

Multiple strategies to improve the therapeutic efficacy of mesenchymal stem cell-derived exosome transplantation in ischemic stroke (Review)

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Abstract. Ischemic stroke (IS) is a common cause of physical disability and death worldwide. Although it has been indicated that mesenchymal stem cells (MSCs) can treat IS through mechanisms such as immunomodulation, angiogenesis and neuroprotection, their clinical application is limited by inherent drawbacks, such as immune rejection risk and tumorigenic potential. Notably, MSC-derived exosomes (MSC-Exos) possess advantages, such as a smaller volume, low immunogenicity and low tumorigenicity, enabling therapeutic effects similar to those of the parent cells. Furthermore, MSC-Exos can carry various bioactive substances and cross the blood-brain barrier (BBB), thus indicating their potential in the diagnosis, treatment and

prognostic assessment of IS. The present review comprehensively elaborates on the principles by which MSC-Exos may treat IS through mechanisms such as inhibiting inflammatory responses, suppressing apoptosis, promoting angiogenesis, regulating ferroptosis and maintaining BBB integrity. However, the therapeutic efficacy of MSC-Exo monotherapy is restricted by its low brain bioavailability, short lesion retention time, insufficient *in vivo* stability and inability to remain at the lesion site for an extended period of time to exert its effect. Furthermore, the review summarizes the recent optimization strategies involving the combination of MSC-Exos with microRNAs, traditional Chinese medicine, hydrogels, exosome pretreatment and electroacupuncture to enhance the efficacy of MSC-Exo therapy for IS, and systematically reviews their synergistic therapeutic mechanisms. In addition, the current status of clinical translation, technical breakthrough obstacles and future development directions for MSC-Exo combination therapies in neurological diseases is outlined, providing a theoretical reference for innovative treatments of IS.

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Abbreviations: BBB, blood-brain barrier; CRP, C-reactive protein; EA, electroacupuncture; HIF-1 α , hypoxia-inducible factor-1 α ; I/R, ischemia/reperfusion; IS, ischemic stroke; miRNA, microRNA; MSC, mesenchymal stem cell; MSC-Exo, MSC-derived exosome; MCAO, middle cerebral artery occlusion; NET, neutrophil extracellular trap; NSC, neural stem cell; NVU, neurovascular unit; NIHSS, National Institutes of Health Stroke Scale; OGD, oxygen-glucose deprivation; PEDF, pigment epithelium-derived factor; TLR4, Toll-like receptor 4; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis protein

Key words: IS, MSC-Exo, miRNA, drug, mechanism

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1. Introduction

Ischemic stroke (IS) occurs as a result of cerebral ischemia and hypoxia caused by multiple factors, leading to irreversible ischemic necrosis or softening of brain tissue (1). Notably, IS has high incidence, mortality and disability rates, with ~6 million cases of IS-associated death and disability occurring globally each year, making it one of the main contributors to the global disease burden (2). Currently, the primary treatments for IS include administration of tissue plasminogen activator and mechanical thrombectomy; however, due to time limitations and the risk of secondary intracerebral hemorrhage, most patients fail to achieve optimal outcomes in recovery of neurological function and quality of life improvement (3). Therefore, exploring novel treatment methods is required.

Mesenchymal stem cells (MSCs) are multipotent stromal cells with promising therapeutic potential for ischemic brain injury via paracrine effects. MSC-derived exosomes (MSC-Exos), the major paracrine products of MSCs, may serve as a candidate therapeutic agent for IS through mechanisms such as inhibiting inflammatory responses, promoting angiogenesis, regulating ferroptosis and maintaining blood-brain barrier (BBB) integrity, leveraging their unique targeting, transport and content-processing characteristics (4). Moreover, as nano-sized cell secretions, MSC-Exos can traverse the BBB and mediate intercellular communication; therefore, they are considered potential therapeutic targets for a number of neurological diseases (5). However, exosomes obtained via different separation methods under varying culture conditions exhibit notable differences in content, purity and function (6). Furthermore, the efficacy of MSC-Exo monotherapy for IS is limited by low exosome yield, low brain bioavailability and its inability to remain at the lesion site for an extended period of time to exert an effect (7). In recent years, numerous studies have shown that combining MSC-Exos with microRNAs (miRNAs/miRs), traditional Chinese medicine, hydrogels, exosome pretreatment and electroacupuncture (EA) can enhance the efficacy of MSC-Exo monotherapy for IS (8,9).

Notably, combining MSC-Exos with various strategies for the treatment of IS is considered a focus of current research, and an in-depth exploration of the therapeutic mechanisms may aid the early diagnosis, treatment and prognosis of IS (10). The present study details the therapeutic effects of MSC-Exos on IS and systematically evaluates the mechanisms by which combining MSC-Exos with different optimization strategies enhances the efficacy of IS monotherapy. In addition, the progress of clinical research on MSC-Exos for the treatment of IS, obstacles in clinical translation and corresponding strategies are discussed, providing a reference for the treatment and scientific research of IS.

For the present review, a comprehensive literature search was performed using databases such as PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science (<https://www.webofscience.com/>) and ScienceDirect (<https://www.sciencedirect.com/>), and search terms including 'ischemic stroke', 'mesenchymal stem cells-exosomes', 'miRNA', 'drug' and 'programmed cell death', with the aim of exploring the mechanisms of MSC-Exo treatment for IS. The retrieval time range was set between January 2013 and June 2026. All retrieved literature was initially screened by title and abstract, followed by full-text screening.

Studies were included if they reported original research or they were review articles focusing on MSC-Exos for IS. The exclusion criteria were as follows: Conference abstracts, case reports, letters, non-English publications, animal studies irrelevant to IS and duplicate publications. Unsuitable literature was progressively excluded, and finally, qualifying literature was compiled and organized for use in the current review.

2. Pathogenesis of IS

IS is primarily caused by narrowing or occlusion of cerebral blood vessels, leading to cerebral ischemia and hypoxia, and subsequently resulting in brain cell death (11). The main causes of IS include the formation and rupture of atherosclerotic plaques, platelet adhesion and aggregation, and the activation of coagulation factors, ultimately leading to thrombus formation and vascular obstruction. In addition, embolisms caused by thrombi detaching from sites such as the heart or carotid arteries, which enter the cerebral circulation via blood flow and obstruct distal small arteries, can also lead to embolic events, namely IS (12).

Following the onset of IS, cerebral tissue initiates a robust inflammatory cascade, accompanied by neuronal apoptosis, autophagy and necrotic cell death (13,14). It has been reported that inflammatory factors, such as C-reactive protein and IL-6, serve important roles in the occurrence, development and prognosis of stroke (15). Oxidative stress is also one of the important mechanisms underlying stroke pathogenesis, and the excessive generation of free radicals has a causal relationship with subsequent neurological dysfunction caused by IS (13). During ischemia/reperfusion (I/R), free radical generation increases, causing lipid peroxidation of cell membranes and inducing cellular ferroptosis (16). Yu *et al* (17) investigated the role of neutrophil extracellular traps (NETs) in a middle cerebral artery occlusion (MCAO)-induced ischemic brain injury mouse model. The results indicated that pharmacological intervention inhibiting peptidyl-arginine deiminase type IV (PAD4) may constitute an effective strategy to reduce neuroinflammation, neuronal apoptosis and brain tissue damage, while enhancing long-term neurobehavioral function in mice. These findings support the pathological role of NETs in IS, suggesting that targeting PAD4 may be a potential direction for IS treatment (17). The pathogenesis of IS may also be associated with multiple risk factors, such as hypertension, diabetes, hyperlipidemia, smoking, alcohol consumption and genetic factors (18). Certain specific diseases (such as cancer) can also increase stroke risk by promoting a hypercoagulable state or vascular lesions (19). Fig. 1 schematically depicts the core pathogenetic mechanisms contributing to the onset and progression of IS.

3. MSC-Exo characteristics, extraction, identification and therapeutic potential in neurological diseases

MSC-Exo characteristics. In recent years, MSCs have demonstrated promising therapeutic effects in neurological diseases, such as IS, intracerebral hemorrhage, spinal cord injury and Alzheimer's disease (20,21). However, multiple limitations of direct MSC transplantation, including potential tumorigenicity, excessive cell dosage and microvascular embolism risk, have impeded their clinical translation. Research has revealed that

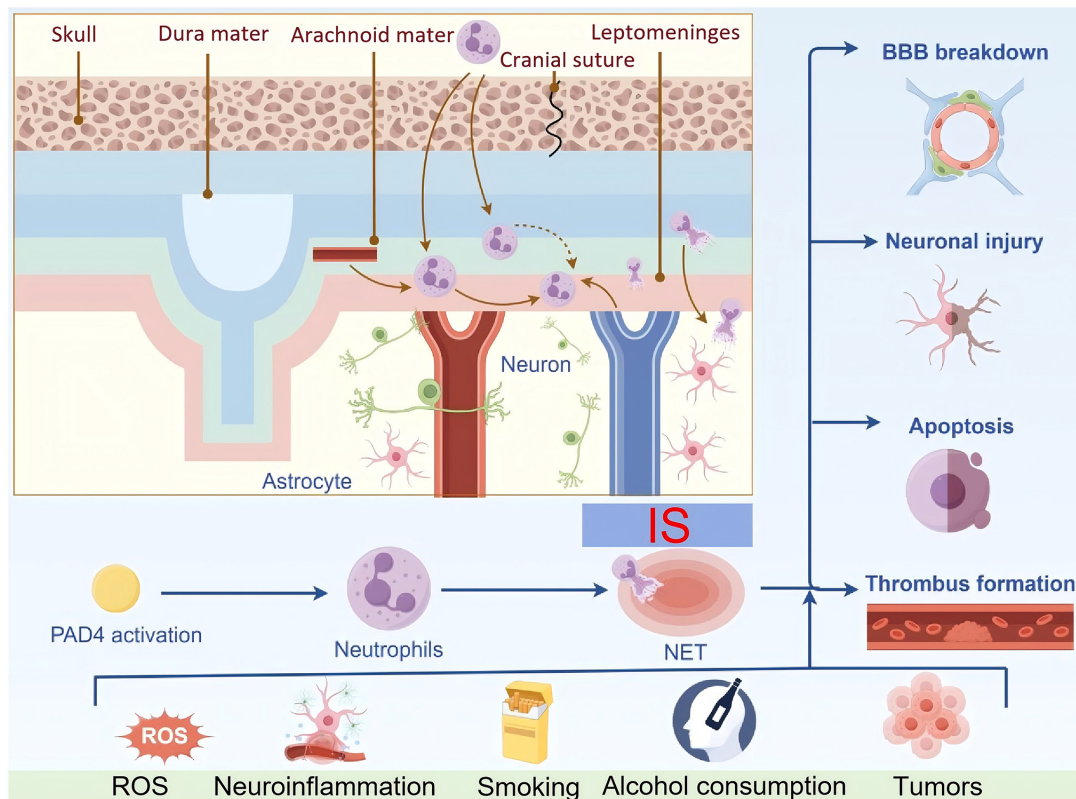


Figure 1. Pathogenesis of IS. The main box illustrates the anatomical layers extending from the skull to the brain parenchyma, including the skull, dura mater, arachnoid mater, cranial sutures and leptomeninges. Neutrophils infiltrate across the meninges into the cerebral parenchyma, where neurons and astrocytes are located. Triggered by the risk factors displayed in the figure: ROS, neuroinflammation, smoking, alcohol consumption and tumors, multiple pathological events take place, such as BBB breakdown, neuronal injury, apoptosis, ferroptosis and thrombus formation. Furthermore, PAD4-dependent NET formation facilitates the progression of IS. Created with Figdraw.com. BBB, blood-brain barrier; IS, ischemic stroke; NET, neutrophil extracellular trap; PAD4, peptidyl-arginine deiminase type IV; ROS, reactive oxygen species.

the paracrine effects of MSCs exert beneficial outcomes that are partly mediated by soluble factors, particularly exosomes (22). MSC-Exos possess marked biological properties, such as good biocompatibility, high stability, low toxicity and efficient exchange of molecular substances, and confer notable benefits on a variety of diseases and injuries including IS, myocardial infarction, cutaneous wounds and osteoarthritis, via bioactive cargos such as growth-associated proteins and miRNAs (23). In addition to possessing biological functions similar to those of parent MSCs, MSC-Exos have been widely utilized due to advantages such as their small volume, low immunogenicity, and ease of acquisition, storage and management. Compared with MSCs, the use of MSC-Exos in the human body offers several potential advantages. i) Nanoscale exosomes can freely traverse various biological barriers without affecting microvascular circulation (24); ii) while the number of transplanted MSCs rapidly decreases, the delivery of MSC-Exos can sustain their therapeutic effect within the body (25); and iii) because MSC-Exos do not exist as living cells *in vivo*, this may reduce the risk of tumor formation (although this does not imply that all tumorigenic risks are eliminated; MSC-Exos may still inherit the carcinogenic cargo from parent cells) (26). Beyond direct disease intervention, MSC-Exos can also serve as drug carriers, working synergistically with medications to achieve therapeutic efficacy (27). Therefore, studies across multiple disease models have indicated that MSC-Exos may serve as an alternative to traditional stem cell therapies.

MSC-Exo extraction. Due to the limitations of traditional separation methods in extracting MSC-Exos (such as cumbersome operation, low efficiency, and low exosome yield and purity), a number of novel separation methods have emerged, including ultrafiltration separation technology, integrated dual-filter microfluidic chips, nano-plasma-enhanced scattering, membrane-mediated exosome separation and chip-based exosome separation (6,28). Table I comprehensively summarizes the advantages and disadvantages of different extraction methods for exosomes. These mainstream separation methods exhibit notable differences in exosome purity, particle yield and impurity content. The differences in technical parameters partially explain the inconsistent therapeutic effects reported in different preclinical studies (28-30). Although advances in new technologies have improved exosome yield and purity to some extent, drawbacks such as high costs and stringent equipment requirements remain. Besides the extraction method, the cultivation method of MSCs, their surrounding environment and the source of MSC-Exos also affect MSC-Exo extraction (30-32). MSC-Exos originating from the bone marrow, adipose tissue and umbilical cord possess distinct cargo compositions, including unique profiles of proteins, cytokines and miRNAs, and therapeutic efficacies against ischemic injury (33); therefore, standardized selection of MSC sources and exosome isolation protocols is crucial for reducing experimental heterogeneity and promoting subsequent clinical translation.

Table I. Advantages and disadvantages of different extraction methods for mesenchymal stem cell-derived exosome.

Authors, year	Extraction method	Advantages	Disadvantages	(Refs.)
Kurian <i>et al</i> , 2021	Ultracentrifugation	High purity	Equipment dependency; the operation is cumbersome and takes a long time	(141)
Huang <i>et al</i> , 2025	Density gradient centrifugation	High purity and few impurities	Operation is complex and time-consuming	(28)
Luo <i>et al</i> , 2019	Ultrafiltration	Fast, easy to operate and scalable for production	Membrane adsorption leads to exosomes loss and a relatively low purity	(142)
Jiawei <i>et al</i> , 2022	Magnetic-activated cell sorting	High specificity and high purity	Low efficiency; the biological activity is easily affected by pH and salt concentration	(143)
Tiwari <i>et al</i> , 2021	Polymer-based precipitation method	Easy to operate; quick procedure	Low purity	(144)
Shirejini <i>et al</i> , 2022	Microfluidic chip technology	High portability and purity; low consumption of reagents and samples	High cost; complex equipment	(145)
Sidhom <i>et al</i> , 2020	Chromatography	Easy to operate; quick procedure; high purity	Small extraction volume; equipment requirements are high	(146)

MSC-Exo identification. MSC-Exo identification is a critical step to ensure the purity, characteristics and reliability of exosomes in research. The International Society for Extracellular Vesicles (ISEV) established minimum experimental requirements for exosome identification in 2018 (34). Regarding biochemical characteristics, the expression of specific positive marker proteins and the absence of contaminating negative markers must be verified. Specifically, at least two transmembrane proteins (any two from CD9, CD63 and CD81) and one cytoplasmic protein (TSG101 or Alix) should be tested. Additionally, calnexin or GRP94 (endoplasmic reticulum proteins), GM130 (Golgi apparatus protein), at least one mitochondrial protein [such as cytochrome *c* (Cyt-C)] and one nuclear protein (such as histone H3) must be detected and excluded as contaminants. Regarding individual characterization, transmission electron microscopy and nanoparticle tracking analysis are used to observe the peak size and morphology of exosomes. Exosomes typically appear as saucer-shaped or oval bilayer membrane structures with varying sizes, featuring a low electron density component in the center, clear boundaries and concentrated distribution. The peak particle size distribution ranges between 30 and 150 nm (35). In summary, the identification of MSC-Exos is an integrative process involving multiple parameters and diverse techniques. Only by comprehensively analyzing physical characteristics (such as size and shape), particle concentration and biochemical characteristics (for example, positive and negative marker proteins), and strictly adhering to the core principles of ISEV guidelines can it be reliably confirmed that the isolated nanoparticles are indeed exosomes and relatively pure, suitable for subsequent functional studies and application development.

Therapeutic potential of MSC-Exos in neurological diseases. To date, extensive research results have confirmed the broad application prospects of MSC-Exos in treating neurological diseases. For example, MSC-Exos have been shown to promote neurovascular remodeling in a rat model of MCAO via miR-133b transfer and to regulate neurite outgrowth (36). Furthermore, when injected intravenously into Alzheimer's disease model mice, MSC-Exos pretreated with hypoxia can effectively increase synaptic proteins (such as PSD-95 and Synapsin-I) expression; downregulate inflammatory factors, such as TNF- α and IL-1 β ; and upregulate anti-inflammatory factors, including IL-4 and IL-10, thereby improving learning and memory function (37). Furthermore, Xu *et al* (27) comprehensively summarized the mechanisms of MSC-Exos in treating spinal cord injury and optimization strategies to enhance therapeutic outcomes, highlighting that combinatorial interventions can further boost the anti-inflammatory and neuroprotective potency of MSC-Exos. In conclusion, MSC-Exos may effectively promote nerve injury repair and functional reconstruction. Compared with MSCs, MSC-Exos have achieved marked success in treating neurological diseases due to their unique advantages, such as improved safety, enhanced stability and greater targeting properties.

4. Mechanisms of IS treatment by MSC-Exos

Research has indicated that MSC-Exos are key effector molecules in the therapeutic action of MSCs, with their internal components exerting complex biological effects on other cells (38,39). MSC-Exos can cross the BBB and demonstrate unique advantages in IS treatment through mechanisms such as inhibiting inflammation, suppressing apoptosis, promoting angiogenesis and regulating ferroptosis (Fig. 2).

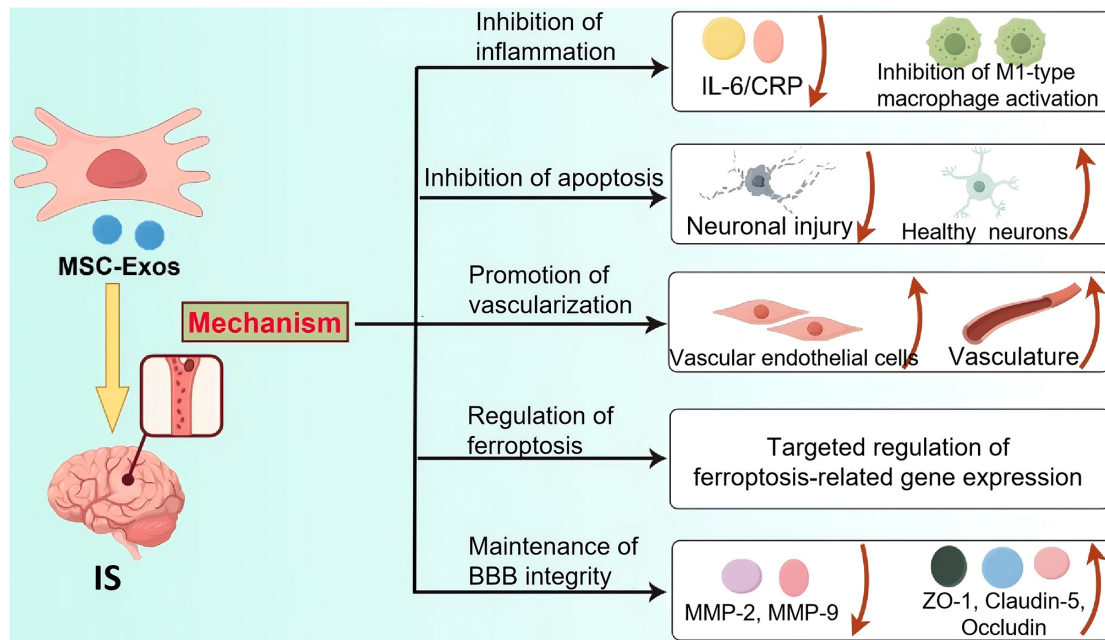


Figure 2. Mechanisms underlying the effects of MSC-Exos on the treatment of IS. i) Inhibition of the expression of inflammatory factors, such as CRP and IL-6, and suppression of the activation of M1-type macrophages. ii) Reduction in apoptosis of nerve cells and promotion of neuronal regeneration. iii) Promotion of vascular regeneration by increasing the number of vascular endothelial cells. iv) Targeted regulation of the expression of genes related to ferroptosis. v) Improvement in BBB integrity by inhibiting the expression of MMP-2 and MMP-9, and enhancing the expression of tight junction proteins (such as ZO-1 and Claudin-5). Created with Figdraw.com. BBB, blood-brain barrier; CRP, C-reactive protein; IS, ischemic stroke; MSC-Exo, mesenchymal stem cell-derived exosome.

Inhibition of inflammatory responses. Neuronal apoptosis in ischemic areas typically induces secondary immune/inflammatory responses, leading to brain tissue damage. Therefore, inhibiting inflammatory responses is an important neuroprotective strategy. MSC-Exos can improve post-IS functional deficits by modulating the activity of immune cells in the IS lesion area and alleviating local inflammation. For example, MSC-Exos can alleviate acute brain injury and microglial inflammation by inhibiting the CysLT2/ERK1/2 signaling pathway and inhibiting M1 macrophage activation (40). Additionally, MSC-Exos can reduce neuronal oxidative stress and inflammation levels by inhibiting ferroptosis and regulating the expression of glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (SLC7A11) (41,42). However, it has been reported that although MSC-Exos mitigate secondary injuries caused by local inflammatory responses through their immunomodulatory effects, it remains unclear as to whether MSC-Exos can promote the repair and regeneration of neural tissue in the lesion area (43).

Inhibition of apoptosis. Due to ischemia and hypoxia following IS, neurological function is impaired and neuronal apoptosis is enhanced. Numerous studies have confirmed that MSC-Exos attenuate brain injury by inhibiting apoptosis. Hao *et al* (44) reported that MSC-Exos markedly reduced the expression levels of Cyt-C and IL-1 β , and the apoptotic index in the neurons of IS rats. Zhao *et al* (45) discovered that when the Toll-like receptor 4 (TLR4)/NF- κ B signaling pathway was inactivated, the expression of the key apoptotic protease caspase-3 was also decreased. The underlying mechanism may involve MSC-Exos modulating the TLR4/NF- κ B signaling pathway to inhibit vascular endothelial cell apoptosis, thereby exerting anti-cerebral ischemic effects. MSC-Exos can also inhibit the activity

of antigen-presenting cells, downregulate the expression of B lymphocytes, T lymphocytes and macrophages at the IS site, participate in the regulation and balance of immune responses and provide a favorable microenvironment for subsequent neural regeneration, thus inhibiting apoptosis (46,47).

Promotion of angiogenesis. Following IS, angiogenesis is a critical step in restoring brain tissue function. Vessel blockage damages the structure of the vessels and alters their permeability, resulting in reduced normal blood supply and oxygen delivery. Notably, it has been shown that MSC-Exos facilitate cerebral tissue remodeling by promoting angiogenesis and neuronal survival (48). Doeppner *et al* (49) demonstrated that when treating adult male rats with MCAO using bone marrow MSC-derived exosomes (BMMSC-Exos), endothelial cell proliferation was markedly increased and a new capillary network was formed, indicating that BMMSC-Exos could promote post-stroke angiogenesis. In addition, Xin *et al* (36) administered MSC-Exos via tail vein injection 24 h after constructing an MCAO rat model, and the results showed a notable increase in vascular endothelial cell numbers, indicating that MSC-Exos can improve post-stroke vascular regeneration.

Regulation of ferroptosis. It has been shown that upon occurrence of IS, due to cerebral tissue hypoxia and reperfusion injury, iron ion levels are markedly increased, thus triggering ferroptosis (50). MSC-Exos protect neurons from damage by targeting and regulating ferroptosis-related pathways through carrying miRNAs, proteins and other bioactive substances. For example, miR-214-3p in MSC-Exos can alleviate neuronal injury in mouse models of cerebral ischemia by modulating the expression of ferroptosis-regulating genes, including GPX4 and SLC7A11 (41). Deng *et al* (51) also revealed that steroid receptor coactivator-3

in MSC-Exos could significantly inhibit lipid peroxidation and ferroptosis in hypoxic glucose-deprived neurons, and reduce infarct volume and brain tissue water content after cerebral ischemia, thereby improving neurological function. Although the specific mechanisms by which MSC-Exos regulate ferroptosis in IS have not been fully elucidated, exosome-mediated regulation of ferroptosis holds promise as a potential therapeutic target for future clinical treatment of IS.

Maintenance of BBB integrity. After IS, disruption of the BBB is an important factor leading to further brain tissue injury. In the early stage of IS, BBB disruption manifests as increased permeability and enhanced destruction, subsequently leading to increased penetration of immune cells and inflammatory substances (52). MSC-Exos can protect the integrity of the BBB by enhancing the expression of tight junction proteins (such as ZO-1 and Claudin-5), and reducing the penetration of inflammatory cells and immune factors. Li *et al* (53) demonstrated that adipose-derived MSC-derived exosomes (ADSC-Exos) could increase the distribution of ZO-1 and Claudin-5 in MCAO rat models, improve the BBB state and alleviate cerebral edema. In addition, MSC-Exos may reduce brain tissue edema and lesion size, lower the levels of blood-derived brain biomarkers (such as S100B and glial fibrillary acidic protein) and enhance BBB integrity by inhibiting the expression and enzymatic activity of MMP-9 (54).

Notably, IS can trigger multiple forms of programmed cell death, not limited to apoptosis and ferroptosis. I/R injury simultaneously activates novel cell death regulatory pathways, such as pyroptosis, necroptosis, PANoptosis, autophagy and cuproptosis. These distinct death pathways interact to amplify oxidative stress and neuroinflammation, collectively resulting in marked neuronal loss in the ischemic penumbra (55,56).

MSC-Exos are capable of successfully crossing the BBB and accumulating at the ischemic lesion site. In addition to the aforementioned effects of suppressing neuroinflammation, antagonizing neuronal apoptosis, regulating iron overload and lipid peroxidation-mediated ferroptosis, and promoting angiogenesis, they can also broadly regulate multiple programmed death pathways by carrying various miRNAs, cytokines and other active substances (55,57,58). Current basic research has also preliminarily suggested that they may improve copper-induced neuronal damage by maintaining mitochondrial homeostasis, although more experimental validation is required (59,60). In conclusion, the synergistic regulation of multiple programmed cell death pathways constitutes an important mechanistic basis for MSC-Exos in improving the prognosis of IS.

5. Different combination strategies for MSC-Exo treatment of IS

Although MSC-Exos show promising therapeutic efficacy in treating IS, they also possess drawbacks such as high heterogeneity, low yield, storage difficulties and rapid clearance *in vivo*, leading to issues including poor organ targeting and insufficient efficacy when used alone for IS treatment (61). Moreover, the mechanisms by which MSC-Exos regulate inflammation, promote angiogenesis and facilitate neuronal regeneration in IS treatment remain unclear. For example, it has been reported

that MSC-Exos can markedly promote functional recovery in MCAO model rats without reducing infarct volume (62). Therefore, combining MSC-Exos with innovative delivery systems and enhancement strategies has become a critical direction for overcoming the obstacles in IS treatment (Fig. 3).

miRNA regulation. Research has shown that a large number of miRNAs in MSC-Exos widely participate in oxidative stress, cellular apoptosis and immune-inflammatory injury after cerebral ischemia, and also serve important roles in vascular remodeling, neuronal regeneration and synaptic plasticity post-ischemia (63,64). Based on core detection indicators and experimental observation focuses from previous studies, the combination of MSC-Exos and miRNAs may promote functional recovery after injury by regulating microglial polarization, alleviating inflammatory responses, promoting angiogenesis and facilitating nerve regeneration. These mechanisms are closely interrelated and mutually reinforcing in the pathological process of ischemic brain injury and inherent biological cross-effects exist (Table II).

Regulation of microglial cell polarization. Numerous studies have indicated that miRNAs can be loaded and transported to specific target cells in the form of exosomes; thus, MSC-Exos carrying miRNAs possess the ability to regulate microglial activation and anti-inflammatory responses (65–67). MSC-Exos loaded with miR-223-3p have been reported to alleviate pro-inflammatory responses mediated by M1 polarization of microglia, reduce cerebral I/R injury and thereby accelerate functional recovery after IS (65). MSC-Exos loaded with miR-126 can also improve functional recovery in IS model rats by promoting neurogenesis and inhibiting microglial activation (66). Furthermore, MSC-Exos loaded with miR-30d-5p may prevent brain injury by inhibiting autophagy-mediated microglial polarization to the M1 phenotype (67). In addition, MSC-Exos can regulate microglia/macrophage polarization via targeted delivery of miR-21a-5p, protecting the brains of newborn mice from hypoxic-ischemic injury (68).

Reducing inflammatory responses. It has been reported that IL-6 is an important cytokine involved in pro-inflammatory responses that is closely associated with immune responses after IS (69). Ji *et al* (70) demonstrated that the concentration of serum exosomes, and the median levels of miR-9 and miR-124 in serum exosomes were notably elevated in patients with IS. In addition, the levels of miR-9 and miR-124 were positively associated with National Institutes of Health Stroke Scale (NIHSS) scores, infarct volume and serum IL-6 concentrations. These results suggested that serum exosomal miR-9 and miR-124 may be promising biomarkers for diagnosing IS and assessing the extent of ischemic injury. Xue *et al* (71) discovered that downregulation of miR-9 inhibited the transcription of inflammatory factors TNF- α and IL-6 by targeting and silencing silent information regulator 1 in the downstream NF- κ B signaling pathway, thereby alleviating I/R injury. Similarly, miR-138-5p within BMMSC-Exos has been shown to promote astrocyte proliferation in the I/R area by targeting lipocalin-2 and to suppress inflammatory responses to mitigate neuronal injury (72). Although inflammatory mediators serve as important targets of MSC-Exos at sites of inflammation, the specific mechanisms by which they promote

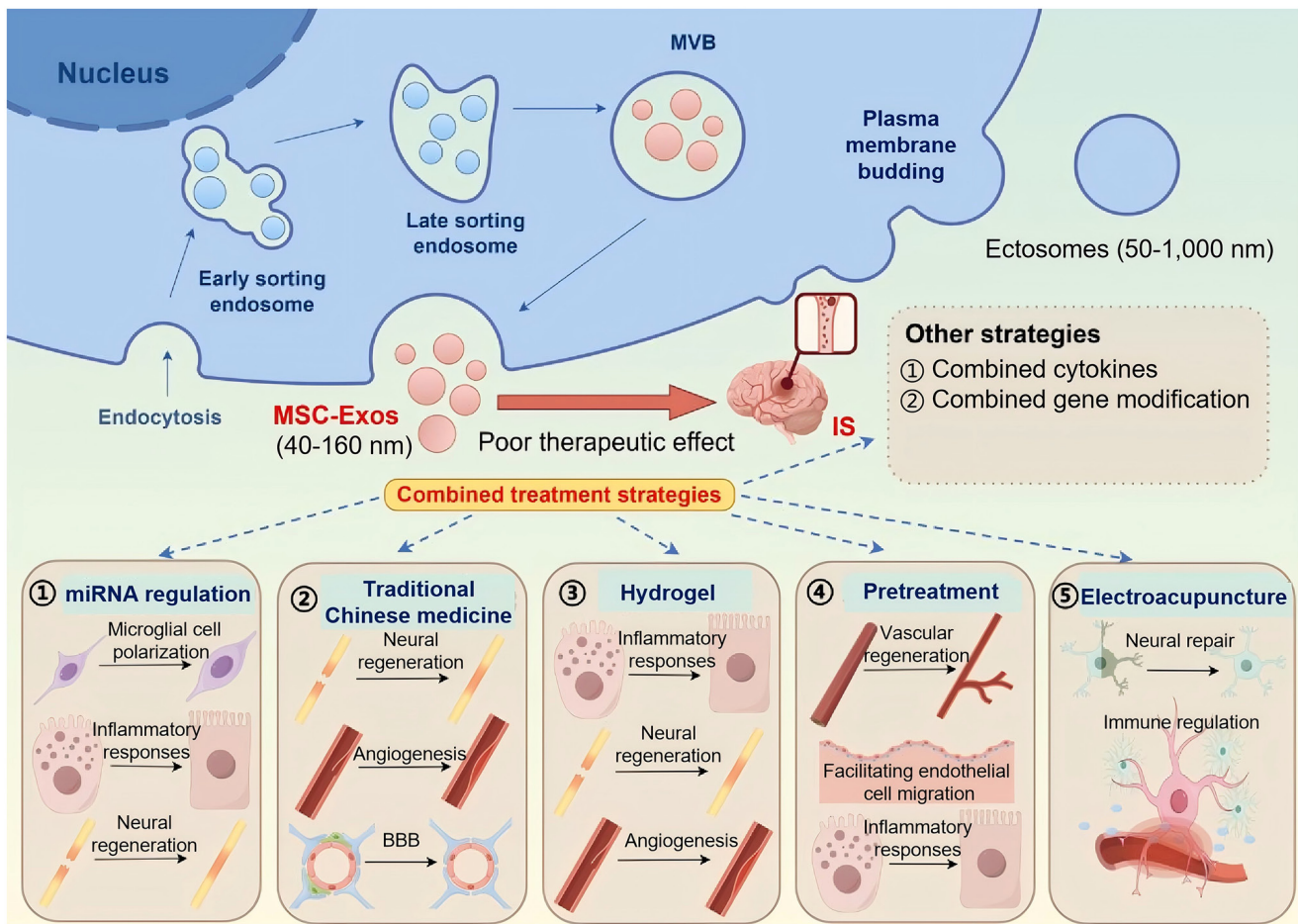


Figure 3. Combination strategies and mechanisms of MSC-Exos in the treatment of IS. i) MSC-Exos combined with miRNAs improves the therapeutic effect of IS by regulating microglial polarization, inhibiting inflammatory responses and promoting nerve regeneration. ii) MSC-Exos combined with traditional Chinese medicine improves the therapeutic effect of IS by promoting nerve regeneration, repairing vascular injury and regulating the blood-brain barrier. iii) MSC-Exos combined with hydrogel improves the therapeutic effect on IS by inhibiting inflammatory response, promoting nerve regeneration and repairing vascular injury. iv) MSC-Exos pretreatment improves the therapeutic effect on IS by promoting vascular regeneration, facilitating endothelial cell migration and inhibiting inflammatory responses. v) MSC-Exos combined with electroacupuncture improves the therapeutic effect on IS by inhibiting neuronal apoptosis and immune regulation. The five boxes at the bottom correspond to the five combinatorial strategies: miRNA regulation, traditional Chinese medicine intervention, hydrogel loading, pretreatment and electroacupuncture. Besides exosomes, extracellular vesicles also include ectosomes released from the plasma membrane. Combined cytokines refers to exogenous cytokine-loaded MSC-Exos for synergistic therapy; combined gene modification refers to genetically engineered MSC-Exos overexpressing target genes to enhance therapeutic potency. Created with Figdraw.com. IS, ischemic stroke; miRNA, microRNA; MSC-Exo, mesenchymal stem cell-derived exosome; MVB, multivesicular body.

an immunosuppressive microenvironment in damaged areas remain unclear.

Promoting neural regeneration. Studies have shown that the number of MSC-Exos in the infarcted hemisphere increases in a dose-dependent manner, and MSC-Exos are rich in various proteins and miRNAs related to neurogenesis (73). In acute IS (AIS), miR-126-3p in MSC-Exos can alter brain plasticity, increase neurite growth, and protect nerve cells from damage and apoptosis (74). Zhang *et al* (75) tracked fluorescence-labeled MSC-Exos and monitored axonal growth of neurons using time-lapse microscopy, revealing that miR-17-92-treated MSC-Exos activated the phosphatase and tensin homolog (PTEN)/mTOR signaling pathway in recipient neurons, further accelerating the growth of cortical neuron axons. Additionally, Hou *et al* (76) observed apoptosis in the neurons of MCAO model rats; however, overexpression of miR-29b-3p or silencing of PTEN reversed these changes. It has also been reported that the use of custom exosomes enriched with the miR-17-92 cluster can enhance neural plasticity and

functional recovery after IS, a mechanism potentially involving targeting PTEN to activate the PI3K/Akt/rapamycin/glycogen synthase kinase 3 β signaling pathway (77).

Regulating the function of brain microvascular endothelial cells. Brain microvascular endothelial cells interact actively with extracellular matrix proteins forming the basal lamina, pericytes, astrocytes and neurons, jointly forming the neurovascular unit (NVU). AIS leads to rapid NVU damage and impairs neurovascular coupling (78). It has been shown that BMMSC-Exos can markedly increase microvascular density after ischemic brain injury by upregulating miR-486 and activating the PTEN/Akt signaling pathway, thereby improving brain ischemic injury (79). Yang *et al* (80) demonstrated that ADSC-Exos can promote the migration and angiogenesis of brain microvascular endothelial cells under *in vitro* oxygen-glucose deprivation (OGD) conditions via the miR-181b/TRPM7 axis. Liu *et al* (81) reported that elevated levels of miR-122 in the blood can reduce neurological deficits and cerebral infarction area in a rat MCAO-induced IS model,

Table II. MSC-Exos combined with miRNAs treat IS through different mechanisms.

Authors, year	Exosome source	miRNA	Biological effect	Mechanism	(Refs.)
Zhao <i>et al</i> , 2020	BMMSC-Exos	miR-223-3p	Regulation of microglia polarization	Inhibition of microglia M1 polarization-mediated pro-inflammatory responses	(65)
Geng <i>et al</i> , 2019	ADSC-Exos	miR-126	Regulation of microglia polarization	Improvement of neurogenesis and inhibition of microglia activation	(66)
Jiang <i>et al</i> , 2018	ADSC-Exos	miR-30d-5p	Regulation of microglia polarization	Inhibition of autophagy-mediated microglia polarization to M1 type	(67)
Xin <i>et al</i> , 2020	MSC-Exos	miR-21a-5p	Regulation of microglia polarization	Regulation of microglia/macrophage polarization and attenuation of brain injury from hypoxic-ischemic injury	(68)
Deng <i>et al</i> , 2019	BMMSC-Exos	miR-138-5p	Reduced inflammatory response	Inhibition of lipocalin-2 to promote astrocyte proliferation after IS	(72)
Zhou <i>et al</i> , 2020	BMMSC-Exos	miR-126-3p	Promotion of nerve regeneration	Altered brain plasticity and increased neural protrusion growth	(74)
Zhang <i>et al</i> , 2017	MSC-Exos	miR-17-92	Promotion of nerve regeneration	Activation of PTEN/ mTOR signaling pathway	(75)
Hou <i>et al</i> , 2020	BMMSC-Exos	miR-29b-3p	Promotion of nerve regeneration	Activation of PTEN-mediated Akt signaling pathway to prevent hypoxic-ischemic injury in rat brain	(76)
Xin <i>et al</i> , 2017	BMMSC-Exos	miR-17-92	Promotion of nerve regeneration	Targeting PTEN to activate the PI3K/Akt/rapamycin/ glycogen synthase kinase 3 β signaling pathway	(77)
Bao <i>et al</i> , 2024	BMMSC-Exos	miR-486	Regulation of cerebral microvascular endothelial cell function	Activation of PTEN/Akt signaling pathway	(79)
Yang <i>et al</i> , 2018	ADSC-Exos	miR-181b	Regulation of cerebral microvascular endothelial cell function	Promotion of endothelial cell movement and angiogenesis via miR-181b/TRPM7 axis <i>in vitro</i>	(80)

This table descriptively summarizes representative individual preclinical studies, without quantitative synthesis, meta-analysis or effect-size evaluation. Study protocols, outcome indicators and detection methods are highly heterogeneous across publications. ADSC-Exo, adipose-derived stem cell-derived exosome; BMMSC-Exo, bone marrow MSC-derived exosome; IS, ischemic stroke; miR/miRNA, microRNA; MSC-Exo, MSC-derived exosome; MSC, mesenchymal stem cell; PTEN, phosphatase and tensin homolog.

by downregulating the expression of cell-adhesion-related genes such as vascular cell adhesion molecule 1 and intercellular cell adhesion molecule 1, which are involved in leukocyte extravasation, while maintaining vascular integrity.

In conclusion, MSC-Exos carrying miRNAs may serve as a means of intercellular communication after cerebral ischemia, providing novel insights into the pathogenesis and treatment of IS using MSC-Exos, and facilitating the

development of MSC-Exos-targeted vectors to improve the therapeutic outcomes for IS. Notably, most preclinical studies rely on young-rodent MCAO models with variable protocols for modelling, exosome preparation and outcome assessment, which may produce conflicting results. Furthermore, small sample sizes and incomplete blinding/randomization raise reproducibility risks. Collectively, the current evidence remains preliminary with low certainty.

Combination with traditional Chinese medicine. The combined application of MSC-Exos with traditional Chinese medicine ingredients also demonstrates favorable therapeutic advantages in the treatment of IS (82). Exosomes have emerged as ideal delivery carriers for herbal active ingredients to target lesion cells and exert therapeutic effects, making them a focus of research (82). Multiple studies have indicated that in the treatment of IS, MSC-Exos loaded with traditional Chinese medicine components such as curcumin, tetramethylpyrazine and gastrodin exert neuroprotective, angiogenic and BBB permeability-regulating effects, with efficacy superior to that of MSC-Exos or drug therapy alone (Table III).

Promotion of neuroprotection. It has been reported that the combination of BMMSC-Exos with tetramethylpyrazine can markedly enhance the neuroprotective effect against IS, a mechanism potentially associated with the inhibition of neuronal apoptosis (83). Furthermore, the combination of BMMSC-Exos with tetramethylpyrazine can notably improve pathological changes in IS, reduce infarct volume, and lower levels of neuronal apoptosis and oxidative stress (84). The combination of BMMSC-Exos with musk ketone has been reported to effectively improve neurological function and reduce infarct volume in MCAO model rats. Further research revealed that this combined strategy can downregulate the expression of pro-apoptotic proteins, such as Bax, and pro-inflammatory factors, including TNF- α and IL-1 β , while promoting the expression of the anti-apoptotic protein Bcl-2, thereby effectively inhibiting neuronal apoptosis and microglial activation in rats (85). Additionally, the combination of MSC-Exos with curcumin exhibits anti-lipid deposition, antioxidant and anti-inflammatory effects, protecting against IS by improving endothelial cell permeability and reducing oxidative stress levels (86). It has also been reported that Xionggu decoction enhances axonal regeneration in rats following BMMSC-Exos intervention in IS by regulating the miR-17-92/PTEN signaling pathway (87). This effect is achieved through the improvement of neurological deficits and brain tissue pathological damage, as well as the promotion of oligodendrocyte differentiation and myelin formation.

Promotion of angiogenesis. Ginsenoside Rg1 is a major active component extracted from ginseng roots. Due to its multiple biological functions, such as anti-inflammatory, immune-regulatory and angiogenesis-promoting effects, it is widely used in the treatment of neurological diseases (88). BMMSC-Exos modified with c(RGDyk) peptide, serving as a multifunctional carrier material, load ginsenoside Rg1 through co-incubation and have been reported to activate the PI3K/Akt pathway in a rat model of IS to promote the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1 α (HIF-1 α), thereby promoting angiogenesis and effectively reducing the volume of cerebral infarction (89). Li *et al* (90) demonstrated that the combination of human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-Exos) with Shennao Fuyuan decoction alleviates OGD/reoxygenation-induced damage to neural stem cells by promoting angiogenesis and inhibiting cell apoptosis, which may be associated with activation of the Wnt3a/ β -catenin pathway. In addition, compared with treatment with BMMSC-Exos alone, the combination of BMMSC-Exos with

Buyang Huanwu decoction shows stronger effects in inducing VEGF secretion by brain tissue endothelial cells and increasing vascular density in cerebral ischemic rats (91). In addition to these drugs, in the treatment of neurological diseases, catalpol can promote post-ischemic neurological recovery, protect the NVU and upregulate VEGF expression (92); however, to the best of our knowledge, whether the combination of catalpol with MSC-Exos is beneficial for angiogenesis after IS has not yet been reported in the literature and requires further exploration.

Regulation of the BBB. Studies have shown that single herbal medicines or prescriptions can regulate the BBB through multiple pathways, targets and effects, thereby exerting antioxidant effects, inhibiting neuronal apoptosis, improving cerebral blood flow and other functions. *In vivo* studies have confirmed that the combination of MSC-Exos and traditional Chinese medicine effectively regulates the permeability of the BBB after cerebral I/R injury, reduces the volume of cerebral infarction and markedly restores neurological function. Gastrodin can alleviate cerebral ischemic injury in mice by enhancing antioxidant and anti-inflammatory activity, and inhibiting the cell apoptosis pathway (93). Nan *et al* (94) demonstrated that gastrodin can increase the content of miR-20a-5p in circulating exosomes; these MSC-Exos may then penetrate the BBB and upregulate the expression of X-linked inhibitor of apoptosis protein (XIAP) in the ischemic hemisphere, inhibit neuronal apoptosis and promote the recovery of neurological function in MCAO model rats. Yao *et al* (95) confirmed that the combination of BMMSC-Exos with Qixiong Zuogui compound may markedly reduce the cerebral infarction area in aged MCAO model rats, improve neurological deficit scores and enhance cognitive function; it may also inhibit the expression of MMP-2 and MMP-9, promote the expression of the tight junction proteins ZO-1 and Occludin, and reduce BBB permeability. In addition, MSC-Exos, which are influenced by scutellarin, can protect homocysteine-induced rat microvascular endothelial cells by upregulating the expression of CD63 and BBB tight junction proteins (96).

Although the combination of MSC-Exos and traditional Chinese medicine exhibits certain advantages in treating IS, most existing preclinical evidence comes from a limited number of research teams and independent verification across different teams is still lacking. Almost all data originate from young-adult rodent MCAO models, and evidence from aged or comorbid animals is scarce. In addition, variability in herbal and exosome protocols hampers cross-study comparison and the pre-clinical evidence certainty remains low. Therefore, these encouraging experimental results should be interpreted with caution and more research is needed to translate them into clinical applications to confirm their reproducibility.

Combination with composite hydrogels. It has been reported that the *in vivo* therapeutic efficacy of natural MSC-Exos is limited by their short half-life, poor lesion accumulation and insufficient tissue targeting ability. Notably, hydrogels loaded with MSC-Exos have attracted widespread attention as an emerging therapeutic strategy in the fields of tissue engineering and regenerative medicine, and are regarded as an effective means to promote nerve regeneration and functional recovery in patients following a stroke (97,98). The good biocompatibility and adjustability of MSC-Exos combined

Table III. MSC-Exos combined with traditional Chinese medicines treat ischemic stroke through different mechanisms.

Authors, year	Exosome source	Drug	Biological effect	Mechanism	(Refs.)
Mao <i>et al</i> , 2023	BMMSC-Exos	Tetramethylpyrazine	Promotion of neuroprotection	Inhibition of neuronal apoptosis	(83)
Zhang <i>et al</i> , 2023	BMMSC-Exos	Tetramethylpyrazine	Promotion of neuroprotection	Inhibition of neuronal apoptosis and attenuation of oxidative stress levels	(84)
Chen <i>et al</i> , 2023	BMMSC-Exos	Musk ketone	Promotion of neuroprotection	Decreased expression of pro-apoptotic proteins and pro-inflammatory factors, and increased expression of the anti-apoptotic protein Bcl-2	(85)
Kalani <i>et al</i> , 2017	MSC-Exos	Curcumin	Promotion of neuroprotection	Improved endothelial cell permeability and reduced levels of oxidative stress	(86)
Liu, 2022	BMMSC-Exos	Xionggui decoction	Promotion of neuroprotection	Modulation of the miR-17-92/PTEN signaling pathway for axonal regeneration	(87)
Luo <i>et al</i> , 2023	BMMSC-Exos	Ginsenoside Rg1	Promotion of angiogenesis	Activation of the PI3K/Akt pathway and upregulation of VEGF and HIF-1 α expression	(89)
Li <i>et al</i> , 2023	hUCMSC-Exos	Shennao Fuyuan decoction	Promotion of angiogenesis	Modulation of the Wnt3a/ β -catenin pathway and attenuation of NSC injury	(90)
Yang <i>et al</i> , 2015	BMMSC-Exos	Buyang Huanwu decoction	Promotion of angiogenesis	Induction of VEGF secretion from endothelial cells and increased vascular density	(91)
Nan <i>et al</i> , 2023	MSC-Exos	Gastrodin	Regulation of the BBB	Upregulation of XIAP expression in the ischemic hemisphere inhibits neuronal apoptosis	(94)
Yao <i>et al</i> , 2024	BMMSC-Exos	Qixiong Zuogui compound	Regulation of the BBB	Inhibition of the expression of MMP-2 and MMP-9, and promotion of the expression of tight junction proteins ZO-1 and Occludin	(95)
Zhong <i>et al</i> , 2019	MSC-Exos	Scutellarin	Regulation of the BBB	Upregulation of CD63 and BBB tight junction protein expression	(96)

This table descriptively summarizes representative individual preclinical studies, without quantitative synthesis, meta-analysis or effect-size evaluation. Study protocols, outcome indicators and detection methods are highly heterogeneous across publications. BMMSC-Exo, bone marrow MSC-derived exosome; HIF-1 α , hypoxia-inducible factor-1 α ; hUCMSC-Exo, human umbilical cord mesenchymal stem cell-derived exosome; miR, microRNA; MSC-Exo, MSC-derived exosome; MSC, mesenchymal stem cell; NSC, neural stem cell; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor.

with hydrogels can effectively deliver exosomes to individual cells, thereby extending their retention time at the site of tissue injury (99).

Regulation of the inflammatory microenvironment. As aforementioned, the main pathogenesis of IS involves ischemia, oxidative stress, inflammatory response and neuronal apoptosis

pathways (100). The novel exosome-hydrogel combination therapy directly delivers exosomes to the injury site, providing a scaffold for tissue and nerve repair, thereby exerting anti-inflammatory, antioxidant, axon growth-stimulating and myelin-regenerating effects (101). Lu *et al* (102) confirmed that the combination of BMMSC-Exos with hydrogel materials can markedly improve neurological functional recovery

in rats with traumatic brain injury and can partially relieve the occurrence of neural inflammation. Hydrogels can also mimic different repair microenvironments after peripheral nerve injury, producing a synergistic effect with MSC-Exos to promote neurite outgrowth and neurological defect repair (103). Li *et al* (104) reported that when using a hydrogel loaded with 3D-MSC-Exos to treat AIS, a 30% Pluronic-F127 hydrogel had good thermosensitivity, was fluid at low temperatures and rapidly transformed into a soft gel near body temperature, and stably released MSC-Exos for >6 days. Notably, this combination can be safely injected into the ischemic core of MCAO model rats, effectively alleviating the inflammatory response in the ischemic hemisphere and promoting the recovery of neurological function in MCAO model rats.

Promotion of vascular and nerve regeneration. Functionally, MSC-Exos not only participate in immune regulation and homeostasis maintenance but also in blood coagulation, nerve regeneration and intercellular signal transduction. Zhang *et al* (22) developed a supramolecular hydrogel capable of effectively sustained release of exosomes. After application in an MCAO mouse model, it promoted nerve repair after stroke. Immunofluorescence staining indicated that the neurological function in MCAO model mice was markedly improved, and angiogenic and neuroregenerative capabilities were enhanced. In behavioral tests, motor coordination and balance abilities of these mice were notably improved and the number of apoptotic cells was also reduced. This demonstrates the efficacy of exosomes combined with hydrogels in promoting post-stroke neurological functional recovery. Furthermore, the latest research reports that hydrogels can precisely deliver bioactive molecules to specific regions of the brain while supporting the survival and proliferation of transplanted stem cell-derived exosomes, thereby improving neuroinflammation, vascular remodeling and sensorimotor functional recovery after IS (105). It has also been reported that injectable adhesive hydrogels composed of catechol-grafted hyaluronic acid, prepared via photocuring, can be combined with exosomes to achieve sustained exosome release, which may be used to improve neurological function and angiogenesis after IS (106).

In summary, the combined application of MSC-Exos and hydrogels represents a revolutionary advancement in the field of IS repair therapy. By addressing the critical obstacle of rapid clearance of exosomes *in vivo*, this approach achieves synergistic effects on neurovascular repair. Current research has transitioned from basic mechanistic exploration to clinical translation, with various innovative hydrogel systems (such as thermosensitive, adhesive and active interface materials) demonstrating unique advantages. It is worth noting that currently, almost all supporting data for hydrogel-assisted MSC-Exos treatment of IS come from rodent models, and such preclinical results cannot be directly applied to human stroke. A number of unresolved issues remain regarding intracranial hydrogel delivery for human IS, including the determination of safe injection volume, optimization of injection sites and assessment of *in vivo* biocompatibility. Risks such as local inflammation and mass effects that may occur after hydrogel implantation also need to be fully validated in large animal models before human trials can be initiated. Limited sample sizes cause reproducibility concerns and these findings cannot be directly extrapolated to humans. In the future, with the

development of engineered exosomes, smart responsive materials and personalized treatment strategies, this field is expected to break through the limitations of neural repair and provide novel solutions for the treatment of disabling IS.

Other combination strategies

Pretreatment. It is well known that hypoxia can activate HIF-1 α , thereby regulating the transcription of angiogenic genes, such as VEGF and VEGF receptors (VEGFRs), and promoting angiogenesis. Hu *et al* (107) reported that, compared with in a normoxia group, MSCs subjected to hypoxic pretreatment and transplanted into brain tissue showed increased survival and migrated towards the ischemic area. This mechanism may be related to the increased expression of CXCR4 chemokine receptor 4 on the surface of MSCs after hypoxic preconditioning. It has also been reported that hypoxic pretreatment of MSC-Exos can be used for IS treatment. Han *et al* (108) revealed that hypoxia-treated hUCMSC-Exos can enhance angiogenesis by regulating the VEGF/VEGFR signal. In addition, BMSC-Exos under low-oxygen conditions promote angiogenesis by activating the HIF-1 α /VEGF/JNK pathway (109).

In addition to hypoxic preconditioning, cholesterol lipid preconditioning of MSCs can enhance the release and survival capacity of MSC-Exos, exerting beneficial effects in stroke models through cerebral blood flow protection (110). Zhang *et al* (111) loaded cholesterol-modified miR-210 onto BMSC-Exos conjugated with RGDyk cyclic peptide; after intravenous injection, these targeted mouse brain tissues, thereby promoting VEGF expression and angiogenesis in the ischemic area. MSC-Exos pretreated with interferon- γ can also induce activated microglia to secrete fewer pro-inflammatory factors, effectively inducing oligodendrocyte differentiation and myelin formation, thereby promoting recovery from stroke (112). Ye *et al* (113) adopted human umbilical vein endothelial cells as an *in vitro* surrogate for cerebral vascular endothelial cells. Using a scratch-wound healing assay, it was demonstrated that infarcted tissue extract-preconditioned hUCMSC-Exos promoted the directional migration of endothelial cells compared with naïve hUCMSC-Exos. In the same study, it was also revealed that hUCMSC-Exos pretreated with infarcted tissue extracts could markedly improve the morphology and viability of endothelial cells, enhance vascular endothelial remodeling and promote neurological functional recovery after stroke (113). Lee *et al* (114) also demonstrated that MSC-Exos treated with brain extracts had therapeutic effects on rat cerebral ischemia models. They enhanced behavioral recovery and reduced the area of cerebral infarction by promoting angiogenesis, neurogenesis and anti-inflammatory effects.

Combination with EA. Studies have shown that EA can enhance the survival, migration and neural differentiation of MSCs, ultimately improving motor function after IS (115). The combination of MSCs and EA can balance pro-inflammatory and anti-inflammatory factors, thereby alleviating brain damage after IS (116). Kim *et al* (117) reported that acupuncture combined with MSC intervention could improve sensorimotor function in IS rats, reduce striatal atrophy caused by ischemia and promote neuronal proliferation. In addition, exosomes act as an important carrier of EA efficacy, and EA

can also regulate the expression and secretion of MSC-Exos. This combined treatment may more effectively regulate neurogenesis than monotherapy through synergistic regulation of brain-derived neurotrophic factor (BDNF), leading to improved therapeutic outcomes. Xu *et al* (118) revealed that EA combined with exosomal miR-210 could promote angiogenesis under hypoxic conditions via the HIF-1 α /VEGF/Notch1 signaling pathway. Moreover, induced pluripotent stem cell-derived small extracellular vesicles combined with EA could reduce infarct volume in mice with IS, improve motor and neural functions, and inhibit neuronal apoptosis. The mechanism may involve regulating IL-33/ST2-mediated inflammation and activating the synergistic neuroprotective effects of microglia and astrocytes against ischemic brain injury. Although both MSCs and MSC-Exos can be combined with EA to correct inflammatory states and exert neuroprotective effects, further research is needed to investigate the impact of factors such as the timing, frequency and intensity of EA intervention on therapeutic efficacy. Current indirect evidence has suggested a potential interaction between EA and MSC-Exos-related pathways; however, the underlying mechanistic links remain largely speculative and require further experimental verification (119). Moreover, this combined strategy is still in the early stages of research. Key parameters such as the dose-response relationship of stimulation, optimal frequency and intervention timing have not been systematically investigated.

Moreover, MSC-Exos can be loaded with exogenous proteins for brain delivery (120). Pigment epithelium-derived factor (PEDF) is a multifunctional protein with anti-inflammatory, antioxidant and neuroprotective properties (121). Huang *et al* (120) combined PEDF overexpression with ADSC-Exos to improve I/R injury by activating autophagy and inhibiting neuronal apoptosis. The results indicated that PEDF may serve a role in preventing exosome-mediated cerebral I/R injury by regulating apoptotic factors and promoting autophagy. Additionally, it has been shown that a novel delivery system, TAT peptide membrane and rabies virus glycoprotein co-modified MSC-Exos carrying a miR-15b-5p inhibitor, can break through the BBB to target the peri-infarct area, markedly inhibiting neuronal apoptosis by regulating the HTR2C/ERK signaling pathway and providing a new strategy for stroke treatment (122). Notably, most studies have only verified the overall neuroprotective effects of MSC-Exos, while evidence revealing dynamic signaling pathways, exosomal cargo sorting mechanisms and specific receptor-ligand interactions mediating biological functions remains limited. A number of studies have also used different stroke models in rodents, but the intervention parameters were inconsistent. Positive results are preferentially reported while neutral or negative data are seldom published, bringing notable reproducibility risks and low-quality pre-clinical evidence.

6. Current status and challenges of clinical translation

Clinical research progress and translational trends. Currently, exosome-based combination therapies have entered early-stage clinical trials. To the best of our knowledge, the ChiCTR2500099772 trial (registered at Chinese Clinical Trial Registry, <https://www.chictr.org.cn/showproj.html?proj=266284>), registered at The First Affiliated Hospital of Xiamen University (Xiamen, China), is the first to explore the safety and efficacy of hUCMSC-Exos nasal spray in post-stroke cerebral infarction following smoking-related diseases. This trial has employed a single-arm design with a planned enrollment of eight patients; the drug is administered via the nasal mucosa to bypass the BBB, and the primary endpoints are improvements in NIHSS and Modified Rankin Scale scores. An investigational stem cell product for chronic stroke developed by Jiuzhitang Maker (Beijing) Cell Technology Co., Ltd. has completed Phase II patient enrollment. The preliminary Phase I data, presented orally at the 2025 Wuzhen Health Congress, showed a 35% improvement in Fugl-Meyer motor score and 28% improvement in Barthel index (Jiuzhitang Maker Cell Technology Co., Ltd., 2025 Wuzhen Health Congress, Wuzhen, 2025). The conference proceedings remain unpublished, and the detailed quantitative data were only presented orally and have not undergone peer review. The company intends to submit an application for conditional marketing approval in 2026. Although some early clinical trials have reported encouraging preliminary results, these should not be over-interpreted. Current clinical evidence is limited to small-sample studies, and there is still a lack of safety and efficacy data from large-scale clinical verification, which restricts the routine clinical application of MSC-Exos in IS.

Clinical transformation obstacles and countermeasures. Although MSC-Exos exert notable neuroprotective effects against IS in preclinical experiments, the overall methodological quality and experimental rigor of current studies remain suboptimal, leading to multiple potential risks of bias and limiting clinical translation (123,124). First, a number of the included preclinical studies share common methodological flaws, including limited sample sizes, incomplete blinding and randomization procedures, and inconsistent exosome identification criteria, which may introduce potential bias risks and undermine the reliability of the experimental results. No unified optimal dosage has been established in either animal or human studies; notably, excessive dosing may trigger off-target inflammatory responses, whereas insufficient dosing fails to achieve satisfactory neuroreparative effects (125). Regarding administration routes, intravenous injection is the most convenient option, but intranasal and intracerebral administration exhibit higher brain targeting efficiency, accompanied by greater operational risks (126-128). The therapeutic time window is another critical factor; early intervention within hours of cerebral ischemia yields a better prognosis, whereas delayed administration during the rehabilitation phase primarily focuses on neuroplasticity (59).

In vivo biodistribution and pharmacokinetic profiles determine the retention time and efficacy of exosomes in the ischemic penumbra (129). Furthermore, standardized good manufacturing practice (GMP)-level production is a prerequisite for clinical trials (130). Differences in cell sources, culture conditions and isolation methods inevitably lead to batch-to-batch variability in exosomes (131). Almost all preclinical studies tend to report positive therapeutic effects, whereas negative or ineffective experimental data are rarely published, resulting in publication bias.

Table IV. Qualitative comparison of different strategies to enhance MSC-Exo therapeutic efficacy in ischemic stroke.

Strategy	Core advantages	Key limitations	Potential application contexts
miRNA-modified MSC-Exos	Targeted regulation of key pathological pathways (inflammation, neuroregeneration, vascular repair); high biological specificity; flexible cargo adjustment	Low <i>in vivo</i> stability of loaded miRNA; technical barriers for efficient loading; risk of off-target effects; mostly validated in rodent models	Mechanistic research; precise molecular intervention; future development of engineered extracellular vesicle therapeutics
Combination with traditional Chinese medicine	Synergistic anti-inflammatory, anti-oxidative and neuroprotective effects; multi-target regulatory characteristics; relatively low cost of herbal components	Complex composition of herbal extracts; undefined active ingredients; poor batch consistency; scarce large animal validation	Pre-clinical combinatorial exploratory research; candidate for adjuvant therapeutic schemes
Hydrogel-loaded MSC-Exos	Prolongs exosome retention at lesion site; provides physical support for neural repair; sustained local release	Invasive intracranial implantation risk; unresolved safety of intracranial hydrogel; problems with clinical translation; almost all data are from rodent studies	Local brain injury repair; pre-clinical exploration for post-stroke neural regeneration; not ready for routine clinical use
Parental cell pretreatment	Simple operational workflow; improves exosome bioactivity without complex genetic modification; enhances targeting to ischemic tissue	Unstable exosome yield after pre-conditioning; unclear optimal pretreatment parameters; large inter batch variation	Easy to implement pre-clinical optimization; low threshold modification for MSC-Exo products
Combined electroacupuncture	Non-invasive physical intervention; can modulate endogenous regenerative microenvironment; good clinical safety record for human patients	Difficult to standardize stimulation parameters; indirect synergistic mechanism; limited mechanistic understanding	Adjuvant rehabilitation therapy; combined use together with cell-derived extracellular vesicles intervention in stroke rehabilitation phase

This table provides a qualitative summary based on existing pre-clinical evidence. Direct head-to-head comparative studies among these strategies are currently lacking; therefore, quantitative comparison of therapeutic efficacy cannot be performed. miR, microRNA; MSC-Exo, mesenchymal stem cell-derived exosome.

Notably, the potential oncogenic risks cannot be ignored. MSC-Exos may encapsulate oncogenic proteins or oncogenic miRNAs derived from parent MSCs; these oncogenic cargoes can be transported to recipient cells, posing potential safety hazards (132). Therefore, strict quality assessment of the parent cell source is obligatory prior to clinical translation. There is a need to establish standardized GMP-level exosome preparation methods, a unified quality control system and strictly standardized *in vivo* experimental protocols to reduce bias and enhance the experimental rigor of future research.

7. Summary and prospects

IS is the most common type of stroke, and the time window for conventional reperfusion therapy is relatively narrow, making it difficult to fully repair neurological damage. Against these conditions, MSC-Exos have shown potential in IS treatment as an emerging therapeutic approach, leveraging their diverse neuroprotective effects. The therapeutic efficacy of MSC-Exos is not determined solely by their intrinsic nucleic acid, protein, lipid carrier contents or membrane substrate structure; exosomes themselves possess notable biological plasticity:

The culture microenvironment of parental stem cells can dynamically regulate exosome secretion levels, surface molecular markers, internal cargo composition and biological functions (133). Hypoxic preconditioning, inflammatory microenvironment stimulation, oxidative stress induction and cytokine intervention can all reshape exosome content, upregulate anti-inflammatory, antioxidant and neuroprotective active components, enhance exosome tropism toward ischemic brain tissue, and thereby achieve endogenous optimization of exosome efficacy without artificial modification. This inherent plasticity also provides a biological basis for the artificial engineering modification of exosomes (134).

Numerous studies have reported that MSC-Exos improve post-IS neurological functional deficits through mechanisms such as neuroprotection, promotion of angiogenesis, regulation of inflammatory responses and maintenance of BBB integrity. The present review systematically summarizes how various optimization strategies, such as miRNA regulation combined with MSC-Exos, traditional Chinese medicine intervention, hydrogel encapsulation, stem cell pretreatment and EA regulation, enhance the therapeutic effects of IS. However, a number of core controversies and knowledge gaps remain unresolved.

For example, details such as the dynamic activation patterns of signaling pathways, cargo sorting during exosome generation, and specific receptor-ligand recognition between exosomes and recipient cells remain unclear (135). Divergent research findings, such as ‘functional improvement but no reduction in infarct volume’, also complicate comparisons across different studies, suggesting that reliance solely on infarct volume metrics may underestimate the therapeutic potential of MSC-Exos (136). Notably, heterogeneity caused by varying cell sources and isolation methods leads to inconsistent therapeutic outcomes across different studies; optimized parameters for EA stimulation, administration routes for traditional Chinese medicine and delivery conditions for hydrogels remain undefined, and there is a lack of large-scale clinical validation and long-term safety assessment. In addition, few direct comparative studies exist between different combination regimens, and most preclinical studies provide only qualitative reports of therapeutic benefits, rarely offering standardized effect sizes suitable for comparing different combination strategies. Due to the absence of unified outcome measures and head-to-head experimental data, it is currently difficult to quantitatively evaluate the advantages and disadvantages of various combined intervention methods or to perform quantitative cross-comparisons based on the available data. Future research should focus on standardizing experimental designs, establishing unified quality control systems, promoting multi-center clinical trials and exploring the potential mechanisms of synergistic effects.

Engineering modification strategies based on exosome plasticity can markedly enhance the efficiency of exosome penetration across the BBB, their ability to target and accumulate at brain lesions and the overall therapeutic efficacy in stroke treatment. Current mainstream modification schemes mainly include four aspects: Pretreatment modification of parent cells, targeted modification of membrane surfaces, exogenous cargo loading and genetic engineering modifications. Current preclinical studies confirm that comprehensive strategies, including exosome surface engineering, combination with anti-inflammatory and neuroprotective drugs, and optimization of administration timing, can enhance the therapeutic effects of MSC-Exos against IS. Among these, targeted-modified exosomes combined with nasal administration have become one of the most promising combination therapies due to their brain-targeting performance and good safety profile (137,138). Kim *et al* (139) demonstrated that engineered MSC-Exos loaded with BDNF, prepared via non-destructive methods, can enhance neuropeptide levels and improve post-stroke recovery after intranasal delivery by promoting neuroregeneration. However, uniform dosage specifications, standardized GMP production processes, stable quality control systems and clear regulatory standards remain major obstacles hindering their widespread clinical application (123,124,131). Of note, the widely referenced Minimal Information for Studies of Extracellular Vesicles guidelines are voluntary expert consensus recommendations rather than mandatory regulatory standards and have not been universally implemented in extracellular vesicle research (140). These guidelines only provide general reporting frameworks and lack unified protocols adaptable to all preclinical experimental scenarios, thereby presenting inherent practical limitations. Therefore, the future development of standardized preparation

systems and research into low-cost, safe modification technologies will become key directions for exosome combination therapy in IS.

In conclusion, MSC-Exo combination therapy offers a novel paradigm for neurological functional recovery in IS. A qualitative overview comparing the combinatorial strategies is provided in Table IV. Through interdisciplinary integration of materials science, genetic engineering and physical therapy, the efficiency and accuracy of MSC-Exo therapy are achieving revolutionary breakthroughs. With the rapid advancement of regenerative medicine technologies, exosome-based combination therapy holds promise as one of the standard treatment approaches for IS neurological repair. If these combination strategies are integrated with traditional vascular recanalization therapies (such as thrombolysis and thrombectomy), they may reshape the landscape of stroke treatment and provide new options for comprehensive patient rehabilitation.

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Availability of data and materials

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Authors' contributions

YX conceptualized the review, designed its methodology, prepared the original manuscript, and reviewed and edited the manuscript. XZ and WZ contributed to the conceptualization of this review. JW and LZ designed the methodology, which referred to the strategy for literature retrieval, inclusion-exclusion criteria and framework construction of this narrative review. LL and ZZ performed the literature search and edited the manuscript. CC, YZ and ZH contributed to reviewing and editing the manuscript. JY conceptualized this review, administered the research project, supervised the whole study and revised the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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