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WORD COUNT	4675	TIME SUBMITTED	23-APR-2023 02:13PM
		PAPER ID	98998435

Name of Journal: *World Journal of Stem Cells*

Manuscript NO: 83096

Manuscript Type: MINIREVIEWS

Potential effects of stem cell exosomes regulating the inflammatory response in ischemic stroke treatment

Running title: Stem cell exosomes in stroke treatment

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Abstract

The high incidence and disability rates of stroke pose a heavy burden on society. Inflammation is a significant pathological reaction that occurs after an ischemic stroke. Currently, therapeutic methods, except for intravenous thrombolysis and vascular thrombectomy, have limited time windows. Mesenchymal stem cells (MSCs) can migrate, differentiate, and inhibit inflammatory immune responses. Exosomes (Exos), which are secretory vesicles, have the characteristics of the cells from which they are derived, making them attractive targets for research in recent years. MSC-derived exosomes can attenuate the inflammatory response caused by cerebral stroke by modulating damage-associated molecular patterns. In this review, research on the inflammatory response mechanisms associated with Exos therapy after an ischemic injury is discussed to provide a new approach to clinical treatment.

Key Words: Mesenchymal stem cell-derived exosome; MicroRNA; Inflammation; Ischemic stroke; Adipose-derived stem cell; Toll-like receptor

Chen N, Wang YL, Sun HF, Wang ZY, Zhang Q, Fan FY, Ma YC, Liu FX, Zhang YK. Potential effects of stem cell exosomes regulating the inflammatory response in ischemic stroke treatment. *World J Stem Cells* 2023; In press

Core Tip: Mesenchymal stem cell-derived exosomes (MSC-Exos) transplantation is a novel treatment method for ischemic stroke that exhibits certain achievements in trials. Here, we review the strategies developed for MSC-Exos in the neuroinflammatory response of patients with stroke and provide potential therapeutic targets. These methods provide new insights for the future clinical application of MSC-Exos in the treatment of ischemic stroke.

INTRODUCTION

Stroke is a common clinical disease that frequently occurs in middle-aged and elderly people and is a global public health problem with high disability and mortality rates; it ranks third in the list of diseases affecting human lifespan [1]. The main goals of stroke treatment are vascular recanalization and reduction of cerebral ischemic injury. At present, the main recanalization methods are intravenous thrombolysis and endovascular mechanical thrombectomy; however, owing to the restricted time window and various comorbidities, few patients can benefit from these procedures [2,3]. Increasing evidence has suggested that inflammatory cytokines promote the migration of immune cells to damaged tissues through the blood-brain barrier (BBB) after stroke, aggravating the inflammatory response and leading to nerve cell injury [4, 5]. However, the exact molecular mechanisms underlying the inflammatory response after stroke remain unclear, hindering the development of effective and specific treatments.

The effectiveness of stem cell transplantation, which can regulate the immune-inflammatory response and the permeability BBB in the treatment of ischemic stroke (IS) has been verified [6,7]. However, pluripotent stem cells are obstructed by the BBB and cannot effectively enter the central nervous system, leading to risks, such as tumorigenicity, thrombosis, and pulmonary embolism, limiting their clinical

application. It has been suggested that the therapeutic mechanism of mesenchymal stem cells (MSCs) may involve secreted exosomes (Exos) rather than the direct replacement of brain cells [8]. MSC-derived extracellular vesicles (MSC-EVs) possess the biological characteristics of cells and can penetrate the BBB, reduce the risk of tumors and pulmonary embolism, considerably improve therapeutic efficiency, and reduce complications, thereby having broad therapeutic prospects. MSC-derived exosomes (MSC-Exos) reduce inflammatory responses after stroke [9-12]. However, the specific mechanism by which Exos alleviate the inflammatory response after stroke has not yet been explored.

Therefore, in this review, we present the current progress in research on the unique biological characteristics of MSC-Exos and the specific mechanism of action of MSC-Exos in the neuroinflammatory response after stroke. This review aims to explore the role of Exos in the neuroinflammatory response in stroke and provide potential therapeutic targets, with the expectation of offering a reference for future clinical treatments.

PATHOLOGICAL CHANGES AFTER CEREBRAL ISCHEMIA

Brain cell death after stroke can lead to a series of pathological processes including cell energy failure, neuronal apoptosis, leukocyte infiltration, inflammatory immune responses, tight junction (TJ) protein breakage and degradation, BBB destruction, and increased permeability [13,14]. The main goals of IS treatment are to restore blood flow and improve functional outcomes as soon as possible [15]. In addition, methods of regulating immune inflammation and oxidative stress responses, anti-apoptosis, and promotion of angiogenesis and neurogenesis are of great significance for the treatment of cerebral apoplexy in ischemic and hypoxic injured brain tissues [16]. The BBB controls the inflow and outflow of biological substances necessary for metabolic activity and neuronal function in the brain; therefore, its structural and functional integrity is essential for maintaining the brain microenvironment. The BBB is mainly comprised of vascular endothelial cells, pericytes, the basement membrane, astrocytes, neurons, and

microglia, which exchange substances that connect the central and peripheral nervous systems. The mechanisms of BBB injury after stroke include modification of tight junction proteins, regulation of transporter expression, and inflammatory damage [17]. The intravascular inflammatory response marks the beginning of BBB disruption and leukocyte infiltration in ischemic brain tissue [18]. The inflammatory response after stroke is an important factor in BBB disruption and nerve cell edema, leading to damage to mental function and even death (Figure 1).

Microglia are the resident immune cells of the brain that polarize into different phenotypes (M1 or M2) [19]. M1 inflamed microglia lead to BBB dysfunction and vascular 'leakage,' whereas M2 microglia have inflammation-inhibiting, immune-regulating, tissue-repair, and damage-eliminating functions; they also protect the BBB [20]. Activated M1 microglia release the pro-inflammatory factors, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), which activate the nuclear factor kappa-B (NF-κB) inflammatory response of reactive astrocytes (A1s) and further amplify this effect [21]. Owing to inflammatory response stimulation, the structure of the neurovascular unit changes, which inhibits central nervous system restoration. This change in the microenvironment stimulates M2 microglia to initiate phagocytosis and secrete transforming growth factor-β (TGFβ), IL-4, IL-10, and the engulfment of immune cells, indirectly protecting against inflammation-induced BBB disruption [22]. M2 polarization promotes the release of anti-inflammatory cytokines and tissue repair, including neurogenesis, axonal remodeling, angiogenesis, and oligodendrogenesis [23, 24]. Activation of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) by microglia after stroke degrades the basement membrane and tight junction proteins, resulting in BBB disruption, leukocyte infiltration, and angioedema, thus aggravating brain injury [17, 25, 26]. Pericytes also release cytokines that play vital roles in maintaining the structural integrity of the BBB. Under pathological conditions, dysfunctional pericytes can cause basement membrane degradation or alter the neurovascular unit (NVU) coordination, leading to BBB instability [17]. In addition, BBB injury along with the activation of TGFβ signaling in astrocytes may be a mechanism to

disrupt NVU structure, as TGFβ overproduction affects the function of pericytes and vascular smooth muscle cells (VSMCs)[27, 28]. Microglial polarization is closely related to stroke progression; M1 microglia promote astrocyte differentiation to the A1 phenotype through a variety of signaling pathways, including the immune inflammatory response, angioedema, BBB disruption, neuronal apoptosis, and glutamate excitotoxicity, thereby exacerbating brain injury caused by IS [29, 30]. The inflammatory reaction of the nervous system is closely related to the polarization of microglia, pericyte-secreted factors, astrocyte differentiation, and leukocyte species. However, the underlying mechanism of action of Exos in the treatment of neuroinflammation in stroke remains unclear.

BIOLOGICAL CHARACTERISTICS OF MESENCHYMAL STEM CELL EXOSOMES

MSCs are pluripotent stem cells with self-renewal, differentiation, secretion, and homing properties. They were first detected in the bone marrow, where they are abundant and easily extractable, and are also found in dental pulp, umbilical cord, hemocytes, and adipose tissue, such as bone marrow MSCs, dental MSCs, umbilical cord-derived MSCs, adipose-derived MSCs, and hematopoietic stem cells [31, 32]. To overcome the problems with primary MSCs, human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and pluripotent stem cells (iPSCs), represent a promising solution [33]. MSCs can differentiate into lipogenic, chondrogenic, osteoblastic, endothelial, neural, and epithelial lineages, both *in vivo* and *in vitro* [34, 35]. MSCs can reduce inflammatory responses, release trophic factors to promote therapeutic effects, induce angiogenesis, promote neurogenesis, reduce infarct volume, and replace damaged cells *via* immunomodulation [36-38]. Extracellular vesicles secreted by MSCs can be divided into three types based on their size and intracellular origin: apoptotic bodies, microvesicles, and exosomes. Apoptotic bodies are ≥ 1,000 nm and microvesicles are 100–1000 nm in diameter. Exos (30–100 nm in diameter) originate from multivesicular bodies and are released by exocytosis, which is dependent on cytoskeletal reorganization, but independent of intracellular Ca^{2+} concentration [39,40]. When multivesicular bodies fuse with the cell membrane, exosomes are released from

the cells. Previously, these vesicles were considered waste products actively excreted by cells; however, studies have shown that exosomes have key functions, such as transmitting information between cells, tissue regeneration, and immune regulation [41] (Figure 2).

Exos are released upon fusion with the cell membrane and trigger the release of different cellular substances. Exos can carry the same bioactive substances as their source cells and are vital for information transmission between cells, such as immune regulation and promotion of cell migration, proliferation, differentiation, and matrix synthesis [42]. Moreover, the exogenous Exos of stem cells express specific surface markers (CD9, CD63, CD81, and CD92), express specific phenotypes of stem cells (CD29, CD44, CD73, and CD90)[43], and carry heat shock proteins (HSP) proteins (HSP8, HSP60, HSP70, and HSP9), signal transduction proteins, and multivesicular production-related proteins. More importantly, they can directly transfer bioactive molecules, including non-coding regulatory microRNAs (miRNAs), messenger RNAs (mRNAs), and proteins from donor cells to recipient cells. MiRNAs are short (approximately 22 nucleotides), single-stranded, non-coding RNAs transcribed in the nucleus by RNA polymerase II from one gene or between two different genes to regulate different cellular processes such as differentiation, proliferation, metabolism, inflammation, stress response, angiogenesis, and signaling pathways [44]. miRNAs mainly affect gene expression by degrading the corresponding miRNAs or suppressing translation [45]. Margaret et al. [46] showed that exosomal miRNAs participate in regulating inflammatory responses; miR-146a-containing Exos can inhibit inflammation, whereas miR-155-containing exosomes promote inflammation following exposure to the same inflammatory stimulus.

Exos contain a variety of active substances that form the basis for disease treatment. These bioactive substances carried by Exos can target specific cells for information transmission and enter the cytoplasm by fusion with receptor cell membranes or endocytosis, thereby changing the target cell function by transmitting proteins, lipids, and nucleic acids [47]. Exos can be effectively isolated from donor cells and protect their

contents from the external environment, ensuring the complete transmission of effective information [48]. Exos act as mediators that facilitate intercellular communication and influence the recipient cell activity by delivering content. DiR-labeled MSC-Exos were injected into a rat model of stroke via the caudal vein, and the *in vivo* tracer showed that Exos could penetrate the BBB and reach the brain tissue. The fluorescence signal peaked on the third day and then gradually decreased [49]. Junichi *et al* [50] also demonstrated that Exos can increase long-term neuroprotective effects after stroke, modulate peripheral immune responses, and increase angiogenesis and axonal dendritic remodeling. Therefore, the use of Exos for the treatment of neurological diseases has great potential [51, 52]. These results suggest that Exos is an important therapeutic target for the treatment of stroke (Figure 3).

MSC-EVS REGULATE INFLAMMATORY RESPONSE IN IS TREATMENT

The potential therapeutic mechanisms of stem cell Exos involve promoting dendritic and axonal growth, repairing nerves, and promoting angiogenesis through direct actions [53,54]. Through indirect action, it can promote the secretion of inflammatory factors by cells by exogenously producing Exos that appear to interact with recipient brain cells, thereby stimulating them to release their own Exos and playing a role in anti-inflammation and neurological repair. Transplantation of MSC-Exos improves inflammatory responses in IS, maintaining BBB function, decreasing brain edema, regulating energy metabolism, and promoting antioxidant, anti-inflammatory, and anti-apoptotic effects [55]. In IS, miRNAs are involved in a variety of cellular functions, such as injured tissue repair, remodeling, and different neuronal activities. Their target genes play a crucial regulatory role in the inflammatory process of post-ischemic reperfusion injury, which explains their potential use as therapeutic targets in IS and is the focus of Exos research [56,57]. According to current research, the main signaling pathways mediated by Exos after cerebral ischemia are as follows (Table 1).

NUCLEAR FACTOR KAPPA B (NF-KB) SIGNALING PATHWAY

The transcription factor NF-κB regulates many aspects of innate and adaptive immunity and plays an important role in the inflammatory response. It is also involved in the migration of immune effector cells to the inflammatory system, thereby allowing the secretion of pro-inflammatory cytokines [66]. Han *et al* [67] showed that MSCs-Exos protect MCAO-injured rats, possibly by regulating the AMP-activated protein kinase (AMPK) and JAK2/STAT3/NF-κB signaling pathways. NF-κB is a central inflammatory mediator responding to many immune receptors. NF-κB mediates the induction of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, in monocytes/macrophages [68]. Fann *et al* [69] confirmed the involvement of NF-κB signaling in the activation of pyrin domain inflammasomes following IS. Preconditioning MSCs with lithium modifies EV secretion patterns, enhancing the therapeutic potential of the derived EVs (Li-EVs) and significantly increasing the resistance of cultured astrocytes, microglia, and neurons to hypoxic injury compared with control and native EVs. Li-EVs reduce the abundance of post-hypoxic and post-ischemic TLR4 (leading to the NF-κB signaling pathway) and decrease proteasomal activity, which together contribute to reduced levels of poststroke encephalitis [70]. The miRNAs carried by Exos play a significant physiological role. Cai *et al* [58] confirmed that MSC-derived exosomal microRNA-542-3p (miR-542-3p) prevented ischemia-induced glial cell inflammatory responses by inhibiting TLR4. Interleukin-1 receptor-associated kinase 1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) may be parts of an NF-κB-induced negative feedback loop [71]. Zhang *et al* [59] found that injected Exos produced by human umbilical cord MSCs (HUMSC-Exos) enter the site of ischemic injury and be internalized by cells, both *in vivo* and *in vitro*. *In vitro*, HUMSC-Exos treatment attenuates microglial inflammation induced by oxygen-glucose deprivation (OGD) vitro *In vivo*, HUMSC-Exos treatment significantly reduced infarct volume, alleviated behavioral deficits, and improved microglial activation three days after transient cerebral ischemia. MiR-146a-5p from HUMSC-Exos can attenuate microglial-mediated neuroinflammation through the IRAK1/TRAF6 pathway and ensuing neurological deficits after IS. NF-κB signaling pathway activation is a 'master regulator' of inflammation and is associated with the generation of free

radicals and the activation of proteolytic enzymes and pro-inflammatory cytokines, playing an important role in regulating apoptosis after stroke [72,73]. Taken together, these results show that NF-κB signaling is essential for the regulation of brain tissue inflammasomes under ischemic conditions. In addition, MSC-Exos treatment decreased the activation of the NF-κB signaling pathway, thereby attenuating inflammasome expression and activation under ischemic conditions. These findings suggest that therapeutic interventions targeting neuroinflammasome activation may provide new opportunities for the treatment of IS.

NLRP DOMAIN-CONTAINING (NLRP3) SIGNALING PATHWAY

NLRP3 plays an important role in mediating the inflammatory responses during cerebral IS [74]. The NLRP3 inflammasome is a multiprotein complex comprising NLRP3 and pyroptosis-related factors (ASC and caspase 1)[75]. The NF-κB and mitogen-activated protein kinase (MAPK) pathways play a major role in the expression and activation of NLRP1 and NLRP3 inflammasomes in primary cortical neurons [69]. Bone marrow MSC-Exos (BMSC-Exos) can reduce brain infarct area and cerebral edema, thus improving neurological function. MSC-Exos can downregulate the expression of NLRP3 inflammasome and pyroptosis-related proteins on the surface of neurons [76]. Moreover, it improved the transition from M1 to M2 phenotype both *in vivo* and *in vitro*. BMSC-Exos relieve cerebral ischemia/reperfusion (I/R) injury by suppressing NLRP3 inflammasome-mediated inflammation and pyroptosis *via* modulation of microglial polarization [76]. Deepaneeta *et al* [77] came to similar conclusions by treating a rat MCAO model with intra-arterial injections of MSCs; the levels of NLRP-1 and NLRP-3 inflammasomes and their related components IL-1β, caspase-1, and ASC were significantly reduced. NLRP3 apoptotic bodies are involved in astrocyte and microglial polarization and are closely related to the development of the inflammatory cascade after stroke, and BMSC-Exos reduce the inflammatory response after stroke by inhibiting NLRP3 activation.

SIRTUINS (SITRS) SIGNALING PATHWAY

SIRTs are NAD+-dependent deacylases with multiple roles in energy metabolism regulation, cell survival, transcriptional regulation, inflammation, circadian regulation, and DNA repair [78]. SIRT-1 and SIRT-3 are both associated with the inflammatory response in stroke patients. Xin *et al* [12] used an *in vivo* male neonatal mouse model of hypoxic-ischemic (HI) injury and induced *in vitro* hypoxia-glucose deficiency, thus simulating microglial BV-2 cells to deliver miR-21a-5p (miR-21a-5p) as a therapeutic intervention through MSC-Exos. Treatment of BV-2 cells with MSC-derived extracellular vesicles (MSC-EVs) increased cell viability and miR-21a-5p levels, which were decreased after glucose-oxygen deprivation. In both *in vitro* and *in vivo* models of HI injury, the effects on microglial polarization and STAT3 phosphorylation decreased when miR-21a-5p levels were reduced in MSC-Exos. These results suggest that MSC-Exos attenuate HI brain injury in neonatal mice by delivering miR-21a-5p, which induces microglial M2 polarization by targeting STAT3. Adipose-derived MSC-derived miR-22-3p reduces infarct volume and apoptosis in a stroke model [79]. Sarmah *et al* [77] demonstrated that intraarterial MSCs increase SIRT-1 to inhibit the NF-κB pathway, reducing inflammasome signaling and apoptosis, thereby exerting a neuroprotective effect. SIRT1 may be an independent risk factor for cerebral infarction, and a high concentration of SIRT1 in cerebral infarction may be associated with disruption of the BBB [80].

CYSTEINYL LEUKOTRIENES (CYSLTS) SIGNALING PATHWAY

Cysteinyl leukotrienes (CysLTs), including leukotriene C4 (LTC4), leukotriene D4 (LTD4), and leukotriene E4 (LTE4), are derived from 5-lipoxygenase metabolites of arachidonic acid after cell necrosis and are effective mediators of inflammation [81]. The effects of CysLTs are mainly mediated by the CysLT1 and CysLT2 receptors (CysLT1R and CysLT2R), which are active in various cell types during pathological brain injury. CysLT2 is expressed in the cerebral cortex, hippocampus, substantia nigra, and lateral ventricle [82]. Zhao *et al* [60, 61] showed that the overexpression of miR-223-3p (miR-223-

3p) in MSC-Exos can reduce MCAO-induced infarction, improve neurological deficits, and promote learning and memorization. MiR-223-3p inhibits the expression of pro-inflammatory factors and promotes the secretion of anti-inflammatory factors in the ischemic cortex and hippocampus. Western blotting and quantitative real-time PCR analyses also showed that exosomal miR-223-3p reduced the mRNA and protein expression of CysLT2R *in vitro* and *in vivo*. Exosomal miR-223-3p from MSCs alleviated cerebral I/R injury by inhibiting the pro-inflammatory response mediated by M1 polarization of microglia, which may be related to the inhibition of CysLT2R by exosomal miR-223-3p.

LIPOCALIN-2 (LCN2)

LCN2, a 25 kDa protein, is a neutrophil gelatinase-associated protein that affects different cellular processes during stroke. The pro-inflammatory mediator LCN2 plays a key role in ischemia-reperfusion injury [83]. Genetic or pharmacological inhibition of these pro-inflammatory mediators (iNOS, IL-6, CCL2, and CCL9) provides neuroprotection against stroke and reduces the expression of inflammatory factors by down-regulating LCN2 [43]. Deng *et al* [43] used a mouse MCAO model to explore the effects of BMSC-derived exosomal miR-138-5p in IS *in vivo*. Overexpression of MiR-138-5p promoted cell proliferation and inhibits apoptosis of OGD-damaged astrocytes, accompanied by decreased expression of inflammatory factors. This was achieved by downregulating LCN2, and the expression of LCN2 protein was subsequently detected by western blotting. More importantly, BMSCs attenuated neuronal injury in IS mice by delivering miR-138-5p to astrocyte Exos. Therefore, the exogenous exosomal miR-138-5p from BMSCs promotes astrocyte proliferation and inhibits the inflammatory response after IS by targeting LCN2, thereby reducing neurological impairment, which may provide a new target for IS treatment.

MAT2B

Methionine adenosyl transferase (MAT) is an enzyme involved in cell cycle regulation. Mammals have three major MAT genes: MAT1A, MAT2A, and MAT2B [62]. TNF-induced activation of MAT2B promotes tumor growth through the NF-κB pathway in hepatoma cells [83]. MiR-21-3p antagomir can control the inflammatory response by inhibiting NF-κB signaling; these functions of miR-21-3p are exerted by directly targeting MAT2B [84]. This interaction forms the basis of the function of miR-21-3p/MAT2B in regulating inflammation. Li *et al* [62] found that miR-21-3p expression was elevated in the MCAO model, and the inhibition of exogenous adipose-derived stem cell Exos miR-21-3p could inhibit the expression of MAT2B in neural cells, thereby improving the BBB status and inhibiting apoptosis and inflammatory responses. MiR-21-3p antagomir could inhibit the expression of pro-inflammatory cytokines (TNF-α, IL-1β, and IL6) and promote the expression of anti-inflammatory cytokines (IL-10). Thus, miR-21-3p can protect neural cells by inhibiting the expression of MAT2B, inhibiting apoptosis, and inflammatory responses.

TOLL-LIKE RECEPTORS (TLRS)

Evidence suggests that TLRs play important roles in the development of ischemic brain injury in adults [85]. TLRs, comprising 13 members (TLR-1 to TLR-13), are type 1 integral membrane proteins responsible for detecting invading pathogens and initiating immune responses [86, 87]. The microglial TLR pathway is activated following cerebral ischemia and inhibition of TLR signaling by exosomal miR-26a-5p decreases cholesterol 25-hydroxylase protein expression, which in turn inhibits microglial M1 polarization and relieves nerve injury after brain injury [63]. The gene expression of many inflammatory mediators, such as TNF-α, IL-1, and IL-6, can reduce the development of nervous system inflammation by inhibiting TLR4 transduction pathway downregulation [88]. Upregulation of miR-326 attenuates IL-10, IL1β, and TNFα pro-inflammatory cytokine expression in response to lipopolysaccharide stimulation by targeting TLR4 [89]. TLR5 activates NF-kB and MAPK pathways that regulate the transcription of genes encoding immune mediators [90]. Qiao *et al* elucidated that TLR5

downregulation is accompanied by alleviated neurological deficits, reduced infarct volume, and reduced edema after IS [91]. Li *et al* [64] validated that BMSC-Exos can improve neurological function and pathological changes, decelerate neuronal apoptosis, and reduce inflammatory factors in MCAO rats. Exosomal miR-150-5p from BMSCs mitigates cerebral I/R injury by inhibiting TLR5 expression. These studies showed that TLRs and their related miRNAs are associated with inflammation after IS.

INFLAMMATORY MEDIATORS

Dabrowska *et al* [92] transplanted human bone marrow stem cells and their secreted Exos into a rat model of local brain injury. The results showed that monocyte chemoattractant protein-1 (MCP-1) expression increased locally after brain injury, whereas MCP-1 expression decreased in the transplanted HUMSCs and Exos groups. In addition, they observed that the infusion of pro-inflammatory cytokines and chemokines with HUMSCs or EV in rats with untreated focal brain injury was associated with reduced microglial/macrophage and astrocyte activation. MSC-Exos therapy can reduce the expression of inflammatory cytokines TNF-α and IL-6, increase the expression of cytokines IL-4 and IL-10, and reduce brain injury. Exos from stem cells can enhance the activation of CD4 + and CD8 + lymphocytes, decrease the number of dendritic cells, regulate peripheral immunosuppression caused by stroke [9], and pass antigens to the immune system through the BBB [93]. IL-4, CD206, and IL-10 are markers of M2 microglial secretion, whereas TNF-α, IL-6, and iNOS are markers of M1 microglial secretion [94]. Yang *et al* [58] found that the MCAO model also verified that Exos intervention reduced the infarct volume and promoted the polarization of microglia to M2 phenotype. These results show that adipose-derived stem cell Exos can prevent stroke by shifting microglia from an M1 to M2 phenotype in the hippocampus. [65].

MICROGLIAL POLARIZATION

Microglia are macrophages of the central nervous system and are important components of innate and adaptive immune responses [95]. The microglial M1 type can secrete pro-inflammatory factors, whereas the M2 type can secrete anti-inflammatory factors; therefore, the fact that MSCs and Exos can promote the polarization of microglia to M2 is notable for the treatment of IS. The M2 type protects nerve cells mainly by engulfing debris and promoting the repair and regeneration of brain tissue. In contrast, inflammatory factors of the M1 phenotype aggravate post-stroke symptoms. Therefore, the microglial response after stroke is an important prognostic factor [96]. Different miRNAs transported by Exos contribute to the differentiation of microglia into distinct phenotypes. Increased levels of miR-124-3p in microglial Exos promote M2 microglial polarization, reduce brain damage, and improve stroke outcomes [97]. Adipose-derived MSCs (ADMSCs) participate in the repair process of tissues through paracrine effects after relieving nerve injury; ADMSCs have similar biological characteristics to MSCs. Stimulation of AMSC-derived Exos with inflammatory factors was found to convert M1 microglia into M2 microglia, suggesting that AMSC-derived Exos promote microglial polarization by activating pro-inflammatory microenvironment signals [98]. miRNAs are critical regulators of genes involved in various biological processes; miR-146a-5p-enriched BMSC-Exos directly target IRAK1 and nuclear factor-activated T cell 5 (NFAT5), which contributes to inflammatory responses and polarize M1 microglia/macrophages [99]. Exos containing miR-216a-5p, miR-124, miR-155, miR-182, miR-17-5p, miR-30d-5p, and miR-223-3p were found to promote microglial M2 polarization [65, 100–103]. BMSC-Exos promote microglial polarization from M1 to M2, inhibit inflammation-related signaling pathways, and reduce endothelial cell injury and neurological impairment caused by IS [104–106]. Although astrocytes may play a role in brain inflammation, little is known about their role in stroke pathology [107].

ACTIVATED TREG CELLS

Changes in Treg cell numbers and function after stroke are accompanied by a decrease in immunosuppressive function, which affects stroke prognosis [108]. The

immunosuppressive function of Tregs is largely impaired during stroke and Treg-derived anti-inflammatory factors, including transforming growth factor-beta (TGF-b) and interleukin-10(IL-10), are reduced [109, 110]. MiRNAs delivered by stem cell exosomes can induce anti-inflammatory polarization as important regulators of Treg homeostasis and function [111, 112]. MSC-Exos induce anti-inflammatory IL-10 and TGF-β transcription, attenuate pro-inflammatory factors IL-1β, IL-6, and TNF-β, and inhibit the differentiation and activation of Tregs [113, 114]. Furthermore, MSC-Exos are absorbed by endothelial cells, impair T-cell function by inhibiting T-cell proliferation *in vitro*, and increase endothelial cell proliferation, migration, and capillary formation in a dose-dependent manner [115]. Chen *et al.* [116] showed that the intravenous injection of MSC-EVs reduced neurological deficits, cerebral infarct volume, brain edema, and neuronal injury in young and old mice. The neuroprotective and anti-inflammatory effects of MSC-EVs were demonstrated through a decrease in leukocyte and, specifically, polymorphonuclear neutrophil, monocyte, and macrophage infiltration in the cerebral ischemic areas of aged mice. In addition, MSC-EVs significantly decreased the number of monocytes and activated Tregs. The expression and phosphorylation of signal transducer and activator 1 (STAT1) are increased in mice with miR-146a deficient Tregs, and miR-125a is involved in the differentiation of Tregs [117]. Exosomal miR-16 and miR-21, derived from bone marrow stem cells, can increase the production of Tregs and exert anti-inflammatory effects [118]. Although breakthroughs have been made in elucidating the working mechanism of Tregs over the past decade, the mechanism by which this minor population of peripheral immune cells has a significant beneficial effect after stroke injury remains largely unknown [108].

CONCLUSION

Brain injury after stroke is a complex pathological process. This review summarizes the recent studies on the mechanism of action of MSC-Exos in regulating inflammatory responses during IS treatment. It was concluded that MSC-Exos regulate microglial polarization through various pathways such as NF-κB, NLRP3, and STATs, indicating

that microglial M1 to M2 phenotype polarization is closely related to the inflammatory response after IS.

However, some essential questions remain unanswered. Stroke-induced brain injury involves multiple mechanisms that cannot be explained by a single one. Immune inflammation plays a crucial role in this process, especially the NF-KB, NLRP, and other signaling pathways. After immune inflammation, microglia, leukocytes, and other inflammatory cells are activated and release many pro-inflammatory factors. Additionally, nerve cells are affected by varying degrees of damage caused by ischemia and hypoxia after stroke.

However, these studies have some limitations. MSC-Exos can mediate different signaling pathways to reduce inflammatory responses after stroke in animal models. However, these results have not been translated into clinical practice. Most studies have focused on exosomal miRNAs, indicating that they play an important role in regulating cellular functions. However, research on other bioactive molecules contained in Exos, such as miRNAs, mRNAs, and proteins, is limited. This does not mean that this mechanism of action of miRNAs can explain how Exos attenuate post-stroke inflammation. The dosage, mode of administration, and duration of action of the Exos were elucidated. Exos are considered ideal biomarkers and drug delivery vehicles, with great potential for overcoming the limitations of stem cell therapy [119]. The use of Exos as drug-loaded systems will facilitate breakthroughs in the research and development of targeted drugs for clinical treatment. Moreover, new directions and methods will be provided for stroke treatment.

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ACKNOWLEDGEMENTS

We would like to thank Editage (www.editage.com) for their linguistic assistance during the preparation of this study.

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