Cellular and Molecular Neurobiology (2025) 45:31
https://doi.org/10.1007/s10571-025-01551-3

REVIEW PAPER

Extracellular Vesicle-Derived miRNAs in Ischemic Stroke: Roles in Neuroprotection, Tissue Regeneration, and Biomarker Potential

Ceren Eyileten[1,2] · Pamela Czajka[1] · Izabela Domitrz[3] · Agata Wierzchowska-Ciok[3] · Aleksandra Gasecka[4] · Dagmara Mirowska-Guzel[1] · Anna Członkowska[1,5] · Marek Postula[1]

Received: 6 December 2024 / Accepted: 20 March 2025

Abstract

Ischemic stroke (IS) is one of the most common causes of death and disability worldwide. Despite its prevalence, knowledge about pathophysiology and diagnostic methods remains limited. Extracellular vesicles (EVs) that are released from cellular membranes constitutively, as well as after activation or damage, may contain various intracellular particles, including microRNAs (miRNAs/miR). miRNAs acting as mRNA transcription regulators are secreted in EVs and may be internalized by other cells. This cellular cross-talk is important for the regeneration of the nervous tissue after ischemic injury. Moreover, miRNAs related to stroke pathophysiology were shown to be differentially expressed after an IS episode. miRNAs associated with various types of stem cell-derived EVs were shown to be involved in post-ischemic neuroprotection and tissue regeneration and may be potential therapeutic agents. Therefore, considering their stability in plasma, they are worth investigating also as potential diagnostic/prognostic biomarkers. The present review summarizes the current knowledge about EV-derived miRNAs in the neuronal injury mechanism and their potential in neuroprotection in IS, and discusses the possibilities of further investigation of their use in preclinical research.

Keywords Ischemic stroke · Exosomes · Microvesicles · MicroRNA · Biomarker · Prognosis · Treatment

Abbreviations

ADSCs	Adipose-derived stem cells
ATF3	Activating transcription factor 3
BMSCs	Bone marrow mesenchymal stem cells
EVs	Extracellular vesicles
EMVs	Endothelial microvesicles
H/R	Hypoxia/reoxygenation
HUCMSCs	Human umbilical cord mesenchymal stem cells
HUVECs	Human umbilical vein endothelial cells
MCAO	Middle cerebral artery occlusion
miRNA/miR	MicroRNA
NPCs	Neural progenitor cells
OGD/R	Oxygen, glucose deprivation/reoxygenation
USCs	Urine-derived stem cells

Ceren Eyileten and Pamela Czajka equally contributed to this work.

✉ Ceren Eyileten
ceyileten@wum.edu.pl

1 Department of Experimental and Clinical Pharmacology, Center for Preclinical Research and Technology CEPT, Medical University of Warsaw, Banacha 1B Street, 02-097 Warsaw, Poland

2 Genomics Core Facility, Centre of New Technologies, University of Warsaw, 02-089 Warsaw, Poland

3 Department of Neurology Faculty of Medicine and Dentistry, Medical University of Warsaw Bielanski Hospital, Warsaw, Poland

4 1st Chair and Department of Cardiology, Medical University of Warsaw, 02-097 Warsaw, Poland

5 2nd Department of Neurology, Institute of Psychiatry and Neurology, 02-957 Warsaw, Poland

Introduction

Ischemic stroke (IS), the most common of all stroke types, is one of the leading causes of disability and death worldwide (Johnston et al. 2009). Despite the notable prevalence of IS, the molecular mechanism of the pathogenesis remains unclear. The diagnosis is based on clinical symptoms and is usually confirmed by either computed tomography or magnetic resonance imaging. However, the limitations of these methods can be seen in the differential diagnosis between stroke and numerous other disorders, including brain tumors, unusual migraines, or encephalitis (Hand et al. 2006; Vilela

Published online: 31 March 2025

2017). Additionally, patients with small infarcts may be underdiagnosed in brain imaging (Powers et al. 2019). There is still a lack of specific biomarkers to assist in the diagnosis of IS. Inflammation is a marked factor in the onset of acute brain ischemia (Simats et al. 2016). The initiation of inflammation occurs within a few minutes after the stroke has occurred (Maida et al. 2020; Araki et al. 2021). It is most likely one of the crucial mechanisms of neural tissue damage during the first few hours of the acute phase, and the increased activity of the immune system persists over subsequent days. The development of inflammation is triggered by cell damage caused by inadequate oxygen and nutrient supply, as well as necrosis. Cell damage leads to the release of a number of molecules called alarmins, which can then activate microglia cells, the immune cells of the brain, via pattern recognition receptors, which trigger an intracellular cascade of signaling pathways in microglia cells (Zhao et al. 2017). This, in turn, leads to the activation of transcription factors, such as NF-κB, which results in the production and release of pro-inflammatory cytokines, notably interleukin (IL)−1β, IL-6 and tumor necrosis factor-α (TNF-α) (Araki et al. 2021). Pro-inflammatory cytokines' presence in the intercellular space leads to migration and activation of microglia that enhance inflammation. Although the primary role of microglia is to repair damaged neural tissue and phagocytosis-damaged neurons and to secrete trophic factors (i.e., brain-derived neurotrophic factor—BDNF) to stimulate subsequent neurogenesis, their collateral over-activation can lead to impaired healing of the brain tissue (Al-Onaizi et al. 2020).

Extracellular vesicles (EVs) are lipid bilayer-delimited particles (range, 30–5000 nm) secreted from cell membranes. EVs are a notable element of intercellular cross-talk and are released constitutively or upon activation under both normal physiological and pathological conditions, including oxidative stress, hypoxia, and apoptosis (Zhang et al. 2021c). There are different subtypes of EVs, such as exosomes, microvesicles, and apoptotic bodies. The smallest EVs, exosomes (range, 30–100 nm), are released by the fusion of cytoplasmic multivesicular bodies with the cellular membrane. Microvesicles (range, 0.1–1.0 μm) are released by outward blebbing of the cellular membrane, especially during cell growth. The largest EVs, apoptotic bodies (range, 1–5 μm), are formed in the late phases of apoptosis (György et al. 2011; Ståhl et al. 2019). EVs may contain proteins, lipids, carbohydrates, membrane receptors, cytokines, and genetic material, including non-coding RNAs (ncRNAs), circular RNAs and microRNAs (miRNAs/miR) (Camussi et al. 2010).

MiRNAs are small ncRNAs that function in the regulation of mRNAs and, ultimately, protein level through cleavage of mRNA transcripts, transcriptional blocking by binding to the 3'-untranslated region, and post-transcriptional silencing through translational disruption (Bartel 2004; Ma et al. 2016). miRNAs can exert control in the cell of origin through secretion into circulation in EVs and subsequent uptake by numerous types of cells, for example, endothelial cells (Valadi et al. 2007; Ge et al. 2014; Kesidou et al. 2020). This unique cellular communication appears to be important for the regeneration of the brain tissue after ischemic/reperfusion injury (Cun et al. 2022). More importantly, circulating miRNAs may be detected in the serum collected from patients with IS, and are stable enough for laboratory measurements (Sabour 2017; Chen et al. 2017). The expression of some circulating miRNAs identified in patients with IS was substantially altered and often associated with stroke severity, suggesting their potential use as both diagnostic and prognostic biomarkers in stroke (Mens et al. 2021; Eyileten et al. 2022a). It is noteworthy that animal and in vitro studies showing the involvement of miRNAs in the pathogenesis of stroke indicate their possible use as therapeutic agents (Xu et al. 2015; Zhang et al. 2015), especially since they can cross the blood–brain barrier and influence gene expression in the damaged brain tissue (Aili et al. 2021).

The present review aims to present in vitro and in vivo studies, including human studies and animal model studies, to summarize the current knowledge about EV-derived miRNAs related to IS pathophysiology and to show future possibilities of their use in clinical settings.

In vitro and Animal Studies

Microglia Activation and Nervous Tissue-Derived Exosomes in Post-ischemic Neuroprotection and Recovery

Microglia, the resident macrophages of the central nervous system (CNS), play a vital role in CNS development, homeostasis, and neurogenesis. Microglia activation and polarization are critical for neuronal damage and compensation following IS, and therefore, microglia can act as a double-edged sword for neurological recovery (Gao et al. 2023). For example, microglia after ischemia can promote the secretion of pro-inflammatory components (TNFα, IL-1β, and IFN-γ) and lead to secondary CNS injury, but they can also release anti-inflammatory cytokines (TGF-β, IL-10) (Zhao et al. 2017; Ip et al. 2017) and CNS protective factors, such as neurotrophic factors (i.e. BDNF), which alleviates ischemic damage (Pöyhönen et al. 2019). Therefore, studies focused on investigating the molecular mechanism of neurogenesis and/or secondary brain damage associated with microglia after ischemia (Jayaraj et al. 2019).

Post-ischemic microglial activity may be regulated by damaged but still viable neurons that release EVs containing miR-98. Once internalized, miR-98 may target PAFR,

which plays a role in the phagocytosis of apoptotic cells, thereby protecting damaged neurons from microglial activity (Yang et al. 2021). The expression of miR-98 in the penumbra remained stable during the first 24 h after IS in a rat model but significantly decreased after three days (Yang et al. 2021). These studies not only highlight novel therapeutic possibilities but also provide insight into the role of EV-derived miRNAs in the pathogenesis of ischemic brain injury.

Exosomes secreted by the nervous tissue are important for IS pathogenesis and post-ischemic repair. The anti-inflammatory effect of the neural progenitor cell (NPC)-derived EVs (ReNcell VM NPC line) was observed both in vitro in lipopolysaccharide (LPS)-stimulated microglial cells, as well as in mice after middle cerebral artery occlusion (using a mouse model of acute stroke-MCAO). It was reported that NPC-derived EVs have an intrinsic activity and decrease the production of pro-inflammatory cytokines in the mimic model. After intravenous infusion, NPC-derived EVs were able to infiltrate the post-ischemic lesion and suppress the inflammatory response (Tian et al. 2021). Further sequencing analysis of the miRNA content in EVs showed that the most notable miRNAs were involved in the suppression of MAPK and other inflammatory pathways, mainly miR-99a-5p, miR-139-5p, miR-98-5p, miR-21-5p, let-7i-5p, let-7 g-5p, and let-7b-5p, all of which were expressed at high levels in NPC-derived EVs and upregulated in LPS-stimulated microglia cells after 24 h of EV treatment (Tian et al. 2021). Another miRNA abundant in neural stem cell (NSC)-derived exosomes is miR-150-3p, which targets *CASP2 mRNA*, a member of the signaling pathway involved in neuronal proliferation (Luo et al. 2022). The neuroprotective effects of NSC-derived exosomes were observed both in vitro in SH-SY5Y cells after oxygen–glucose deprivation/reoxygenation (OGD/R) and in MCAO rats. Treatment with NSC-derived exosomes derived from anti-miR-150-3p-transfected (inhibitor of miR-150-3p) NSCs inhibited both neuroproliferation and attenuation of neuronal apoptosis. The reported influence of NSC-derived exosomes on neural tissue regeneration is mediated by miR-150-3p affecting *CASP2*, which suggests that miR-150-3p may be an important player in post-ischemic tissue regeneration (Luo et al. 2022).

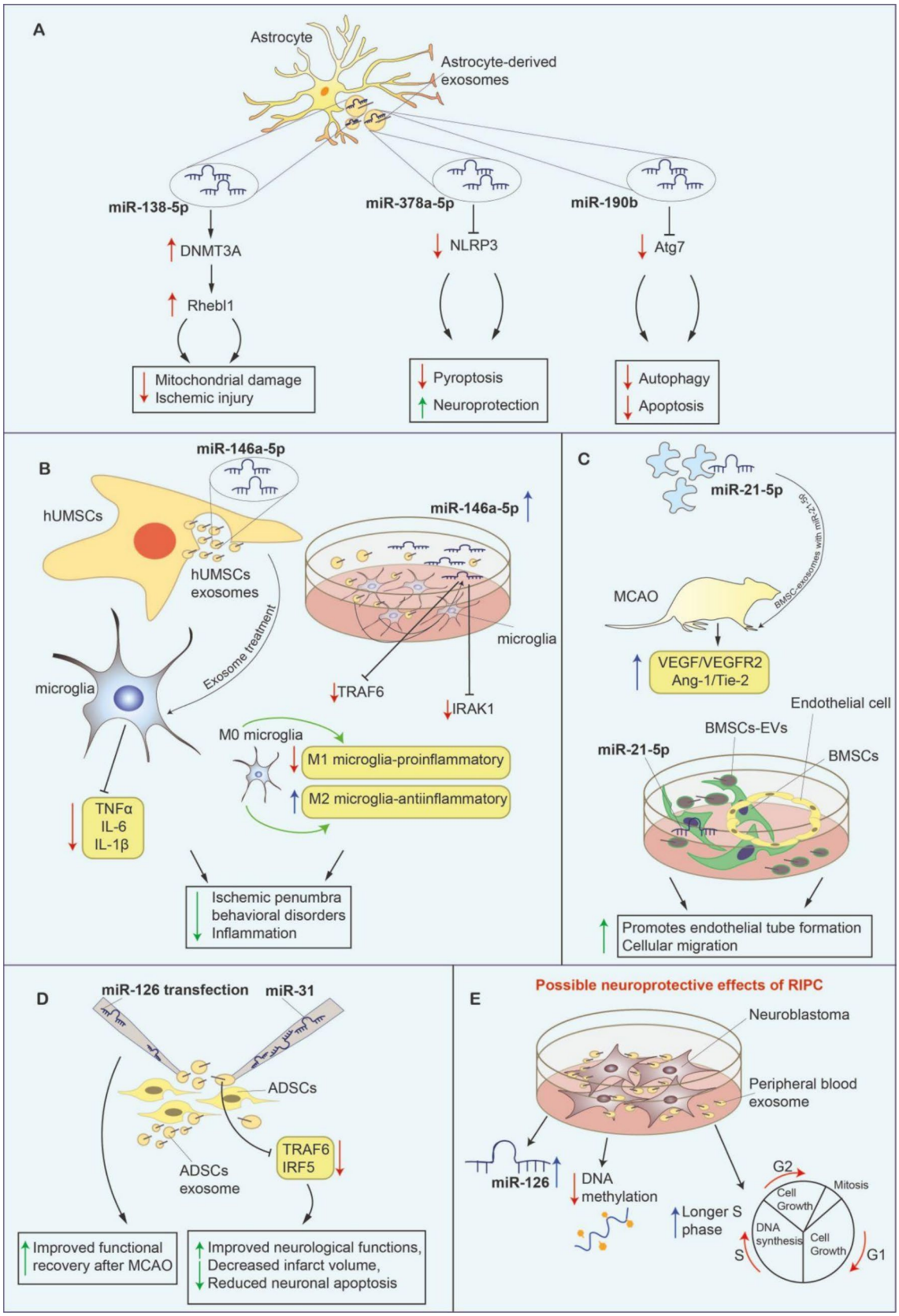
Astrocyte-Derived Exosomal miRNAs in Ischemia

One of the latest study investigated the role of astrocyte-derived exosomes in neuroprotection following cerebral ischemia. Primary astrocytes and neurons were isolated and cultured, and astrocyte-derived exosomes were extracted via ultracentrifuge. RNA-sequencing revealed that miR-378a-5p played a key role in regulating neuroinflammation, with further luciferase reporter assays confirming its direct binding to *NLRP3* mRNA-3'UTR. Astrocyte-derived exosomes engineered with miR-378a-5p mimics and inhibitors showed that inhibiting miR-378a-5p exacerbated neuronal injury, whereas its upregulation mitigated OGD-induced damage. Overall, findings from both the rat MCAO model and the primary neuronal OGD model demonstrated that these exosomes were internalized by neurons, and astrocyte-derived exosomes treatment can decrease pyroptosis and have neuroprotective effects (Sun et al. 2024).

A previous methodologically well-established study explored the neuroprotective role of astrocyte-derived exosomes delivering miR-138-5p to damaged neurons both via in vitro and in vivo analysis. Infarct size was assessed in an MCAO rat model, and results showed that astrocyte-derived exosomes promote neuronal mitophagy and protect against ischemic injury. The study found that miR-138-5p regulates mitophagy via the DNMT3A/Rhebl1 axis as a key regulator of neuroprotection. Notably, intravenous administration of engineered microglial vectors produced therapeutic effects comparable to intraventricular injection in rats, which suggested the potential of cell-vector-targeted delivery systems for IS treatment (Zhu et al. 2024). Additionally, Wang et al. (Wang et al. 2023) used an in vitro stroke model with primary mouse astrocytes and analyzed small RNA deep sequencing from astrocyte-derived exosomes. Seven differentially expressed miRNAs (miR-92b-3p, miR-122-5p, miR-370-3p, miR-485-3p, miR-664-5p, miR-1306-5p, miR-1843b-3p) were validated using RT-qPCR. Their findings suggested that astrocyte-derived exosomal miRNAs contribute to neuroprotection by supporting neuronal signaling, neurotrophic interactions, and apoptosis mitigation (Wang et al. 2023).

MiR-190b was found to be significantly upregulated in astrocyte-derived exosomes compared to primary astrocytes in 2017 (Jovičić and Gitler 2017). Later on, bioinformatic analysis identified autophagy-related gene 7 (*Atg7*) as a potential target of miR-190b, and further experiments demonstrated that astrocyte-derived-mediated transfer of miR-190b inhibits OGD-induced neuronal apoptosis by regulating autophagy. It suggested that astrocyte-derived exosomes play a neuroprotective role by suppressing neuronal apoptosis through miR-190b-mediated autophagy inhibition. Therefore, the study indicated that modulating miR-190b expression in astrocyte-derived exosomes may represent a potential therapeutic strategy for IS (Pei et al. 2020).

Taken together, these studies highlighted the critical role of astrocyte-derived exosomes in neuroprotection following cerebral ischemia through the regulation of key miRNAs that modulate neuroinflammation, mitophagy, and apoptosis. The findings suggest that targeting specific exosomal miRNAs, such as miR-378a-5p, miR-138-5p, and miR-190b, may provide novel therapeutic strategies for IS (Fig. 1A).


A
Astrocyte
Astrocyte-derived exosomes
miR-138-5p
miR-378a-5p
miR-190b
DNMT3A
Rhebl1
NLRP3
Atg7
Mitochondrial damage
Ischemic injury
Pyroptosis
Neuroprotection
Autophagy
Apoptosis
B
miR-146a-5p
hUMSCs
hUMSCs exosomes
Exosome treatment
microglia
TRAF6
IRAK1
M0 microglia
M1 microglia-proinflammatory
M2 microglia-antiinflammatory
TNFα
IL-6
IL-1β
Ischemic penumbra
behavioral disorders
Inflammation
C
miR-21-5p
BMSC-exosomes with miR-21-5p
MCAO
VEGF/VEGFR2
Ang-1/Tie-2
Endothelial cell
BMSCs-EVs
BMSCs
Promotes endothelial tube formation
Cellular migration
D
miR-126 transfection
miR-31
ADSCs
ADSCs exosome
TRAF6
IRF5
Improved functional recovery after MCAO
Improved neurological functions,
Decreased infarct volume,
Reduced neuronal apoptosis
E
Possible neuroprotective effects of RIPC
Neuroblastoma
Peripheral blood exosome
miR-126
DNA methylation
Longer S phase
G2
Mitosis
Cell Growth
DNA synthesis
Cell Growth
S
G1

◂**Fig. 1** Mechanism of miRNAs and EVs in ischemic injury. **A** Astrocyte-derived exosomal miRNAs. **B** Effect of HUCMSC-derived EVs on microglial activity. **C** Influence of BMSC-derived EVs on the neurons after ischemic damage. **D** Mechanism of action of ADSC-derived EV-associated miRNAs in IS. **E** Possible neuroprotective action of RIPC. Abbreviation: ADSCs, adipose-derived stem cells; BMSCs, bone marrow mesenchymal stem cells, EVs, extracellular vesicles; HUCMSCs, umbilical cord mesenchymal stem cells; IL-1β, interleukin 1β; IL-6, interleukin 6; MCAO, middle cerebral artery occlusion; OGD, oxygen–glucose deprivation; RIPC, remote post-ischemic conditioning; TNF-α, tumor necrosis factor α

Effect of EV-Related miRNAs Derived from Bone Marrow and Stem Cells on Post-ischemic Neural Injury

Mesenchymal Stem Cells (MSCs)

Preclinical studies revealed that stem cell treatment might improve neurological deficits and quality of life after IS (Ouyang et al. 2019; Zhang et al. 2021a). Due to this, the effect of human umbilical mesenchymal stem cells (HUCMSC)-derived exosomes on microglia-mediated inflammation after IS was studied in a murine model (Zhang et al. 2021d). Treatment with HUCMSC-derived exosomes notably reduced the volume of ischemic penumbra, attenuated behavioral disorders, and decreased the expression of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β in ischemic injured brain tissue. To validate the in vivo results, microglia cells were cultured with HUCMSC-derived exosomes, which altered the pattern of their activation after OGD/R. The treatment decreased the number of classically activated, pro-inflammatory M1 cells and increased alternatively activated M2 microglia, which are responsible for efferocytosis and neurotrophic factor secretion. Moreover, in the exosomes-treated cells, the levels of IRAK1/TRAF6 signaling pathway proteins and the IL-1β, IL-6, and TNF-α cytokines were decreased. The miR-146a-5p content in microglia was markedly increased after exosome administration. Moreover, a miR-146a-5p knockdown reversed the inhibition of pro-inflammatory cytokine expression (Zhang et al. 2021d). Both in vivo and in vitro complex results suggest the potential role of the exosome-derived miR-146a-5p in post-ischemic/reperfusion neuroinflammation via inhibition of the IRAK1/TRAF6 pathway (Zhang et al. 2021d) (Fig. 1B). Recently, Wu et al. (Wu et al. 2024) investigated the role of hypoxia-treated MSCs and their derived exosomes (Hypo-Exo) in ischemia, compared to exosomes from normoxic MSCs (Norm-Exo). Using a mouse model of MCAO/R, researchers intravenously administered exosomes from both oxygen conditions to assess their therapeutic effects. MiRNA microarray analysis revealed that Hypo-Exo was enriched with miR-214-3p. MiR-214-3p plays a crucial role in suppressing the phosphatidylinositol signaling pathway by directly inhibiting *PTEN/Akt* pathway. The knockdown of miR-214-3p abolished the beneficial effects of Hypo-Exo. Therefore, study suggested that hypoxia-treated MSCs enhance neuroprotection after ischemia via miR-214-3p/PTEN axis (Wu et al. 2024).

Bone Marrow-Derived Stem Cells (BMSCs)

BMSC-derived EVs that have been shown to be protective in myocardial ischemia/reperfusion injury (Chen et al. 2020) were previously reported as differentially expressed in patients with IS, and may be a neuroprotective factor (Jia et al. 2015; Shan et al. 2021). MiR-221-3p targets the activation of transcription factor 3 (ATF3), a regulator of the progression of inflammatory processes after ischemic stress-induced injury (Lin and Cheng 2018). BMSC-derived exosomes containing miR-21-5p may mediate the promotion of angiogenesis in ischemic damaged tissues. Treatment with BMSC-derived exosomes reduced the infarct volume and decreased the neurological deficits in MCAO mice. Brain tissue analysis showed increased proliferation of microvesicles, as well as VEGF/VEGFR2 and Ang-1/Tie-2 protein levels, in the peri-infarct zone in exosome-treated mice. Also, the expression of miR-21-5p in the ischemic boundary zone was notably higher than that in controls. In vitro experiments demonstrated that BMSCs may transfer miR-21-5p into endothelial cells, enhancing proliferation, cellular migration, and tube formation (Hu et al. 2022a). To confirm this, cells transfected with anti-miR-21-5p, an inhibitor of miR-21-5p, inhibited the pro-angiogenic activity of BMSC-derived exosomes (Hu et al. 2022a). Since angiogenesis is essential for post-IS tissue regeneration, exosome-derived miR-21-5p should be studied further as a potential therapeutic agent (Fig. 1C).

Besides the miR-21, miR-34 family was investigated in one of the latest research. Using an MCAO model in adult mice, researchers analyzed the effects of IS on the BM microenvironment and EV. IS induced alteration in the BM microenvironment, particularly increasing pro-inflammatory cytokines such as TNF-alpha and MCP-1. BM cells also exhibited higher levels of inflammatory markers (IL-6, TNF-alpha, TLR-4) and senescence markers (p21, p16). The study did not find a significant change in the size or concentration of EVs. However, an increase in miR-141-3p and miR-34a was observed within the EVs, along with alterations in their protein cargo (Patel et al. 2024).

Adipose-Derived Stem Cells (ADSCs)

Congruous results were observed in IS rats treated with ADSC-derived exosomes. Before administration, ADSC-derived exosomes were transfected with either miR-126 (miR-126^{+}) or miR-126 inhibitor (miR-126^{-}) or were

non-transfected (naïve). In the miR-126 + exosome-treated group, functional recovery after MCAO improved despite no significant reduction in infarct volume. In addition, miR-126^{+}-exosome therapy increased the expression of the Von Willebrand factor and that of a doublecortin-neuroblastic marker, indicating enhanced vasculogenesis and neurogenesis in the peri-infarct zone (Geng et al. 2019). Likewise, ADSC-derived exosome treatment attenuated microglia activation repressed the production of the pro-inflammatory cytokines TNF-α and IL-1β and reduced the activation of the pro-apoptotic caspase-3 in affected neural tissue in both in vitro and in vivo studies (Liu et al. 2022; Tang et al. 2023). The role of miR-126 in neuroprotection is confirmed by the fact that ischemia signs were decreased by miR-126^{+}-exosome treatment, and inflammatory cytokines were increased by miR-126^{-} exosome treatment compared to naïve exosome administration (Geng et al. 2019).

Another miRNA related to the anti-apoptotic effect of ADSCs is miR-31, which was shown to be a negative regulator of *TRAF6* and *IRF5* expression, leading to a reduction of the apoptosis-related factors, caspase-3 and Bax (Lv et al. 2021). Mice treated with ADSC-derived EVs containing miR-31, showed improved neurological functions, decreased infarct volume, and reduced neuronal apoptosis (Fig. 1D) (Lv et al. 2021). It is noteworthy that ADSC-derived EVs containing miR-22-3p were also studied as a potential regulator of post-ischemic neuronal damage. The mechanism is related to the inhibition of apoptotic pathways by targeting *KDM6B* involved in activating caspase-3 and Bax (Zhang et al. 2021b). KDM6B also promotes the expression of BCL-2 modifying factor (BMF), a factor enhancing apoptosis induced by OGD/R (Zhang et al. 2021b). In conclusion, targeting the miR-31/TRAF6/IRF5 and miR-22-3p/KDM6B/BMP2/BMF axes should be studied further as promising therapeutic strategies in IS (Table 1).

Table 1 MiRNAs associated with EVs and exosomes that derived from various types of stem cells and astrocytes, and their possible genetic targets

Types of stem cells/astrocyte-derived exosomes	EV/exosome-associated miRNA	Possible targeted gene
HUCMSC-derived EVs	miR-146a-5p	*IRAK/TRAF* pathway
BMSC-derived EVs	miR-221-3p	*ATF3*
	miR-21-5p	*VEGF/VEGFR2* *Ang-1/Tie-2*
ADSC-derived-EVs	miR-126	n/a
	miR-31	*TRAF6* *IRF5*
	miR-22-3p	*KDM6B*
MSC-derived EVs	MiR-214-3p	*PTEN/Akt*
Astrocytes-derived exosomes	miR-378a-5p	*NLRP3*
	miR-138-5p	*DNMT3A/Rhebl1*
	miR-190b	*Atg7*

HUCMSCs human umbilical mesenchymal stem cells, *BMSCs* bone marrow mesenchymal stem cells, *ADSCs* adipose-derived stem cells, *EVs* extracellular vesicles, *MSCs* Mesenchymal stem cells, miR/miRNA microRNA, n/a not applicable

Influence of Remote Ischemic Post-Conditioning on Neuroprotection via *EV-Related miRNAs*

Another miRNA, miR-126, widely known as an endothelial-specific miRNA, was related to the mechanism of remote ischemic post-conditioning (RIPC) phenomenon, an additive therapy that may improve clinical outcomes in patients with acute IS (Hougaard et al. 2014). The human neuroblastoma cells SH-SY5Y were cultured with peripheral blood exosomes collected from healthy volunteers after controlled transient limb ischemia. In ischemic conditions (post-RIPC), the levels of the exosome-derived miR-126 in plasma were increased. Reduced DNA methylation, increased P21 protein concentration, and an extended S phase of the cell cycle were observed in SH-SY5Y cells cultured with post-RIPC exosomes. In addition, the expression and activity of DNA methyltransferase (DNMT) 3B was decreased when compared with that in cells cultured without post-RIPC exosomes. These results were further confirmed in SH-SY5Y cells infected with lentiviral vectors carrying miR-126. RIPC exosomes exerted neuroprotective effects by down-regulating the expression of *DNMT* in neural cells through the upregulation of serum exosomal miR-126 (Cui et al. 2020). Thus, inhibition of neuronal *DNMT* expression through the upregulation of exosome-derived miR-126 in serum may be responsible for the neuroprotective effects of RIPC (Cui et al. 2020) (Fig. 1E).

The development of ischemic tolerance after RIPC was enhanced by the involvement of both miR-126 and miR-199a-5p. Hypoxia/reoxygenation (H/R) conditions increased miR-199a-5p expression in human umbilical vein endothelial cells (HUVECs) and HUVEC-derived exosomes. Coincidentally, in neural cells exposed to H/R in vitro, administration of HUVEC-derived exosomes resulted in suppression of inflammation and apoptosis. Increased levels of miR-199a-5p in neural cells were shown to decrease stress on the endoplasmic reticulum (Yu et al. 2020). The inhibition of miR-199a-5p notably reduces the protective effect of HUVEC-derived exosomes (Yu et al. 2020). The effect of urine-derived stem cell (USC)-derived exosome treatment in IS was assessed both in animal and in in vitro models. The intravenous infusion of USC-derived exosomes reduced the infarct volume and notably improved functional recovery following MCAO in rats and enhanced proliferation and differentiation of neural stem cells, leading to neurogenesis in the infarct boundary zone (Yu et al. 2020). Moreover, it

was reported that the underlying mechanism is associated with an increased level of miR-26a, which promotes NSC proliferation and differentiation via inhibition of histone deacetylase 6. The transfer of the exosome-derived miR-26a is a plausible explanation for the positive effect of USC-derived exosomes on neurogenesis (Ling et al. 2020).

Cuomo et al. investigated the neuroprotective role of exosomal miRNAs in remote limb ischemic postconditioning (RLIP) in an animal model. They found that miR-702-3p and miR-423-5p were significantly upregulated in the temporoparietal cortex of ischemic rats treated with plasmatic exosomes. MiR-702-3p and miR-423-5p play crucial role in modulating NOD1 and NLRP3 genes and regulates neuroinflammation and neuronal apoptosis. The administration of plasmatic exosomes isolated from plasma of RLIP rats effectively attenuated cerebral ischemia–reperfusion injury and improved neurological function for up to three days post-stroke. They suggested that plasmatic exosomes may act as a neuroprotective therapy by modulating neuroinflammatory pathways (Cuomo et al. 2024).

Taken together, in vitro and animal studies reflect the promising role of exosomes as potential therapeutic agents in post-ischemic brain injury; the described miRNAs could be the key to explaining the mechanism of action (Fig. 2).

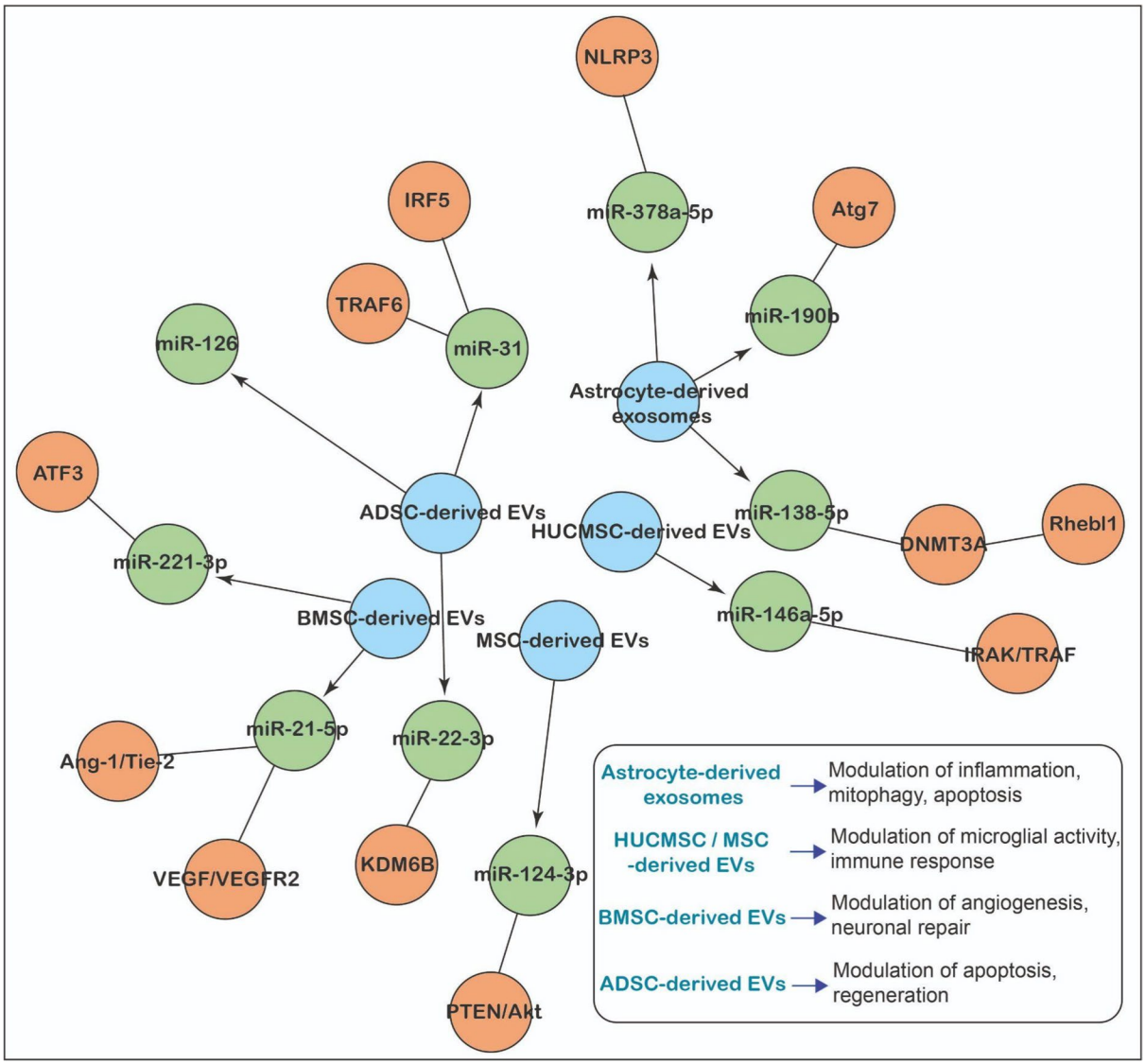


Fig. 2 Network of stem cell-derived EVs and astrocyt-derived exosomal miRNAs and their target genes. Abbreviations: HUVECs, human umbilical vein endothelial cells; BMSCs, bone marrow mesenchymal stem cells; ADSCs, adipose-derived stem cells; EVs, extracellular vesicles; MSCs, Mesenchymal stem cells; miR/miRNA, microRNA

Human Studies

Exosomes

Exosomes secreted by numerous cells, including microglia, endothelial cells, and leukocytes, may be found in peripheral blood, making them a valuable diagnostic material in clinical practice (Hazrati et al. 2022). The influence of IS on the expression of several exosome-derived miRNAs, previously observed in animal and in in vitro studies, has also been detected in human research (Alexander et al. 2015). Exosome-derived miRNAs are considered to be more sensitive and specific biomarkers compared with total cellular miRNAs or free miRNAs, as exosomes are secreted into extracellular space (Aimaletdinov and Gomzikova 2022). MiR-223 is one of the most common exosome-derived miRNAs found in the plasma of healthy individuals (Shi et al. 2023). Conversely, altered miR-223 expression has been observed in patients with diseases that typically increase the risk of IS, including atherosclerosis (You et al. 2022), diabetes mellitus (Eyileten et al. 2022b), and enhanced platelet reactivity (Czajka et al. 2021; Li et al. 2021). It was reported that the miR-223 levels were notably upregulated in the acute phase of IS when compared with those in non-stroke controls (Chen et al. 2017). In the study group, the expression levels were positively associated with the National Institutes of Health Stroke Scale (NIHSS) score (Adams et al. 1999), as well as with the neurological outcome assessed 3 months after stroke occurrence. However, there was no association with the infarct volume (Chen et al. 2017) (Tables 2, 3).

Previous studies indicate that the abundant expression of miR-126 in the endothelium is a neuroprotective factor after ischemic injury in both in vitro and animal studies (Geng et al. 2019). These results are confirmed in patient studies where the levels of miR-126 were notably reduced in the acute phase of IS when compared with those in controls (Geng et al. 2019). It is noteworthy that miR-126 expression was positively associated with the Barthel scale (Quinn et al. 2011), indicating that higher miR-126 levels in plasma are associated with better clinical condition and improved daily living activities (Geng et al. 2019).

Altered expression associated with IS was also noted for miR-152-3p, which was notably downregulated in patients with IS compared to controls. MiR-152-3p levels were also associated with clinically observed severity of stroke, assessed using the NIHSS scale (Luo et al. 2019). Additionally, it was revealed that the expression of miR-152-5p was lowest in patients with large artery atherosclerosis compared with other stroke etiologies, including large artery atherosclerosis (LAA) patients compared to that in small vessel occlusion (SAA), cardioembolism (CE) and stroke of undetermined etiology (SUE) group (Song et al. 2020).

Increased expression of miR-134 was observed in patients with IS compared with that in non-stroke controls within 24 h of the stroke onset. Exosome-derived miR-134 levels were positively associated with stroke severity, as measured using the NIHSS score, infarct volume, and worse prognosis in the modified Rankin scale (Zhou et al. 2018). No notable changes were observed for the level of exosome-derived miR-134 at the time point of 24, 48 and 72 h after the IS. As the decision on stroke therapy is made in the first hours after its occurrence, a more detailed investigation is needed to uncover the manner of miR-134 expression within 24 h after IS onset (Zhou et al. 2018). These results also found an association between miR-134 expression and plasma concentrations of c-reactive protein (CRP) and IL-6, which are well-known markers of inflammation (Zhou et al. 2018). As shown in animal research, increased levels of inflammatory cytokines (TNF, IL-1, IL-6, and IL-8). A previous study showed that miR-134 promoted ischemic injury-induced neuronal cell death by targeting the cAMP response element-binding protein and inhibiting the expression of the anti-apoptotic gene Bcl-2 and that of BDNF (Huang et al. 2015). One of the advantages offered by the use of both exosomes and EVs and their derived miRNAs as disease biomarkers, is their ability to predict the severity of disease (Eyileten et al. 2022a). BDNF is a member of the neurotrophin family of growth factors that plays an important role in the maturation of the nervous system while also supporting the survival of existing neurons and instigating neurogenesis (Castrén and Antila 2017). It is a crucial biomarker and has potential as a pharmaceutical agent in neurodegenerative disorders including Alzheimer's Disease, Parkinson's disease as well as diabetes mellitus (Eyileten et al. 2017, 2021; Gao et al. 2022). However, the protective effect of BDNF on neurogenesis at the molecular level remains unclear. BDNF expression induces the synthesis of BDNF protein in ribosomes through targeted coding, a process regulated by non-coding RNAs. Protein-RNA interaction searching tools and techniques are useful in understanding how BDNF and miRNAs behave in neurodegeneration and neuroregeneration (Shi 2015; Eyileten et al. 2021). As limited studies have aimed to investigate the BDNF-miRNAs axis and the regulation of BDNF signaling by exosome/EV-derived ncRNAs, further studies are required.

The other miRNAs related to IS reported by (Wang et al. 2018; Graham 2020), miR-21-5p and miR-30-5p, were assessed in all phases of IS, including hyperacute (within 6 h), acute (divided on days 1–3 and 4–7), subacute (days 7–14) and recovery (days > 14). The miR-21-5p expression levels were notably upregulated in the subacute and recovery phases compared with those in the acute phase. The miR-30a-5p expression was notably higher in the first 6 h compared with that in all other phases, and its levels were decreased in acute IS (days 1–3) compared with those

Table 2 Human studies focused on miRNAs associated with EVs in IS

miRNA/source (exosome- or extracellular vesicle-derived)	Related genes	Material	Patients with IS, n	Materials and methods	Results	Conclusion	(Refs.)
MiR-21-5p, MiR-30a-5p	TMEM49 (miR-21); RhoB, beclin-1 (miR-30a)	Human blood	HIS ($n=15$), AIS ($n=65$), SIS ($n=31$), RIS ($n=32$), and non-stroke control ($n=24$) were included	Exosome isolation, exoRNeasy kit (Qiagen, Inc.); nanoparticle tracking; flow cytometry ($CD63^+$ or $CD81^+$); PCR detection of miRNAs via cDNA was synthesized using a Mir-XTM miRNA First-Strand Synthesis Kit was used	MiR-21-5p in SIS ($P<0.05$) and RIS ($P<0.01$) was significantly higher than that in controls. MiR-30a-5p was significantly higher in HIS group ($P<0.05$) and significantly lower in AIS compared to controls ($P<0.05$)	The study concluded that miR-21-5p and miR-30a-5p can assist in the early-stage diagnosis of IS and IS subgroups	(Wang et al. 2018; Graham 2020)
MiR-134	n/a	Human blood	AIS ($n=50$), cardioembolism ($n=23$), large artery atherosclerotic stroke ($n=10$), small artery stroke ($n=17$), non-stroke control ($n=50$) were included	Exosome isolation, ExoQuick exosome precipitation solution; Exosome RNA Purification Kit; RNA extraction, NanoDrop™ ND-2000 (Thermo Fisher Scientific, Inc.), RT-qPCR miRNA detection; Western blotting, RIPA lysis buffer, and antibodies against $CD9^+$, $CD63^+$, β-actin and Tsg101 were used	MiR-134 was significantly increased in patients with AIS within 24 h of stroke onset compared with that in the control group ($P=6.0\times10^{-5}$) and higher abundance of the exosome-derived miR-134 was found in patients with IS and poor prognosis ($P=7.0\times10^{-5}$). Plasma levels of hs-CRP ($P=8.3\times10^{-5}$) and IL-6 ($P=6.5\times10^{-5}$) were significantly increased in patients with AIS and positively associated with miR-134 level	The study concluded that exosome-derived miR-134 is a possible novel biomarker for the diagnosis and prognosis of AIS	(Zhou et al. 2018)

Table 2 (continued)

miRNA/source (exosome- or extracellular vesicle-derived)	Related genes	Material	Patients with IS, n	Materials and methods	Results	Conclusion	(Refs.)
Exosome-derived miR-30d-5p	Beclin-1 Atg5	Human blood in vitro and in vivo (rats)	AIS ($n=70$) and healthy controls ($n=35$) were included	Nanoparticle tracking analysis; RNA isolation TRIzol reagent, RT PrimeScript™ RT Master Mix; RT-PCR Power SYBR Green PCR Master Mix; ELISA kit was used	Expression of miR-30d-5p was lower in patients with AIS, and the levels of the inflammatory factors TNF-α, IL-6, and iNOS were upregulated, while the anti-inflammatory factors IL-4 and IL-10 were downregulated compared to controls. Treatment with the exosome-derived-miR-30d-5p reduced AIS-induced brain injury in the in vitro *model*	The study concluded that exosome-derived miR-30d-5p can reverse ischemia-induced, autophagy-mediated brain injury (M2 microglia/macrophage polarization) and has a greater effect in suppressing autophagy and can be a promising therapeutic strategy for cerebral injury mitigation through inflammatory response inhibition	(Jiang et al. 2018)
Exosome-derived miR-223	n/a	Human blood	Patients with AIS within the first 72 h ($n=50$) and matching controls ($n=33$) were included	Exosome isolation, ExoQuick exosome precipitation solution, Exosome identification (TEM), protein extraction and blotting, RNA isolation miRNeasy Mini Kit, RT universal cDNA synthesis kit qRT-PCR SYBR Green master mix were used	The positive association between exosome-derived miR-223 expression and NIHSS score was found ($p=0.03$). Stroke patients who had poor outcomes were shown to have a greater expression of exosomal miR-223 than patients who had good outcomes ($p=0.036$)	The study concluded that increased exosome-derived miR-223 expression was associated with AIS occurrence, stroke severity, and short-term outcomes	(Chen et al. 2017)

Table 2 (continued)

miRNA/source (exosome- or extracellular vesicle-derived)	Related genes	Material	Patients with IS, n	Materials and methods	Results	Conclusion	(Refs.)
Exosome-derived miR-126	n/a	Human blood in vitro and in vivo (rats)	Patients with AIS ($n=13$), and matching controls ($n=17$) were included	Exosome isolation, ExoQuick™ Exosome Precipitation Solution, RNA extraction Trizol reagent, RT Prime Script TM Master Mix, qPCR SYBR Green Mix were used	The level of miR-126 level was notably lower in patients with AIS compared with that in controls. A positive association was observed between the barthel index and the miR-126 level ($P=0.034$), indicating that the patients with a higher miR-126 level in plasma have improved daily life activities	The study concluded that the level of miR-126 in the plasma of patients with IS was notably lower than that in controls and associated with improved daily life activities	(Geng et al. 2019)
Exosome-derived miR-144a and miR-125b-2-3p	n/a	Human blood	Patients with IS ($n=55$); patients with AIS (days 1–3), ($n=27$), patients with SIS (days 4–14) ($n=28$), and matching controls ($n=25$) were included	Exosome isolation and RT-qPCR was used	The levels of plasma exosome-derived miR-422a and miR-125b-2-3p were significantly decreased in the subacute phase group ($P<0.001$). The miR-422a levels were significantly increased in the acute phase group ($P<0.005$) compared to the controls. The expression of miR-422a and miR-125b-2-3p was significantly decreased in the subacute phase group compared with the acute phase group ($P<0.001$)	The study concluded that plasma exosome-derived miR-422a and miR-125b-2-3p may be considered as future IS diagnostic biomarkers, with plasma exosome-derived miR-422a showing the best diagnostic value	(Li et al. 2017)

Table 2 (continued)

miRNA/source (exosome- or extracellular vesicle-derived)	Related genes	Material	Patients with IS, n	Materials and methods	Results	Conclusion	(Refs.)
EMV-derived miR-155	n/a	Human blood	Patients with IS ($n=93$), acute ($t \leq 24$ h) ($n=40$), subacute (24 h $< t \leq 2$ weeks) ($n=35$), chronic ($t > 2$ weeks) ($n=18$), large artery atherosclerosis ($n=35$), cardioembolism ($n=24$), small vessel disease ($n=25$), other etiology ($n=8$), matching controls ($n=70$) were included	Nanoparticle tracking analysis, microvesicle size and morphology analysis TEM, RNA isolation, TRIzol reagent, miRNeasy Mini Kit, qRT-PCR SYBR Premix Ex Taq TM were used	Positive associations between levels of EMVs, EMV-derived miR-155 and infarct volume were found. The levels of EMVs and EMV-derived miR-155 were also positively associated with the NIHSS score. The levels of EMVs and EMV-derived miR-155 were higher in large artery atherosclerosis and cardioembolic subtypes of IS. The diagnostic value of EMV-derived miR-155 was higher when using their combination	The study concluded that plasma microvesicles and microvesicle-derived miR-155 are promising biomarkers for IS	(Zhang et al. 2020)

Table 2 (continued)

miRNA/source (exosome- or extracellular vesicle-derived)	Related genes	Material	Patients with IS, n	Materials and methods	Results	Conclusion	(Refs.)
Circulating EV-miRNA microarray profile		Human blood	Patients with AIS ($n=5$) and matching healthy controls ($n=5$) were included	Purification of EVs was performed by size exclusion chromatography-based Izon original 35 nm Smart columns, EV-rich samples was determined by Pierce BCA Protein Assay Kit; Isolation of total RNA (including miRNA) was performed from the concentrated pooled EV-rich samples was achieved using miRNeasy Serum/Plasma Kit from Qiagen; Affymetrix® GeneChip® miRNA 4.0 Arrays were used for microarray analysis	Let-7b-5p, miR-16-5p, miR-320c were found upregulated, while miR-548a-3p, miR-6808-3p were found downregulated in AIS group	Five differentially expressed miRNAs—hsa-let-7b-5p, hsa-miR-16-5p, hsa-miR-320c, hsa-miR-548a-3p, and hsa-miR-6808-3p identified and can regulate multiple genes and pathways relevant to stroke	(Pir et al. 2024)

IS ischemic stroke, *EVs* extracellular vesicles, *miRNA/mir* microRNA, *HIS* hyperacute ischemic stroke, *AIS* acute ischemic stroke, *SIS* subacute ischemic stroke, *RIS* recovery ischemic stroke, *RT-qPCR* reverse transcription-quantitative *PCR*, *EMVs* endothelial microvesicles, n/a not applicable, high-sensitive C-reactive protein, *CRP*, *NIHSS* National Institutes of Health Stroke Scale, *TEM* Transmission electron microscopy, *EMVs* Endothelial microvesicles, *ECs* endothelial cells

Table 3 Evaluation of the potential of miRNAs as biomarkers in patients with IS

miRNA	IS phase	AUC	*P*-value	(Refs.)
miR-21-5p	SIS	0.714	0.007	(Wang et al. 2018)
	RIS	0.734	0.003	
miR-30-5p	HIS	0.826	0.001	
	AIS	0.438	0.516	
miR-134	AIS	0.834	7 × 10–5	(Zhou et al. 2018)
miR-30d-5p	IS	n/d	n/d	(Jiang et al. 2018)
miR-223	AIS	0.859	< 0.001	(Chen et al. 2017)
miR-126	AIS	n/d	n/d	(Geng et al. 2019)
miR-144a	AIS	0.769	< 0.001	(Li et al. 2017)
miR-144a	SIS	0.971	< 0.001	
miR-125b-2-3p		0.889	< 0.001	
miR-155	IS	0.851	< 0.05	(Zhang et al. 2020)

miRNA/miR microRNA; *IS* Ischemic stroke, *HIS* hyperacute ischemic stroke, *AIS* acute ischemic stroke, *SIS* subacute ischemic stroke, *RIS* recovery ischemic stroke, *AUC* area under curve

in the controls. Thus, the combined exosome-derived miR-21-5p and miR-30a-5p assessment may be promising for IS diagnosis and stratifying the hyperacute, subacute, and recovery phases. Therefore, small RNAs may appear to be a useful biomarker for the diagnosis of the hyperacute phase, which is essential in clinical practice, as the hyperacute phase represents the critical window of opportunity to prevent irreversible brain damage and improve patient outcomes (Wang et al. 2018; Graham 2020). Besides the human studies, a previous bioinformatic prediction analysis demonstrated the putative target genes of miR-21, including the oncogenes homo sapiens v-ski sarcoma viral oncogene homolog, *RAB6A*, and *RAB6C*, both of which are members of the RAS oncogene family, and RAS homolog gene family member B (*RhoB*) in breast cancer (Yan et al. 2008). *RhoB* gene contribution to cancer research opened the investigation of other small RNA targets, such as miR-335, especially in hepatocellular carcinoma (Thapa et al. 2023). Exosome-based miR-335 delivery prevented metastasis and showed cytotoxicity in cancer cells (Thapa et al. 2023). By contrast, there is no study available that investigated the impact of EV-associated miR-335 in IS, showing the need for future research. miR-21 was previously described as associated with atherosclerosis and IS (Tsai et al. 2013), where it may play a protective role via upregulation of the anti-apoptotic protein Bcl-2 (Zhou and Zhang 2014). By contrast, miR-30a was seen as an apoptosis promoter and inhibitor of autophagy via the negative regulation of the Beclin-1 protein (Fu et al. 2012; Long et al. 2013).

As in animal studies, macrophage polarization may be a key factor for brain tissue recovery after ischemia (Zhang et al. 2019; Jiang et al. 2020). In the group of patients with acute IS, miR-30d-5p and the anti-inflammatory cytokines IL-4 and IL-10 were downregulated, whereas the pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and iNOS were upregulated. Pro-inflammatory cytokines are markers of M1 macrophages, while IL-4 and IL-10 are markers of alternatively activated M2 macrophages, which are involved in neurotrophic factor secretion (Jiang et al. 2018). It was therefore suggested that miR-30d-5p may be involved in the inflammatory response regulation following IS (Jiang et al. 2018).

Two other notable exosome-derived miRNAs, miR-125b-2-3p and miR-422a, were also studied as potential IS biomarkers. In a group of patients with IS, both miRNAs were markedly decreased in the subacute phase (days 4–14) compared with the healthy controls. In addition, the expression of miR-422a was higher in the acute IS phase (days 1–3) compared with that in healthy controls (Li et al. 2017). These results suggest that both miRNAs may be useful to distinguish patients in the subacute IS phase from non-stroke individuals; however, only the levels of miR-422a could potentially serve as a biomarker of acute IS (Li et al. 2017). MiR-422a, known to be brain-specific, was shown in a previous animal study to reflect the onset of ischemic IS and has proven to have diagnostic value, as it consistently alters during the acute phase of stroke, regardless of age, stroke severity, or confounding metabolic complications (Sepramaniam et al. 2014). It is also expressed in abundance in atherosclerotic plaque and may participate in the genetic regulation of the development and progression of atherosclerosis, a key factor leading to IS (Hansson and Libby 2006; Vemuganti 2013). The mechanism of miR-125-2-3p involvement is potentially related to inflammatory reactions following ischemic brain injury (Chaudhuri et al. 2011; Bidzhekov et al. 2012).

Microvesicles and Other Types of EVs

Microvesicles are vesicular structures released from the plasma membrane by various types of cells, especially platelets, erythrocytes, leukocytes, and endothelial cells (Ståhl et al. 2019). Endothelial dysfunction is one of the key pathophysiological factors in the occurrence and progress of IS. Endothelial microvesicles (EMVs) and the level of their miR-155 content were assessed in patients with IS in acute, subacute, and chronic phases (Zhang et al. 2020). The levels of EMVs and the expression of miR-155 were elevated in acute and subacute IS compared with those in the controls. The EMV-derived miR-155 expression was notably higher in the acute stage than in the chronic stage. The study showed a positive association between EMVs and miR-155 and NIHSS score, as well as infarct volume, which suggests its potential future use as a prognostic biomarker. Both EMVs and EMV-miR-155 expressions were associated with the type of IS according

to the TOAST criteria (Zhang et al. 2020). Higher concentrations were detected in patients with large artery atherosclerosis and cardioembolic etiology compared to those with ischemic stroke related to small vessel disease (Adams et al. 1993). In conclusion, both EMVs and EMV-miR-155 may be further investigated as sensitive biomarkers to identify those patients with IS from the non-stroke population. However, EMV-miR-155 demonstrated improved specificity and therefore greater diagnostic value than EMVs as a whole (Zhang et al. 2020). Likewise, previous studies have revealed that miR-155 is an important player in oxidative stress and apoptosis. Inhibition of miR-155 decreases apoptosis and ROS production and promotes NO generation in the brain microvessel endothelium (Liu et al. 2015; Hu et al. 2022b). MiR-155 also takes part in inflammation and is seen as differentially expressed in numerous diseases related to inflammation, such as asthma, lung cancer, lymphoblastic leukemia, and neurodegenerative diseases (Tables 2, 3) (Gao et al. 2014; Liu et al. 2019; El-Khazragy et al. 2019; Karam and Abd Elrahman 2019).

As previously shown, the exclusion of intracranial hemorrhage is an important step in the diagnosis of IS, enabling the initiation of fibrinolytic therapy (Kalani et al. 2020). Kalani et al. (Kalani et al. 2020) revealed that EV-associated miRNAs could have potential diagnostic value and be useful in distinguishing between ischemic and hemorrhagic stroke types. This study involved three subgroups of patients with different types of stroke, including IS, aneurysmal subarachnoid hemorrhage, and spontaneous intraparenchymal hemorrhage (Kalani et al. 2020). The in silico analysis revealed that 67 EV-associated miRNAs were notably dysregulated across the stroke subgroups. Consequently, 13 EV-associated miRNAs demonstrated a high value in distinguishing between ischemic and hemorrhagic stroke. Therefore, the study showed the potential use of blood-based, EV-associated miRNAs as diagnostic biomarkers to distinguish between stroke subtypes (Kalani et al. 2020).

A recent study utilized microarray analysis on plasma-EVs samples from IS patients. The study not only analyzed newly generated microarray data but also reanalyzed publicly available datasets, integrating bioinformatic analysis for a comprehensive evaluation. The study compared 5 acute IS patients with 5 matched healthy controls. Microarray analysis identified 383 miRNAs, with 245 shared between both groups, while 90 were unique to controls and 48 were exclusive to acute IS patients. The most abundant miRNAs in the stroke group belonged to the let-7b, miR-106, miR-16, and miR-320 families. Gene Ontology (GO) analysis revealed enrichment in pathways related to extracellular exosomes, BMP signaling regulation, apoptosis, and outflow tract morphogenesis. When comparing circulating EV-miRNA profiles between acute IS patients and healthy individuals, let-7b-5p, miR-16-5p, and miR-320c were found to be upregulated, while miR-548a-3p and miR-6808-3p were downregulated in the acute IS group (Pir et al. 2024).

Lately, neuronally derived extracellular vesicles (L1CAM-positive EVs-L1EVs) have been studied in acute IS. Patients with acute IS and control patients with at least three cardiovascular risk factors (free of stroke) were included. Blood samples underwent centrifugation, L1EV isolation, RNA extraction, and sequencing to analyze small RNA content. The sequencing revealed 62 miRNAs and 76 small non-coding RNAs significantly altered in acute IS patients. Using weighted gene correlation network analysis. Researchers grouped these miRNAs into modules linked to stroke severity and type. A machine learning-based random forest model was developed, identifying an 8-small RNA signature (particularly, LINC01359, LINC02116, LINC02256, miR-181a-5p, miR-221-3p, miR-27b-3p, miR-154-3p, miR-376a-3p) that classified acute IS patients with 87.5% sensitivity and 83.3% specificity. The study's findings suggest that L1EVs carry distinct small RNA profiles that could serve as biomarkers for stroke diagnosis (Manwani et al. 2024).

Limitations and Challenges of EVs and Exosomal miRNAs in IS

Standardization and Reproducibility of miRNA Studies

One of the biggest challenges in the field of EVs and exosomal miRNAs is the lack of standardization of the methodologies, particularly EVs, exosome isolation, and quantification. Unstandardized isolation techniques, including ultracentrifugation, size-exclusion chromatography, cytometry, and precipitation methods cause unreproducible laboratory results. Additionally, the absence of consensus regarding normalization strategies in miRNA quantification further complicates cross-study comparisons (Li et al. 2019; Sidhom et al. 2020).

Specificity/Sensitivity of miRNAs as Biomarkers

Although circulating miRNAs hold promise as potential biomarkers for IS, their specificity and sensitivity remains a concern. Many miRNAs implicated in stroke pathology were ofund also associated with other neurodegenerative and cardiovascular diseases, which cause challenges in distinguishing stroke from other conditions (Li et al. 2023). Moreover, while specific miRNAs demonstrate altered expression following ischemic events, their dynamic changes over different stroke phases need better characterization for improved clinical applicability. For example, identifying miRNAs that can be utilized in the hyperacute phase of stroke (within the

first hours of onset) is crucial, yet most studies focus on later stages (Wechsler et al. 2023). This gap in research limits the potential for miRNA-based diagnostics and therapeutics for stroke.

Lack of Large-Scale Clinical Validation

The abovementioned studies on EVs and exosomal-miRNAs in IS rely on small sample sizes or animal models/in vitro analysis. While these preliminary findings are valuable, large-scale, multicenter clinical trials are needed to validate the diagnostic and prognostic potential of miRNAs in stroke patients. Additionally, the heterogeneity of IS subtypes requires broad patient cohort studies to establish stroke-specific miRNA profiles.

Cost and Feasibility of Implementation

Currently, the use of miRNA-based diagnostics and therapies in clinical settings is still quite costly. In particular, the isolation of small RNAs from EVs and exosomes, followed by sequencing, is still expensive and requires specialized laboratory infrastructure in hospitals (Xiong et al. 2022; Llorens-Revull et al. 2023). Developing cost-efficient, rapid, and scalable miRNA detection methods is essential for clinical use. In oncology, multi-omic analysis for drug response, including genomic sequencing, is increasingly becoming a standard clinical practice (Kan et al. 2025). As the cost of next-generation and single-cell RNA-sequencing continues to decrease, the implementation of small RNA analysis in stroke care is expected to become more common, potentially improving clinical stroke management.

Conclusions

In conclusion, miRNAs are expressed in abundance in numerous types of tissues and maybe shed in EVs, internalized by other cells, and remotely regulated gene expression. To date, various EV-associated miRNAs are involved in nearly every aspect of IS pathogenesis, including endothelial function, coagulation, inflammatory response, microglial activity, apoptosis and cell proliferation. Thus, these may be widely investigated as potential diagnostic and prognostic biomarkers as well as therapeutic targets. The present review focused on the latest knowledge relating to EV-associated miRNAs in IS, showing that while available data are limited so far, they may prove useful prognostic indicators. Human studies (Li et al. 2017; Zhang et al. 2020) revealed that some types of miRNAs may be considered potentially useful clinical biomarkers, but additional research conducted in larger patient cohorts is required. Moreover, due to the dynamics of ischemic lesion development, as well as the specificity of the IS treatment, further studies should consider changes in miRNA expression not only in the first days and weeks but also in the first hours after stroke onset. However, this would be challenging since the onset of symptoms is often elusive, and numerous patients are admitted to the hospital several hours later.

Despite the promising role of EVs and exosomal miRNAs in IS, several limitations and challenges that were also mentioned above should be addressed before their clinical application. Improving the standardization of laboratory methodologies, identifying specific and sensitive biomarkers that differentiate stroke from other neurodegenerative and cardiovascular diseases, conducting large-scale clinical trials, and overcoming delivery challenges are key areas that need further exploration. Addressing these obstacles will enhance miRNA-based innovations in stroke diagnosis, prognosis, and therapy.

Ethical Approval

Not applicable.

Consent for Publication

Not applicable.

Acknowledgements This work was written by the members (MP, DMG, CE) of the International and Intercontinental Cardiovascular and Cardiometabolic Research Team (I-COMET; www.icomet.science).

Author Contributions CE prepared the original draft. PC, ID, AWC, AG, DMG, AC, MP, and CE wrote and edited the manuscript. CE, visualized the paper. DMG, AC and MP supervised the study. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Funding The present review was supported by the 'OPUS', National Science Center, Poland (Grant No. 2018/31/B/NZ7/01137) and the Medical University of Warsaw (Grant No. 1M9/2/M/MBM/N/21).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

References

Adams HP Jr, Bendixen BH, Kappelle LJ et al (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. Stroke 24:35–41. https://doi.org/10.1161/01.str.24.1.35

Adams HP Jr, Davis PH, Leira EC et al (1999) Baseline NIH stroke scale score strongly predicts outcome after stroke: a report of the trial of Org 10172 in acute stroke treatment (TOAST). Neurology 53:126–131. https://doi.org/10.1212/wnl.53.1.126

Aili Y, Maimaitiming N, Mahemuti Y et al (2021) The role of exosomal miRNAs in glioma: biological function and clinical application. Front Oncol 11:686369. https://doi.org/10.3389/fonc.2021.686369

Aimaletdinov AM, Gomzikova MO (2022) Tracking of extracellular vesicles' biodistribution: new methods and approaches. Int J Mol Sci. https://doi.org/10.3390/ijms231911312

Alexander M, Hu R, Runtsch MC et al (2015) Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat Commun 6:7321. https://doi.org/10.1038/ncomms8321

Al-Onaizi M, Al-Khalifah A, Qasem D, ElAli A (2020) Role of microglia in modulating adult neurogenesis in health and neurodegeneration. Int J Mol Sci. https://doi.org/10.3390/ijms21186875

Araki T, Ikegaya Y, Koyama R (2021) The effects of microglia- and astrocyte-derived factors on neurogenesis in health and disease. Eur J Neurosci 54:5880–5901. https://doi.org/10.1111/ejn.14969

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297. https://doi.org/10.1016/s0092-8674(04)00045-5

Bidzhekov K, Gan L, Denecke B et al (2012) microRNA expression signatures and parallels between monocyte subsets and atherosclerotic plaque in humans. Thromb Haemost 107:619–625. https://doi.org/10.1160/TH11-09-0607

Camussi G, Deregibus MC, Bruno S et al (2010) Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 78:838–848. https://doi.org/10.1038/ki.2010.278

Castrén E, Antila H (2017) Neuronal plasticity and neurotrophic factors in drug responses. Mol Psychiatry 22:1085–1095. https://doi.org/10.1038/mp.2017.61

Chaudhuri AA, So AY-L, Sinha N et al (2011) MicroRNA-125b potentiates macrophage activation. J Immunol 187:5062–5068. https://doi.org/10.4049/jimmunol.1102001

Chen Y, Song Y, Huang J et al (2017) Increased circulating exosomal miRNA-223 is associated with acute ischemic stroke. Front Neurol 8:57. https://doi.org/10.3389/fneur.2017.00057

Chen Q, Liu Y, Ding X et al (2020) Bone marrow mesenchymal stem cell-secreted exosomes carrying microRNA-125b protect against myocardial ischemia reperfusion injury via targeting SIRT7. Mol Cell Biochem 465:103–114. https://doi.org/10.1007/s11010-019-03671-z

Cui J, Liu N, Chang Z et al (2020) Exosomal MicroRNA-126 from RIPC serum is involved in hypoxia tolerance in SH-SY5Y cells by downregulating DNMT3B. Mol Ther Nucleic Acids 20:649–660. https://doi.org/10.1016/j.omtn.2020.04.008

Cun Y, Jin Y, Wu D et al (2022) Exosome in crosstalk between inflammation and angiogenesis: a potential therapeutic strategy for stroke. Mediators Inflamm 2022:7006281. https://doi.org/10.1155/2022/7006281

Cuomo O, Anzilotti S, Brancaccio P et al (2024) Systemic administration of blood-derived exosomes induced by remote ischemic post-conditioning, by delivering a specific cluster of miRNAs, ameliorates ischemic damage and neurological function. J Cereb Blood Flow Metab 44:1459–1471. https://doi.org/10.1177/0271678X241270284

Czajka P, Fitas A, Jakubik D et al (2021) MicroRNA as potential biomarkers of platelet function on antiplatelet therapy: a review. Front Physiol 12:652579. https://doi.org/10.3389/fphys.2021.652579

El-Khazragy N, Noshi MA, Abdel-Malak C et al (2019) miRNA-155 and miRNA-181a as prognostic biomarkers for pediatric acute lymphoblastic leukemia. J Cell Biochem 120:6315–6321. https://doi.org/10.1002/jcb.27918

Eyileten C, Kaplon-Cieslicka A, Mirowska-Guzel D et al (2017) Antidiabetic effect of brain-derived neurotrophic factor and its association with inflammation in type 2 diabetes mellitus. J Diabetes Res 2017:2823671. https://doi.org/10.1155/2017/2823671

Eyileten C, Sharif L, Wicik Z et al (2021) The relation of the brain-derived neurotrophic factor with MicroRNAs in neurodegenerative diseases and ischemic stroke. Mol Neurobiol 58:329–347. https://doi.org/10.1007/s12035-020-02101-2

Eyileten C, Jakubik D, Shahzadi A et al (2022a) Diagnostic performance of circulating miRNAs and extracellular vesicles in acute ischemic stroke. Int J Mol Sci. https://doi.org/10.3390/ijms23094530

Eyileten C, Wicik Z, Keshwani D et al (2022b) Alteration of circulating platelet-related and diabetes-related microRNAs in individuals with type 2 diabetes mellitus: a stepwise hypoglycaemic clamp study. Cardiovasc Diabetol 21:79. https://doi.org/10.1186/s12933-022-01517-5

Fu L-L, Wen X, Bao J-K, Liu B (2012) MicroRNA-modulated autophagic signaling networks in cancer. Int J Biochem Cell Biol 44:733–736. https://doi.org/10.1016/j.biocel.2012.02.004

Gao F, Chang J, Wang H, Zhang G (2014) Potential diagnostic value of miR-155 in serum from lung adenocarcinoma patients. Oncol Rep 31:351–357. https://doi.org/10.3892/or.2013.2830

Gao L, Zhang Y, Sterling K, Song W (2022) Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. Transl Neurodegener 11:4. https://doi.org/10.1186/s40035-022-00279-0

Gao C, Jiang J, Tan Y, Chen S (2023) Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets. Signal Transduct Target Ther 8:359. https://doi.org/10.1038/s41392-023-01588-0

Ge Q, Zhou Y, Lu J et al (2014) miRNA in plasma exosome is stable under different storage conditions. Molecules 19:1568–1575. https://doi.org/10.3390/molecules19021568

Geng W, Tang H, Luo S et al (2019) Exosomes from miRNA-126-modified ADSCs promotes functional recovery after stroke in rats by improving neurogenesis and suppressing microglia activation. Am J Transl Res 11:780–792

Graham M (2020) Burying our mistakes: dealing with prognostic uncertainty after severe brain injury. Bioethics 34:612–619. https://doi.org/10.1111/bioe.12737

György B, Szabó TG, Pásztói M et al (2011) Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci 68:2667–2688. https://doi.org/10.1007/s00018-011-0689-3

Hand PJ, Kwan J, Lindley RI et al (2006) Distinguishing between stroke and mimic at the bedside: the brain attack study. Stroke 37:769–775. https://doi.org/10.1161/01.STR.0000204041.13466.4c

Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 6:508–519. https://doi.org/10.1038/nri1882

Hazrati A, Soudi S, Malekpour K et al (2022) Immune cells-derived exosomes function as a double-edged sword: role in disease progression and their therapeutic applications. Biomark Res 10:30. https://doi.org/10.1186/s40364-022-00374-4

Hougaard KD, Hjort N, Zeidler D et al (2014) Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. Stroke 45:159–167. https://doi.org/10.1161/STROKEAHA.113.001346

Hu H, Hu X, Li L et al (2022a) Exosomes derived from bone marrow mesenchymal stem cells promote angiogenesis in ischemic stroke mice via upregulation of MiR-21-5p. Biomolecules 12:883. https://doi.org/10.3390/biom12070883

Hu J, Huang S, Liu X et al (2022b) MiR-155: An important role in inflammation response. J Immunol Res 2022:7437281. https://doi.org/10.1155/2022/7437281

Huang W, Liu X, Cao J et al (2015) miR-134 regulates ischemia/reperfusion injury-induced neuronal cell death by regulating CREB signaling. J Mol Neurosci 55:821–829. https://doi.org/10.1007/s12031-014-0434-0

Ip WKE, Hoshi N, Shouval DS et al (2017) Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 356:513–519. https://doi.org/10.1126/science.aal3535

Jayaraj RL, Azimullah S, Beiram R et al (2019) Neuroinflammation: friend and foe for ischemic stroke. J Neuroinflammation 16:142. https://doi.org/10.1186/s12974-019-1516-2

Jia L, Hao F, Wang W, Qu Y (2015) Circulating miR-145 is associated with plasma high-sensitivity C-reactive protein in acute ischemic stroke patients. Cell Biochem Funct 33:314–319. https://doi.org/10.1002/cbf.3116

Jiang M, Wang H, Jin M et al (2018) Exosomes from MiR-30d-5p-ADSCs reverse acute ischemic stroke-induced, autophagy-mediated brain injury by promoting M2 microglial/macrophage polarization. Cell Physiol Biochem 47:864–878. https://doi.org/10.1159/000490078

Jiang C-T, Wu W-F, Deng Y-H, Ge J-W (2020) Modulators of microglia activation and polarization in ischemic stroke (Review). Mol Med Rep 21:2006–2018. https://doi.org/10.3892/mmr.2020.11003

Johnston SC, Mendis S, Mathers CD (2009) Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. Lancet Neurol 8:345–354. https://doi.org/10.1016/S1474-4422(09)70023-7

Jovičić A, Gitler AD (2017) Distinct repertoires of microRNAs present in mouse astrocytes compared to astrocyte-secreted exosomes. PLoS ONE 12:e0171418. https://doi.org/10.1371/journal.pone.0171418

Kalani MYS, Alsop E, Meechoovet B et al (2020) Extracellular microRNAs in blood differentiate between ischaemic and haemorrhagic stroke subtypes. J Extracell Vesicles 9:1713540. https://doi.org/10.1080/20013078.2020.1713540

Kan Z, Bonato V, Wen J (2025) Real-world clinical multi-omics analyses reveal bifurcation of ER-independent and ER-dependent drug resistance to CDK4/6 inhibitors. Nat Commun 16:932–935

Karam RA, Abd Elrahman DM (2019) Differential expression of miR-155 and Let-7a in the plasma of childhood asthma: potential biomarkers for diagnosis and severity. Clin Biochem 68:30–36. https://doi.org/10.1016/j.clinbiochem.2019.04.007

Kesidou D, da Costa Martins PA, de Windt LJ et al (2020) Extracellular vesicle miRNAs in the promotion of cardiac neovascularisation. Front Physiol 11:579892. https://doi.org/10.3389/fphys.2020.579892

Li D-B, Liu J-L, Wang W et al (2017) Plasma exosomal miR-422a and miR-125b-2-3p serve as biomarkers for ischemic stroke. Curr Neurovasc Res 14:330–337. https://doi.org/10.2174/1567202614666171005153434

Li X, Corbett AL, Taatizadeh E et al (2019) Challenges and opportunities in exosome research-perspectives from biology, engineering, and cancer therapy. APL Bioeng 3:011503. https://doi.org/10.1063/1.5087122

Li X, Yao Q, Cui H et al (2021) MiR-223 or miR-126 predicts resistance to dual antiplatelet therapy in patients with ST-elevation myocardial infarction. J Int Med Res 49:3000605211016209. https://doi.org/10.1177/03000605211016209

Li S, Lei Z, Sun T (2023) The role of microRNAs in neurodegenerative diseases: a review. Cell Biol Toxicol 39:53–83. https://doi.org/10.1007/s10565-022-09761-x

Lin H, Cheng C-F (2018) Activating transcription factor 3, an early cellular adaptive responder in ischemia/reperfusion-induced injury. Ci Ji Yi Xue Za Zhi 30:61–65. https://doi.org/10.4103/tcmj.tcmj_37_18

Ling X, Zhang G, Xia Y et al (2020) Exosomes from human urine-derived stem cells enhanced neurogenesis via miR-26a/HDAC6 axis after ischaemic stroke. J Cell Mol Med 24:640–654. https://doi.org/10.1111/jcmm.14774

Liu Y, Pan Q, Zhao Y et al (2015) MicroRNA-155 regulates ROS production, NO generation, apoptosis and multiple functions of human brain microvessel endothelial cells under physiological and pathological conditions. J Cell Biochem 116:2870–2881. https://doi.org/10.1002/jcb.25234

Liu D, Zhao D, Zhao Y et al (2019) Inhibition of microRNA-155 alleviates cognitive impairment in Alzheimer's disease and involvement of neuroinflammation. Curr Alzheimer Res 16:473–482. https://doi.org/10.2174/1567205016666190503145207

Liu H, Shi M, Li X et al (2022) Adipose mesenchymal stromal cell-derived exosomes prevent testicular torsion injury activating PI3K/AKT and MAPK/ERK1/2 pathways. Oxid Med Cell Longev 2022:8065771. https://doi.org/10.1155/2022/8065771

Llorens-Revull M, Martínez-González B, Quer J et al (2023) Comparison of extracellular vesicle isolation methods for miRNA sequencing. Int J Mol Sci 24:12183. https://doi.org/10.3390/ijms241512183

Long G, Wang F, Li H et al (2013) Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. BMC Neurol 13:178. https://doi.org/10.1186/1471-2377-13-178

Luo S, Ding S, Liao J et al (2019) Excessive miR-152-3p results in increased BAFF expression in SLE B-cells by inhibiting the KLF5 expression. Front Immunol 10:1127. https://doi.org/10.3389/fimmu.2019.01127

Luo H, Ye G, Liu Y et al (2022) miR-150-3p enhances neuroprotective effects of neural stem cell exosomes after hypoxic-ischemic brain injury by targeting CASP2. Neurosci Lett 779:136635. https://doi.org/10.1016/j.neulet.2022.136635

Lv H, Li J, Che Y (2021) miR-31 from adipose stem cell-derived extracellular vesicles promotes recovery of neurological function after ischemic stroke by inhibiting TRAF6 and IRF5. Exp Neurol 342:113611. https://doi.org/10.1016/j.expneurol.2021.113611

Ma H, Wu Y, Yang H et al (2016) MicroRNAs in oral lichen planus and potential miRNA–mRNA pathogenesis with essential cytokines: a review. Oral Surg Oral Med Oral Pathol Oral Radiol 122:164–173. https://doi.org/10.1016/j.oooo.2016.03.018

Maida CD, Norrito RL, Daidone M et al (2020) Neuroinflammatory mechanisms in ischemic stroke: focus on cardioembolic stroke, background, and therapeutic approaches. Int J Mol Sci. https://doi.org/10.3390/ijms21186454

Manwani B, Brathaban N, Baqai A et al (2024) Small RNA signatures of acute ischemic stroke in L1CAM positive extracellular vesicles. Sci Rep 14:13560. https://doi.org/10.1038/s41598-024-63633-4

Mens MMJ, Heshmatollah A, Fani L et al (2021) Circulatory MicroRNAs as potential biomarkers for stroke risk: the rotterdam study. Stroke 52:945–953. https://doi.org/10.1161/STROKEAHA.120.031543

Ouyang Q, Li F, Xie Y et al (2019) Meta-analysis of the safety and efficacy of stem cell therapies for ischemic stroke in preclinical and clinical studies. Stem Cells Dev 28:497–514. https://doi.org/10.1089/scd.2018.0218

Patel S, Khan MB, Kumar S et al (2024) The impact of ischemic stroke on bone marrow microenvironment and extracellular vesicles: a study on inflammatory and molecular changes. Exp Neurol 379:114867. https://doi.org/10.1016/j.expneurol.2024.114867

Pei X, Li Y, Zhu L, Zhou Z (2020) Astrocyte-derived exosomes transfer miR-190b to inhibit oxygen and glucose deprivation-induced autophagy and neuronal apoptosis. Cell Cycle 19:906–917. https://doi.org/10.1080/15384101.2020.1731649

Pir GJ, Zahid MA, Akhtar N et al (2024) Differentially expressed miRNA profiles of serum derived extracellular vesicles from patients with acute ischemic stroke. Brain Res 1845:149171. https://doi.org/10.1016/j.brainres.2024.149171

Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC (2019) Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American heart association/American stroke association. Stroke 50(12):e344–e418

Pöyhönen S, Er S, Domanskyi A, Airavaara M (2019) Effects of neurotrophic factors in glial cells in the central nervous system: expression and properties in neurodegeneration and injury. Front Physiol 10:486. https://doi.org/10.3389/fphys.2019.00486

Quinn TJ, Langhorne P, Stott DJ (2011) Barthel index for stroke trials: development, properties, and application. Stroke 42:1146–1151. https://doi.org/10.1161/STROKEAHA.110.598540

Sabour S (2017) The diagnostic value of serum miRNA-221-3p, miRNA-382-5p, and miRNA-4271 in ischemic stroke: methodological issue to avoid misinterpretation. J Stroke Cerebrovasc Dis 26:1161

Sepramaniam S, Tan J-R, Tan K-S et al (2014) Circulating microRNAs as biomarkers of acute stroke. Int J Mol Sci 15:1418–1432. https://doi.org/10.3390/ijms15011418

Shan Y, Hu J, Lv H et al (2021) miR-221 exerts neuroprotective effects in ischemic stroke by inhibiting the proinflammatory response. J Stroke Cerebrovasc Dis 30:105489. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105489

Shi J (2015) Regulatory networks between neurotrophins and miRNAs in brain diseases and cancers. Acta Pharmacol Sin 36:149–157. https://doi.org/10.1038/aps.2014.135

Shi M, Lu Q, Zhao Y et al (2023) miR-223: a key regulator of pulmonary inflammation. Front Med 10:1187557. https://doi.org/10.3389/fmed.2023.1187557

Sidhom K, Obi PO, Saleem A (2020) A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int J Mol Sci 21:6466. https://doi.org/10.3390/ijms21186466

Simats A, García-Berrocoso T, Montaner J (2016) Neuroinflammatory biomarkers: from stroke diagnosis and prognosis to therapy. Biochim Biophys Acta 1862:411–424. https://doi.org/10.1016/j.bbadis.2015.10.025

Song P, Sun H, Chen H et al (2020) Decreased serum exosomal miR-152–3p Contributes to the progression of acute ischemic stroke. Clin Lab. https://doi.org/10.7754/Clin.Lab.2020.200106

Ståhl A-L, Johansson K, Mossberg M et al (2019) Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases. Pediatr Nephrol 34:11–30. https://doi.org/10.1007/s00467-017-3816-z

Sun R, Liao W, Lang T et al (2024) Astrocyte-derived exosomal miR-378a-5p mitigates cerebral ischemic neuroinflammation by modulating NLRP3-mediated pyroptosis. Front Immunol 15:1454116. https://doi.org/10.3389/fimmu.2024.1454116

Tang L, Xu Y, Wang L, Pan J (2023) Adipose-derived stem cell exosomes ameliorate traumatic brain injury through the NLRP3 signaling pathway. NeuroReport 34:677–684. https://doi.org/10.1097/WNR.0000000000001941

Thapa N, Chwae YJ, Yoo KH et al (2023) Exosomal delivery of TRAIL and miR-335 for the treatment of hepatocellular carcinoma (Review). Int J Mol Med. https://doi.org/10.3892/ijmm.2022.5206

Tian T, Cao L, He C et al (2021) Targeted delivery of neural progenitor cell-derived extracellular vesicles for anti-inflammation after cerebral ischemia. Theranostics 11:6507–6521. https://doi.org/10.7150/thno.56367

Tsai P-C, Liao Y-C, Wang Y-S et al (2013) Serum microRNA-21 and microRNA-221 as potential biomarkers for cerebrovascular disease. J Vasc Res 50:346–354

Valadi H, Ekström K, Bossios A et al (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654–659. https://doi.org/10.1038/ncb1596

Vemuganti R (2013) All's well that transcribes well: non-coding RNAs and post-stroke brain damage. Neurochem Int 63:438–449. https://doi.org/10.1016/j.neuint.2013.07.014

Vilela P (2017) Acute stroke differential diagnosis: Stroke mimics. Eur J Radiol 96:133–144. https://doi.org/10.1016/j.ejrad.2017.05.008

Wang W, Li D-B, Li R-Y et al (2018) Diagnosis of hyperacute and acute ischaemic stroke: the potential utility of exosomal MicroRNA-21-5p and MicroRNA-30a-5p. CED 45:204–212. https://doi.org/10.1159/000488365

Wang Z, Xu F, Zhao X et al (2023) Expression analysis and targets prediction of microRNAs in OGD/R treated astrocyte-derived exosomes by smallRNA sequencing. Genomics 115:110594. https://doi.org/10.1016/j.ygeno.2023.110594

Wechsler LR, Adeoye O, Alemseged F et al (2023) Most promising approaches to improve stroke outcomes: the stroke treatment academic industry roundtable XII workshop. Stroke 54:3202–3213. https://doi.org/10.1161/STROKEAHA.123.044279

Wu Q, Wu J-H, Ye Z-Y et al (2024) Exosomes from hypoxia-treated mesenchymal stem cells: promoting neuroprotection in ischemic stroke through miR-214-3p/PTEN mechanism. Mol Neurobiol 61:7611–7626. https://doi.org/10.1007/s12035-024-04056-0

Xiong Y-H, Fan X-G, Chen Y-Y et al (2022) Comparison of methods of isolating extracellular vesicle microRNA from HepG2 cells for High-throughput sequencing. Front Mol Biosci 9:976528. https://doi.org/10.3389/fmolb.2022.976528

Xu L-J, Ouyang Y-B, Xiong X et al (2015) Post-stroke treatment with miR-181 antagomir reduces injury and improves long-term behavioral recovery in mice after focal cerebral ischemia. Exp Neurol 264:1–7. https://doi.org/10.1016/j.expneurol.2014.11.007

Yan L-X, Huang X-F, Shao Q et al (2008) MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 14:2348–2360. https://doi.org/10.1261/rna.1034808

Yang J, Cao L-L, Wang X-P et al (2021) Neuronal extracellular vesicle derived miR-98 prevents salvageable neurons from microglial phagocytosis in acute ischemic stroke. Cell Death Dis 12:23. https://doi.org/10.1038/s41419-020-03310-2

You D, Qiao Q, Ono K et al (2022) miR-223–3p inhibits the progression of atherosclerosis via down-regulating the activation of MEK1/ERK1/2 in macrophages. Aging 14:1865–1878. https://doi.org/10.18632/aging.203908

Yu Y, Zhou H, Xiong Y, Liu J (2020) Exosomal miR-199a-5p derived from endothelial cells attenuates apoptosis and inflammation in

neural cells by inhibiting endoplasmic reticulum stress. Brain Res 1726:146515. https://doi.org/10.1016/j.brainres.2019.146515

Zhang L, Li Y-J, Wu X-Y et al (2015) MicroRNA-181c negatively regulates the inflammatory response in oxygen-glucose-deprived microglia by targeting Toll-like receptor 4. J Neurochem 132:713–723. https://doi.org/10.1111/jnc.13021

Zhang H, Lu M, Zhang X et al (2019) Isosteviol sodium protects against ischemic stroke by modulating microglia/macrophage polarization via disruption of GAS5/miR-146a-5p sponge. Sci Rep 9:12221. https://doi.org/10.1038/s41598-019-48759-0

Zhang H, Chen G, Qiu W et al (2020) Plasma endothelial microvesicles and their carrying miRNA-155 serve as biomarkers for ischemic stroke. J Neurosci Res 98:2290–2301. https://doi.org/10.1002/jnr.24696

Zhang X-L, Zhang X-G, Huang Y-R et al (2021a) Stem cell-based therapy for experimental ischemic stroke: a preclinical systematic review. Front Cell Neurosci 15:628908. https://doi.org/10.3389/fncel.2021.628908

Zhang Y, Liu J, Su M et al (2021b) Exosomal microRNA-22-3p alleviates cerebral ischemic injury by modulating KDM6B/BMP2/BMF axis. Stem Cell Res Ther 12:111. https://doi.org/10.1186/s13287-020-02091-x

Zhang Y, Tan J, Miao Y, Zhang Q (2021c) The effect of extracellular vesicles on the regulation of mitochondria under hypoxia. Cell Death Dis 12:358. https://doi.org/10.1038/s41419-021-03640-9

Zhang Z, Zou X, Zhang R et al (2021d) Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. Aging 13:3060–3079. https://doi.org/10.18632/aging.202466

Zhao S-C, Ma L-S, Chu Z-H et al (2017) Regulation of microglial activation in stroke. Acta Pharmacol Sin 38:445–458. https://doi.org/10.1038/aps.2016.162

Zhou J, Zhang J (2014) Identification of miRNA-21 and miRNA-24 in plasma as potential early stage markers of acute cerebral infarction. Mol Med Rep 10:971–976. https://doi.org/10.3892/mmr.2014.2245

Zhou J, Chen L, Chen B et al (2018) Increased serum exosomal miR-134 expression in the acute ischemic stroke patients. BMC Neurol 18:198. https://doi.org/10.1186/s12883-018-1196-z

Zhu X, Liu Q, Zhu F et al (2024) An engineered cellular carrier delivers miR-138-5p to enhance mitophagy and protect hypoxic-injured neurons via the DNMT3A/Rhebl1 axis. Acta Biomater 186:424–438. https://doi.org/10.1016/j.actbio.2024.07.059