinculin and ZO-1 by binding to DOCK-1, syntenin-1, claudin-1, and annexin-2 genes	Lopez-Ramirez et al. 2014
	miR-125a-5p	hCMEC/D3	MiR-125a-5p increases brain endothelial cell barrier function and the expression level of 107 miRNAs	Regulating claudin-5 and ZO-1	Reijerkerk et al. 2013
	miR-126/ miR-126*	hCMEC/D3	MiR-126/miR-126* reduces leukocyte adhesion to endothelial cells	Reducing monocytic and T cell shear-resistant adhesion by binding to VCAM1, CCL2, E selection, and CCL7	Cerutti et al. 2017
	Positive miR-98 and let-7g*	Mouse	MiR-98 and let-7g* diminish BBB permeability	Reducing pro-inflammation by directly binding to CCL5 gene	Rom et al. 2015
	Brain infection Negative				

Diseases	miRNAs	Study subject	Proposed contribution to BBB	Target gene/Mechanism	Refs
	miR-101	hBMVECs	MiR-101 impairs BBB integrity and permeability	Inhibiting claudin-5 expression by directly binding to VE-cadherin	Mishra et al. 2013
	miR-155	Mouse	MiR-155 impairs BBB integrity	Targeting Ang/Tie2 axis	Barker et al. 2017
	miR-1303	Human umbilical vein ECs	MiR-1303 increases the BBB permeability	Degrading junction proteins claudin-4, claudin-5, VE-cadherin, and ZO-1 by binding to MMP-9	Song et al. 2018
Others	Negative				
	miR-29b	Mouse	MiR-29a mediates the opening of the BBB	Directly binding to DNMT3 β and subsequently regulating MMP-9	Kalani et al. 2014
	miR-143	Mouse	MiR-137 increases BBB permeability	Inducing the activation of NF- κ B and p53 transcription factors by binding to PUMA, consequently regulating the expression of TJ-related proteins	Bai et al. 2016
	Positive				
	miR-203	Z310 cells (immortalized murine choroidal epithelial cells)	MiR-203 preserves the lead-induced BCB integrity	Directly binding to tricellulin	Su et al. 2015