

MicroRNAs: Possible Regulatory Molecular Switch Controlling the BBB Microenvironment

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The human brain is separated and restricted from circulatory arrangement through an extremely proficient blood-brain barrier (BBB). The BBB is formed by endothelial cells that provide selective impermeability to the BBB, maintaining the finest microenvironment in the central nervous system (CNS). The BBB, a unique anatomical and physiological barrier, is responsible for protecting the CNS.^{1,2} It has been acknowledged that various components in the brain communicate with each other via exchanging signaling molecules through gap junctions and signaling pathways involving various kinds of receptors and exosomes.³ The BBB prevents entry of blood-derived pathogens, toxic substances, and unwanted substances in the brain from brain blood capillaries.^{4,5} The BBB is also responsible for the transport of different substrates, such as oxygen, nutrients, energy metabolites, etc., from the blood to the brain.³ Conversely, it also helps to eliminate metabolic waste from the brain back into the circulatory system.⁶

Scientists have made efforts to understand the molecular structure of the BBB for a long time. In a recent review by Chow and Gu,⁷ various molecular determinants and key cellular pathways that provide BBB characteristics to endothelial cells of the CNS were discussed. Another paper by Abbott et al.⁸ illustrated the structure and functions of BBB and summarized that BBB is a neurovascular unit that manages molecular exchange and cellular traffic at the blood-CNS interface. Ballabh et al.⁹ defined brain microvasculature as being composed of the BBB with endothelial cells, pericytes, and astrocyte endfeet. The tight junctions that are present amid the cerebral endothelial

cells constitute a diffusion barrier. The neural microenvironment regulates the development and function of the BBB and controls BBB development through Wnt and Sonic hedgehog signaling pathways.³ Zhao and Zlokovic¹⁰ recently reported that exosomes are one of the regulating components of the BBB along with microRNAs (miRNAs). However, until today, molecular switches that controlled the BBB were still not correctly and appropriately understood.

Current studies show that cells liberate endosome-derived microvesicles, which are called exosomes. These are double membrane extracellular vesicles (EVs) and are tiny (50–100 nm size). Every cell type produces exosomes, which are enriched with signaling proteins and lipids and released into the extracellular space.¹¹ Exosomes have very distinct physiologic functions. However, the role of exosomes may differ depending on their cellular origin, such as from blood coagulation, immune regulation, cell differentiation, cell migration, and cell-to-cell communication.¹² It has been acknowledged that exosomes contain specific proteins and RNA to interact with neighboring cells.¹³ In the nervous system, microglia-derived microvesicles have also been reported,¹⁴ and these microvesicles (exosomes) can be influenced by neurotransmitters.¹⁵ These microvesicles have been reported to regulate synaptic plasticity and neuron-glia communication.^{13,16,17} Along with the protein, exosomes also carry RNAs, including miRNAs.¹³ These transported miRNAs within the exosomes have an essential role in intercellular communication¹⁸ and a range of physiological functions.¹⁹

miRNAs are single-stranded, small noncoding RNAs (ncRNAs) containing a small number of nucleotides (21–23 nucleotides) and can silence gene expression. These small ncRNAs can control gene expression through a post-transcriptional gene regulation process.²⁰ In the CNS microenvironment, miRNAs play a regulatory role in gene expression during neural development.^{21,22} Cao et al.²³ suggested that miRNAs are critical regulators in the CNS and are spatially expressed in the brain, implicating their role in neural development and function.²⁴ It has been reported that two miRNAs are essential for the physiological process of brain function (miRNA-941 and miRNA-1202). miR-941 inhibits the expression of the host gene, DNAJC5, in both regions of the brain. These genes interact with two other genes (RAB3A and WDR7; directly with RAB3A and indirectly with WDR7). This phenomenon is related to neurotransmitter release.²⁴ A decreased expression of miR-1202 was observed in the brain samples with a depressed state when compared to the standard control brain samples. When the miR-1202 gene is expressed, the quantity of metabotropic glutamate receptor-4 (GRM4) expression is enhanced, which demonstrates antidepressant effects. GRM4 expression is observed throughout the brain, and the expression is associated with the release of various neurotransmissions, such as

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**Table 1. miRNAs that Control the BBB Microenvironment**

No.	miRNAs	Remark	Reference
1	miR-132	it control the BBB integrity with exosome	28
2	miR-1303	it regulates BBB permeability	29
3	miR-130a	it control the cerebral ischemia-induced BBB permeability	31
4	miR-210	it disrupts BBB integrity during hypoxic-ischemic brain injury	33
5	miR-285	miR-285-Yki/Mask regulatory circuit controls the mechanism of BBB integrity	34
6	miR-143	it protects the BBB integrity	35
7	miR-155	it regulations the leukocyte adhesion at the inflamed BBB	36
8	miR-27a-3p	upregulated AQP11 during intracerebral hemorrhage (ICH)	37
9	miR-212/132	targets Jam3, Cldn1, and Tjap1, affecting BBB property and integrity	38

dopaminergic, glutamatergic, serotonergic and, GABAergic.²⁵ Recent work by Nowakowski et al.²⁶ indicates that the miRNA network regulates cell-type-specific transcriptomes during human brain development. One such example is an interaction between miR-2115 and radial-glia-enriched ORC4, which emerges to control radial glia propagation rates during brain development. A study showed that overexpression of let-7 and miR-98 reduced adhesion and migration of leukocytes, decreased the release of pro-inflammatory cytokines, and increased tightness in the BBB during neuroinflammatory conditions.²⁷ Taken together, these two miRNAs help in protecting (preventing leakiness) the BBB under neuroinflammatory diseases.²⁷ miRNAs are essential molecular switches that control the BBB microenvironment.

In a recent study, a group of researchers examined the function of miR-132 to understand vascular integrity and brain function.²⁸ Vascular integrity is vital for the proper development and function of the CNS. It also assists in maintaining the brain microenvironment to the state of homeostasis. In this study, the researchers utilized zebrafish as a model system and found that miR-132 is mostly expressed in neurons and is highly conserved. Neurons secrete miRNA through exosomes to the endothelial cells and help maintain the brain vascular integrity. When

miR-132 was antagonized with morpholino antisense oligonucleotides, a severe intracranial hemorrhage was observed, causing damage to the BBB integrity. It was actually a result of the leakage of red blood cells into the brain ventricles. This discovery was confirmed by CRISPR-Cas9 (a genomic editing system). To support the intercellular exosome transfer hypothesis, the researchers used transgenic zebrafish larvae, filled neuronal exosomes with green fluorescent protein (GFP), and labeled brain endothelial cells with red fluorescent protein (RFP). Researchers obtained confocal images to explain the uptake of GFP-positive exosomes through the RFP-positive endothelial cells.²⁸ Simultaneously, they also performed an experiment where they showed the uptake of purified neuronal exosomes by the brain endothelial cells of mice. For this, miR-132 was transferred to the co-cultured endothelial cells, and the transfer uptake was studied. From this experiment, they concluded that this mechanism is conserved throughout the mammalian group.²⁸ They observed that miR-132 regulates VE-cadherin (vascular endothelial cadherin) expression. It is a critical adherens junction protein that directly targets the eukaryotic eef2k (elongation factor 2 kinase) required for brain vascular integrity. In conclusion, disruption of the miR-132 expression or exosome secretion or overexpression of vascular eef2k damages VE-cadherin expression and

ultimately the brain vascular integrity. Therefore, the study implies that miR-132 acts as a signal mediator of neural regulation for brain vascular integrity. It also suggests that the neuronal exosome with miRNAs is a novel way for neurovascular communication through the BBB.²⁸ Xu et al.²⁸ have shown a new direction of thinking for the role of exosomes and miRNAs in controlling the BBB microenvironment in healthy states and disease states. Zhao and Zlokovic¹⁰ concluded from the work of Xu et al.¹⁰ that exosomes and miRNAs could remotely regulate BBB integrity and the controlling switch for BBB function. It will open a new line for new therapeutic strategies for brain diseases.

Song et al.²⁹ stated that miR-1303 regulates BBB permeability. In this study, they showed that miR-1303 may control BBB permeability by targeting a brain protein called matrix metalloproteinase 9 (MMP-9). MMP-9 is a member of a family of zinc-dependent endopeptidases and is a critical CNS protein that is required during brain development.³⁰ A recent study by Wang et al.³¹ stated that miR-130a controls cerebral ischemia-induced BBB permeability. They found that miR-130a was mainly from brain microvascular endothelial cells and that ischemia considerably increased the level of miR-130a. Conversely, the use of antagomir-130a, an antagonist of miR-130a, brain edema can be attenuated by lowering the BBB permeability. Recently, Almutairi et al.³² reviewed and concluded that miRNAs are indeed essential factors that control the BBB permeability. Ma et al.³³ found that miRNA-210 disrupts BBB integrity during hypoxic-ischemic brain injury in rats by suppressing junction proteins. They demonstrated that inhibition of miR-210 by miR-210-LNA (complementary nucleic acid oligonucleotides) preserved the expression of junction proteins. Recently, in the *Drosophila* model, Li et al.³⁴ found that the miR-285-Yki/Mask regulatory circuit controls the mechanism for BBB maintenance and thus BBB integrity. miR-143 is a primary miRNA that plays a significant role in various cellular processes. A recent study showed that silencing miRNA-143 protects the BBB integrity.³⁵ miR-155 is a brain endothelial miRNA that is upregulated in



multiple sclerosis and by inflammatory cytokines in the human brain. miR-155 regulates leukocyte adhesion at the inflamed BBB.³⁶ In addition, another miRNA, miR-27a-3p, was downregulated and AQP11 was upregulated during intracerebral hemorrhage (ICH), contributing to the disruption of the BBB.³⁷ It was observed that the miR-27a-3p mimic targeted AQP11 and protected against ICH.³⁷ Burek et al.³⁸ observed that miR-212/132 was overexpressed in hypoxic brain microvascular endothelial cells (BMECs) in both mouse and human brains. miR-212/132 targets Jam3, Cldn1, and Tjap1, affecting the BBB properties and integrity.³⁸ Therefore, it is clear that miRNA can control the BBB microenvironment (Table 1). Similar to the BBB, the blood-tumor barrier (BTB) restricts paracellular diffusion. It was found that miR-181a targets Kruppel-like factor 6 (a transcription factor) and increases the permeability of the BTB, indicating the therapeutic potential of miR-181a against gliomas.³⁹

The BBB microenvironment is an essential protecting structure for the CNS. Our article will open the door for novel thinking and innovation related to the miRNA controlled BBB microenvironment. Studies related to the role of miRNA controlling the BBB microenvironment are still in the infancy stages, and several questions should be answered as to their potential as a critical regulatory unit in controlling the BBB microenvironment. More research grants and participation are needed to understand the underlying mechanism and to achieve a therapeutic reply for neurological diseases.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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