

# Exosomal miRNA Research Technology for Combating Ischemic Stroke

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## ABSTRACT

Neurological dysfunction caused by ischemic stroke has brought a heavy economic burden to society and families. There is still a lack of specific treatment methods for ischemic stroke in clinical treatment. Exosomes have good compatibility and are easy to cross the blood-brain barrier. At the same time, exosomes can deliver contents to recipient cells and affect the biological function of recipient cells, which may provide new ideas for the treatment of ischemic stroke. As a part of exosome contents, miRNAs have attracted extensive attention. This article reviews the research progress of exosome miRNA in the treatment of ischemic stroke and the potential of targeted delivery of exosome miRNA in the treatment of ischemic stroke to provide a reference for the future research direction and the selection of therapeutic targets for haemorrhagic stroke.

**KEYWORDS:** Exosomes; Mirna; Ischemic stroke

## INTRODUCTION

Stroke is the leading cause of mortality and disability worldwide and the second leading cause of death after ischemic heart disease [1]. Stroke can be divided into ischemic stroke and haemorrhagic stroke. Ischemic stroke is the most common type, accounting for about 62% of the total number of strokes worldwide and more than 80% of the total number of ischemic strokes in China [1,2]. Due to the limitation of the treatment time window, most patients fail to receive adequate treatment in the acute phase of ischemic stroke, resulting in severe neurological deficits. There is still a lack of adequate clinical treatment for patients with ischemic stroke beyond the treatment window, so it is urgent to seek new treatment methods. The blood-brain barrier makes safe and effective drug delivery a significant difficulty in treating ischemic stroke. Exosomes have attracted extensive attention in preclinical studies due to their unique characteristics. MicroRNA (miRNA), as one of the exosome's genetic materials, has become a potential therapeutic target for treating ischemic stroke.

## TREATMENT STATUS OF ISCHEMIC STROKE

Ischemic stroke refers to the reduction or interruption of blood supply to the brain due to various reasons, leading to ischemia and hypoxia of brain tissue, resulting in neuronal death, activation of microglia and astrocytes and a series of changes [3]. The pathology of ischemic stroke includes the formation of the ischemic core and penumbra. The key to stroke treatment is rescuing the ischemic penumbra neurons. Currently, the most effective treatment for ischemic stroke is intravascular reperfusion. Methods to achieve reperfusion after stroke include recombinant tissue plasminogen activator, intravenous thrombolytic therapy with RT-PA, and mechanical endovascular thrombectomy [4]. However, RT-PA has a strict time window ( $\leq 4.5$ h) limitation, thrombolysis contraindications, and the risk of bleeding transformation. However, endovascular mechanical thrombectomy applies only to patients with large artery occlusion and some patients with thrombolysis contraindications [5,6]. Therefore, it is necessary to find a new

### Quick Response Code:



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**Received:** August 18, 2022

**Published:** October 19, 2022

**How to cite this article:** Wirda ZMR, Asral F, Ahmad A, Rabia A. Exosomal miRNA Research Technology for Combating Ischemic Stroke. 2022- 4(5) OAJBS.ID.000502. DOI: [10.38125/OAJBS.000502](https://doi.org/10.38125/OAJBS.000502)

specific treatment for patients outside the acute treatment window to promote the recovery of neurological function. Exosomes have attracted more attention in treating ischemic stroke due to their characteristics such as carrying genetic material, low immunity against phytophthora, good histocompatibility, high delivery efficiency and easy passage through the blood-brain barrier [7].

### OVERVIEW OF EXOSOMES AND mRNA

Exosomes are single-layer membrane vesicles with diameters ranging from 30 to 200 nm secreted by living cells, containing proteins, lipids, nucleic acids, glycoconjugates, etc. Exosomes are a kind of extracellular vesicles [8]. Exosomes have stable bimolecular phospholipid structures, which can protect the biological activities of their contents from destruction. Exosomes play an essential role in immune response, tumor progression and neurodegenerative diseases by delivering their contents to neighboring or distant receptor cells and regulating the function of receptor cells [9]. At the same time, exosomes are expected to provide new treatment ideas for ischemic stroke due to their excellent compatibility and easy passage through the blood-brain barrier [10].

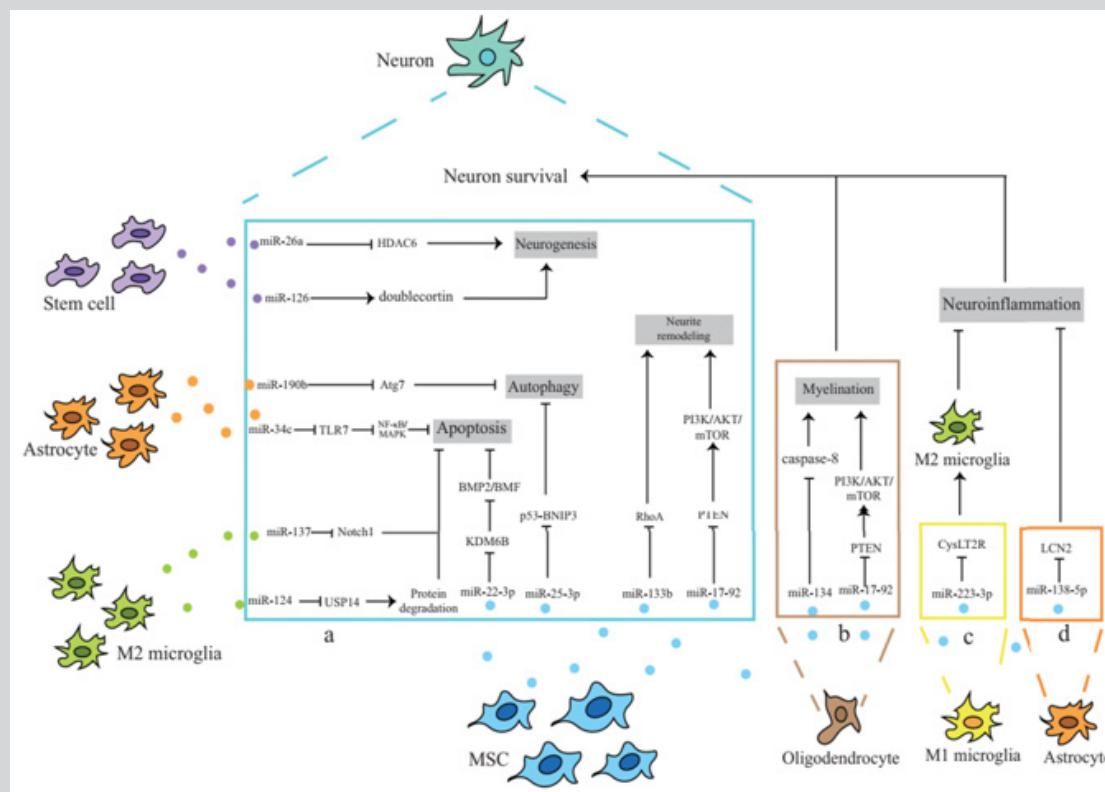
MiRNA is an endogenously produced single-stranded non-coding RNA molecule with a length of 21-23 nucleotides [11], which can regulate gene expression at the post-transcriptional level by inhibiting the expression of messenger RNA (mRNA) or promoting mRNA down-regulation [12]. MiRNA plays an important role in cell growth, proliferation, differentiation, apoptosis and other biological processes. In recent years, with the deepening of research, miRNAs in exosomes have attracted more and more attention. In animal

models, miRNAs can be packaged into exosomes or microcapsules and secreted into the extracellular microenvironment for long-distance intercellular communication [13]. Many studies have shown that miRNAs in exosomes play an important role in diagnosing, treating and treating diseases [14-16].

Exosomes are highly heterogeneous in size, contents, function and cell origin. Changes in cell microenvironment and intrinsic biology may affect the content of exosomes and their contents [7]. Exosome packaging miRNA is not a random process and has an active sorting mechanism [15]. Studies have shown that exosomes' miRNA expression profiles differ from those of parental cells, and highly enriched exosomal miRNAs may play an essential role in disease progression and treatment [9]. The expression level of miRNA in exosomes also changes under different physiological conditions. These characteristics of exosomes and the miRNAs they carry lay the foundation for the miRNAs in exosomes to become potential therapeutic targets for ischemic stroke.

### miRNAs IN EXOSOMES

Treatment of ischemic stroke Exosome-loaded miRNAs have become a hot topic for treating central nervous system diseases. Exosomes can transport miRNAs with biological functions, and because of their inherent biological characteristics, exosomes have become a new therapeutic tool. After ischemic stroke, exosome miRNAs exert neuroprotective effects in different ways (including inhibiting inflammatory response, apoptosis, autophagy, and promoting neurite remodelling); (Figure 1).



**Figure 1:** Different cell-derived exosomal miRNAs inhibit apoptosis and autophagy by acting on target genes, promote neurite remodeling and neurogenesis, and exert neuroprotective effects after ischemic stroke. B: miRNAs in MSCS exosomes play a protective role by promoting myelination. C: miRNAs in MSCS exosomes can reduce neuroinflammation and secondary nerve injury by inducing microglia to transform from M1 phenotype to M2 phenotype. D: MSC exosomes inhibit the inflammatory response of astrocytes through miRNA.

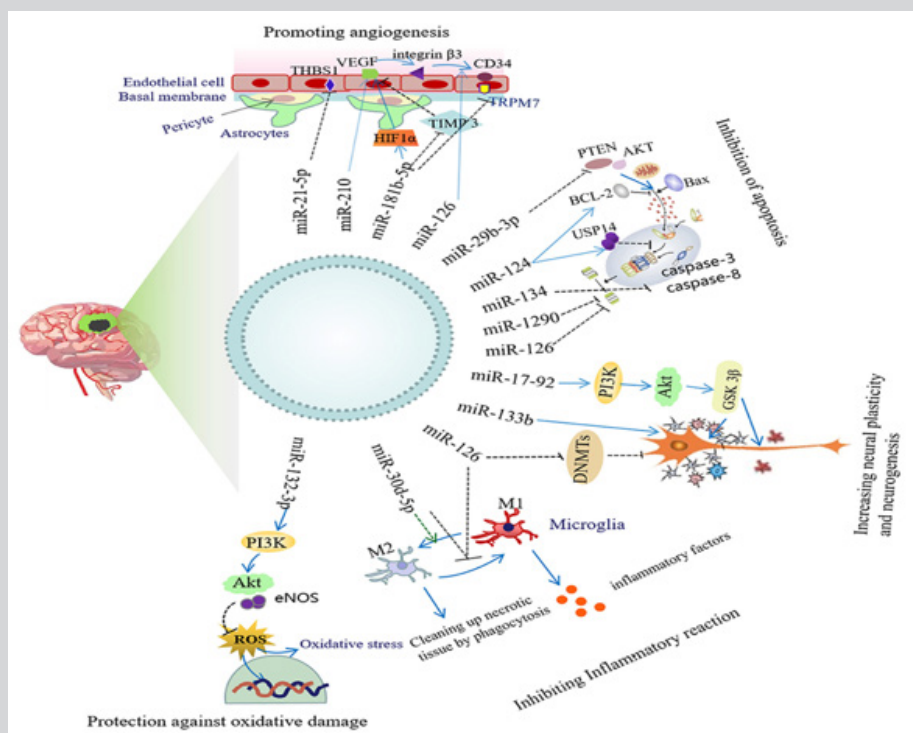
## Inhibition Of Inflammatory Response

Neuroinflammation plays an important role in the occurrence and development of ischemic stroke. The inflammatory response has two aspects: on the one hand, activated inflammatory cells can engulf dead cells or debris and promote tissue repair; On the other hand, the overactivated inflammatory response will further aggravate the damage of neurons [17]. After the haemorrhagic stroke, Toll-like receptor 4 (TLR4) is activated on inflammatory cells, leading to neuroinflammation and secondary nerve damage. Mesenchymal stem cells (Mesenchymal stem cells), Mir-542-3p carried by exosomes of Mesenchymal stem cells can reduce the glial inflammatory response and neuronal apoptosis by inhibiting TLR4 [18]. In addition, Mir-221-3p carried by exosomes of bone marrow mesenchymal stem cells (BMSC) can reduce activated transcription factor 3 (ATF3), Reduce inflammation and neuronal injury in brain tissue after ischemic stroke in middle cerebral artery occlusion (MCAO) mice [19].

After ischemic stroke, microglia, the innate immune cells of the central nervous system, can differentiate into the “classically activated” pro-inflammatory M1 phenotype and the “vicarious activated” anti-inflammatory M2 phenotype, thus playing a role in nerve injury or protection [20]. Scientist found that MSC exosomes loaded with Mir-223-3p could induce microglia to transform from M1 phenotype to M2 phenotype by inhibiting the expression of Cysteinyl leukotriene receptor 2 (CysLT2R) [21]. It then suppresses the inflammatory response. In addition, Mir-30D-5p carried in

exosomes of Adipose-derived stem cells (ADSC) can promote the transformation of microglia into M2 phenotype by inhibiting autophagy and reducing inflammatory response after ischemic nerve injury [22]. After oxygen and glucose deprivation (OGD), ADSC-derived exosomes overexpressing Mir-126 were applied to BV2 microglia. Exosomes were found to inhibit the activation of BV2 cells and the expression of inflammatory factors. Promote the recovery of neurological function after haemorrhagic stroke [23]. In addition, Scientist found that Mir-146A-5p in human umbilical cord mesenchymal stem cells and exosomes produced by mesenchymal stem cells can reduce microglia-mediated neuroinflammation, reduce infarct size and improve neurobehavioral defects after ischemic stroke by inhibiting IRAK1/TRAF6 signaling pathway [24].

After ischemic stroke, astrocytes are activated as reactive astrocytes. Although the anti-toxic effect of reactive astrocytes limits the expansion of lesions and releases nutrients, the inflammatory response of reactive astrocytes to ischemic stroke can aggravate ischemic lesions, and the formation of glial scars also hinders the remodeling of neurites [25]. Mir-138-5p carried by BMSC-derived exosomes inhibits astrocyte inflammation and attenuates ischemic brain injury by down-regulating Lipocalin 2 (LCN2) [26]. In addition, Scientist found that Mir-181c-3p in cortical neuron exosomes down-regulated the expression of C-X-C motif ligand 1 (CXCL1) through *in vivo* and *in vitro* experiments [27,28]. It can inhibit the inflammatory response of astrocytes and has a protective effect on ischemic brain injury as shown in Figure 2.



**Figure 2:** Neuroprotective mechanism of exosomal miRNA

## Inhibition Of Apoptosis

Apoptosis is the main cause of neuronal death in ischemic penumbra after stroke. Scientist demonstrated through *in vivo* and *in vitro* experiments that Mir-22-3p in ADMSC-derived exosomes could inhibit the BMP2/BMF axis by inhibiting the expression of KDM6B, thereby reducing neuronal apoptosis after ischemic stroke

[29]. Scientist found that compared with exosomes derived from human neural stem cells, interferon- $\gamma$  (IFN- $\gamma$ ) -induced exosomes derived from human neural stem cells are functionally superior and can significantly reduce neural cell apoptosis and promote cell proliferation and survival [30]. It plays a protective role after ischemic stroke, which may be related to exosomal miRNAs (such as Mir-206, Mir-133A-3p and Mir-3656). researcher found

that in exosomes derived from M2 microglia, Mir-124 enhanced proteasome activity by inhibiting the expression of downstream target ubiquitin-specific protease 14(USP14) [31]. Increased degradation of misfolded proteins can significantly reduce neuronal apoptosis and promote survival after stroke. M2 microglia can also inhibit the expression of Notch1 through Mir-137 in its exosomes, thereby reducing neuronal apoptosis, improving neurological deficits, and alleviating cerebral ischemia-reperfusion injury [32]. In addition, Mir-34c in astrocytes-derived exosomes can down-regulate NF-κB/MAPK signaling pathway by inhibiting TLR7, thereby inhibiting the apoptosis of N2a cells (neuroblastoma cell line), promoting the proliferation of N2a cells, and alleviating cerebral ischemia-reperfusion injury [33].

### Inhibition of Autophagy

The lack of oxygen and sugar supply caused by cerebral ischemia can lead to the activation of the autophagy process, and an appropriate autophagy response can degrade or recycle misfolded proteins and damaged organelles. In contrast, an over-activated autophagy response will further aggravate cell damage [34]. Studies have shown that astrocyte-derived exosomes can improve neuronal injury after ischemic stroke by inhibiting autophagy, thus playing a neuroprotective role [35]. Scientist found that the expression of Mir-190b in astrocyte-derived exosomes was increased, and it could inhibit neuronal autophagy by targeting autophagy-related gene 7(Atg7), thereby reducing neuronal apoptosis induced by OGD [36]. In *in vitro* OGD primary neuron model and *in vivo* mouse MCAO model, Scientist found that adipose-derived mesenchymal stem cells, Mir-25-3p in exosomes secreted by ADMSC inhibit autophagy flux in damaged neurons after ischemic stroke by down-regulating p53-BNIP3 signaling. In pathway, in thus in inducing in neuroprotective effect [37,38].

### Enhance Neurite Remodelling

After ischemic stroke, many neurons die and denervate. Promoting neurite outgrowth and establishing new synaptic connections is the basis for a gradual recovery of nerve function. Scientist found that the expression level of Mir-133b in exosomes increased after multipotent mesenchymal stem cells (MSC) were exposed to rat ischemic brain tissue extracts [39]. Mir-133b was transferred into neurons and astrocytes by exosomes, and transfected Mir-133b significantly increased the number and length of neurite branches. *In vivo* studies, the team further found that Mir-133b carried by MSC exosomes inhibited connective tissue growth factors in astrocytes, inhibited the expression of CTGF and Ras homologous family member A(RhoA) in the ischemic border zone, and inhibited the release of exosomes from astrocytes that promote neurite outgrowth. It significantly enhances axonal plasticity after ischemic stroke and promotes synaptic remodelling in the ischemic border area [40,41]. Scientist also found that MSC exosomes rich in Mir-17-92 cluster could activate the PI3K/AKT/mTOR signaling pathway by inhibiting the expression of phosphatase and tensin homolog (PTEN) [42]. Enhance the extension of axons after cerebral ischemia and promote the recovery of neurological function.

### Promote Myelination

Oligodendrocytes are myelinating cells in the central nervous system and are vulnerable to injury during ischemic stroke. Although neuronal plasticity causes brain tissue to undergo a large amount of activity-dependent functional reorganization, leading to partial recovery of neurological function in patients, myelin repair is essential for the normal function and reorganization

of neuronal networks [43]. Mir-134 in BMSC-derived exosomes inhibits apoptosis of rat oligodendrocytes by negatively regulating caspase-8, thus exerting neuroprotective effects [44]. Scientist found that MSC exosomes rich in Mir-17-92 cluster activates the PI3K/AKT/mTOR signaling pathway by inhibiting PTEN, thereby enhancing myelination and promoting the nervous system recovery after stroke [42]. Mir-126 in mouse brain endothelial exosomes can also improve neurocognitive function in type 2 diabetic mice with stroke by increasing myelin density [45]. However, the specific mechanism is not completely clear and needs to be further studied.

### Promote Neurogenesis

The injury caused by a stroke can cause neurogenesis of ischemic penumbra in the infarct area. However, this endogenous neurogenesis process is slow, so improving its neurogenesis is a promising treatment for ischemic stroke [46]. Histone deacetylase 6(HDAC6) is a member of the Histone deacetylase family, which plays an important role in chromosome structure modification and gene expression regulation. Inhibition of HDAC6 after ischemic stroke can significantly promote neurogenesis and alleviate neurological deficits [47]. researcher found that Mir-26a in exosomes derived from human urine-derived stem cells could promote the proliferation of neural stem cells and neuronal differentiation by inhibiting HDAC6, thus improving the neurological deficit after ischemic stroke [48]. In addition, Scientist found that ADSC-derived exosomes overexpressing Mir-126 promoted neurogenesis by increasing the expression of biarticular proteins (a sign of migrating neuroblasts) in the periinfarct region, thereby promoting neurological recovery after ischemic stroke [23].

### Promote Angiogenesis

After ischemic stroke, the promotion of angiogenesis contributes to the recovery of neurological function and improves the survival rate of stroke patients [49]. Studies have shown that Mir-181b-5p in ADSC-derived exosomes promotes the migration and angiogenesis of brain microvascular endothelial cells after OGD by inhibiting transient receptor potential melastatin 7(TRPM7). Therefore, it has a protective effect after ischemic stroke [50-52]. In addition, Mir-126 carried by ADSC exosomes can promote angiogenesis by upregulation of the expression of vWF, a marker of cerebrovascular endothelial cells in the peripheral area of infarction, and then improve neurological function recovery after ischemic stroke [23]. Interleukin-4 (IL-4) can differentiate BV2 microglia into M2 phenotype. Compared with the control group, the expression of Mir-26a in exosomes secreted by M2 BV2 cells is higher, which can improve the injury caused by ischemic stroke by promoting angiogenesis [53,54]. Scientist found that endothelial progenitor cells are rich in Mir-126. Exosomes released by EPC can increase cerebral blood flow and cerebrovascular density by down-regulating c-caspaseE3 and up-regulating vascular endothelial growth factor receptor 2(VEGFR2) [55-57]. It promotes angiogenesis and neurogenesis, which is beneficial to the recovery of neurological function in diabetic ischemic stroke mice.

## ENGINEERED EXOSOMAL MIRNA TARGETED DELIVERY FOR THE HEALING OF ISCHEMIC STROKE

In conclusion, miRNAs in exosomes have good potential in treating ischemic stroke, which provides the experimental basis for their clinical application in treating ischemic stroke. However, although exosomes can cross the BBB, the brain targeting ability of natural exosomes produced by cells is insufficient, which limits their clinical application [58-60]. Studies have found that



modification of exosome membrane can improve its targeting ability [61]. Studies have found that exosomes binding RGDyk cyclic peptide could bind integrin  $\alpha\beta 3$  of the BBB and deliver Mir-210-loaded exosomes to ischemic brain tissue [60-62]. To promote the expression of vascular endothelial growth factor (VEGF) in ischemic stroke lesions and improve angiogenesis after ischemic stroke. In addition, rabies virus glycoprotein (RVG) and lysosome-associated membrane glycoprotein 2B (Lamp2b) can bind to the acetylcholine receptor of the blood-brain barrier and effectively deliver Mir-124 to the infarct site of ischemic stroke. Thus, the neurogenesis of neural precursor cells is promoted, and the prognosis of stroke is improved [63-66]. Therefore, the in-depth study of engineered exosomes may provide new strategies for treating ischemic stroke.

## SUMMARY AND PROSPECT

Patients with ischemic stroke are often accompanied by neurological dysfunction, which seriously affects their quality of daily life and brings a huge burden to the family and society. There is currently no effective treatment for ischemic stroke patients who have exceeded the treatment time window, so new treatments need to be found. miRNAs in exosomes can be delivered to recipient cells and thus affect the function of recipient cells. Meanwhile, the exosomes coated with them have good compatibility, easily cross the blood-brain barrier, and can be modified by engineering. Therefore, exosomal miRNAs are expected to be effective therapeutic targets for ischemic stroke. However, although several studies have demonstrated the great potential of exosomal miRNAs in the treatment of ischemic stroke, the optimal cellular source of exosomes has not been identified, and the mechanism by which exosomal miRNAs exert their protective effect on ischemic stroke is not fully understood. At the same time, the research of exosomes in the treatment of ischemic stroke is still in the early stage, and large-scale and multi-center clinical trials are needed to ensure the safety and efficacy of exosomes before clinical application. Although facing many problems, using miRNA in exosomes for treating ischemic stroke still holds great promise. It is expected that the further development of exosome research may become a new direction of gene therapy for ischemic stroke.

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