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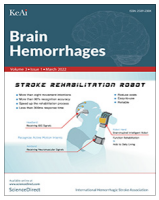

Review article

Effect of mesenchymal stem cell-derived exosomes on the inflammatory response after stroke

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ABSTRACT

Stroke, characterized by sudden onset and significant mortality rates, represents a critical challenge in effectively treating neuroinflammation to improve treatment efficacy. In this context, mesenchymal stem cell (MSC)-derived exosomes have attracted significant attention in scientific research due to their diverse cellular origin, tiny size, and huge number of bioactive molecules. Recent studies have shed light on the remarkable potential of MSC-derived exosomes to not only suppress the inflammatory responses of microglia and astrocytes, but also enhance their neuroprotective functions. Moreover, these exosomes have demonstrated a remarkable ability to modulate various immune cells and inflammatory mediators, thereby exerting profound mitigating effects on neuroinflammation. Through a thorough examination of the role and underlying mechanisms of MSC-derived exosomes in mitigating neuroinflammation after stroke, this review aims to provide comprehensive information and recommendations for the development of innovative therapeutic strategies aimed at significantly improving the treatment of stroke.

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Introduction

Over the past two decades, ischemic stroke has surged to become the second leading cause of mortality globally, with approximately 75 % to 85 % of all stroke cases attributed to its ischemic form.[1] Despite this alarming prevalence, the clinical arsenal for treating ischemic stroke remains limited to tissue plasminogen activator (tPA), which must be administered via injection, and thrombectomy. However, therapeutic efficacy tPA is confined to a narrow window of just 4.5 h for rescuing ischemic brain tissue. Given the restrictive time constraints associated with thrombolytic therapy, a substantial portion of patients remains without access to effective interventions.[2] Thus, the imperative to seek out alternative pharmacological agents capable of effectively managing ischemic stroke is underscored as a matter of utmost importance.

The cornerstone of treating ischemic stroke lies in initiating treatment promptly following the onset of symptoms to minimize neuronal apoptosis.[3] Neuroinflammation and oxidative stress have consistently been identified as key contributors to inducing neuronal apoptosis.[4–5] When neurons undergo apoptosis in the ischemic region, it often sets off secondary immune and inflammatory responses, characterized by the activation of glial cells and the production of cytokines.[6] Consequently, strategies aimed at inhibiting the activation of glial cells and regulating the balance of cytokines emerge as pivotal approaches for promoting neuroprotection.

Mesenchymal stem cells (MSCs) have garnered extensive attention from researchers due to their diverse capabilities, including promoting blood vessel formation, preventing cell death, and regulating immune responses, particularly in addressing ischemic stroke.[7] Among the components of MSC therapy, MSC-derived exosomes (MSC-Exo) have emerged as pivotal agents.[8] Exosomes, minute vesicles secreted by cells, possess the remarkable ability to penetrate the blood–brain barrier, rendering them promising therapeutic targets for various neurological conditions (Fig. 1).

The process of MSC-exo formation involves several key steps:

- Endosomal System Involvement: The process begins in the endosomal system of the cell. Early endosomes are formed by the inward budding of the plasma membrane, which then mature into late endosomes. During this maturation process,

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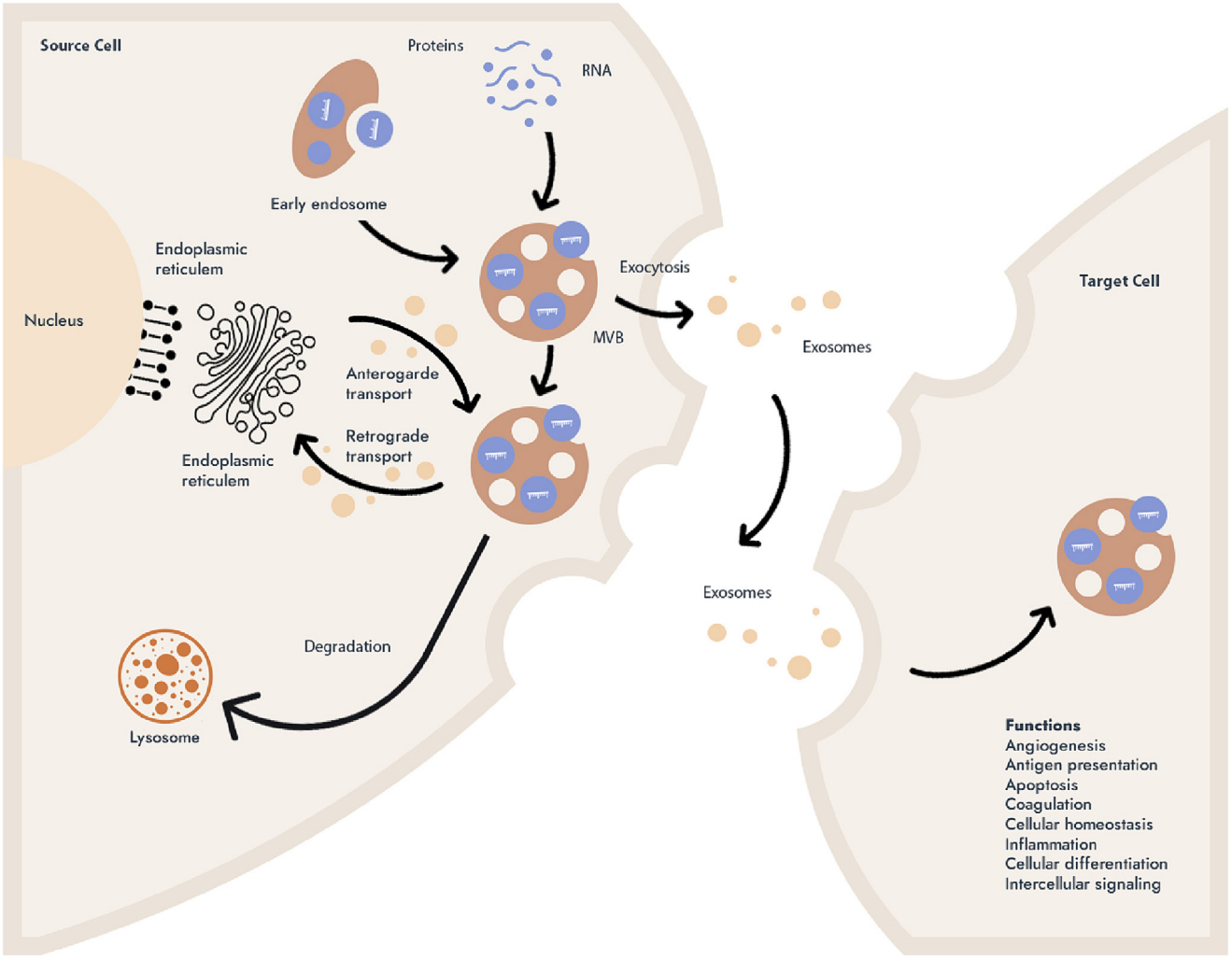

Fig. 1. Endosomal formation of exosomes involves the creation of multi-vesicular bodies (MVBs) within endosomes. These MVBs transport various molecules, including RNA and proteins, with some of these cargos being selectively incorporated. The fate of MVBs can diverge; they may either be broken down by lysosomes or fuse with the plasma membrane to release their contents into the extracellular environment. Upon release, exosomes can interact with and activate various membrane receptors on target cells. Alternatively, they can be taken up by cells, with their contents discharged internally. Through these interactions, exosomes have the potential to influence a wide range of physiological processes.

small vesicles form inside the endosomes by inward budding into the lumen of the endosome. These vesicles are called intraluminal vesicles (ILVs).

- Multivesicular Body Formation: As more ILVs accumulate, the late endosome becomes a multivesicular body (MVB). MVBs are characterized by their content of ILVs and are an integral part of the pathway that leads to the formation of exosomes.
- Exosome Secretion: MVBs are then transported to the plasma membrane. Upon reaching the plasma membrane, MVBs can fuse with it, leading to the release of ILVs into the extracellular space. Once released, these ILVs are referred to as exosomes.
- Molecular Sorting: During the formation of ILVs within MVBs, certain molecules are selectively sorted into the vesicles. This sorting process is mediated by various mechanisms, including the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, lipid-based mechanisms, and tetraspanin-enriched microdomains. The cargo of MSC-exo can include a wide range of molecules, such as proteins, mRNAs, microRNAs, and other non-coding RNAs, reflecting the physiological state and the therapeutic potential of the parent MSCs.
- Therapeutic Implications: The unique composition of MSC-exo and their ability to transfer molecules from MSCs to recipient cells make them an attractive tool for regenerative medicine and drug delivery. They have been studied for their potential in promoting tissue repair, modulating immune responses, and treating various diseases.

Studies have highlighted the indispensable role of exosomes in facilitating communication between neurons and glial cells, with MSC-Exo offering a strategic intervention in this complex process for therapeutic benefits.[9] This review provides an in-depth exploration of the intricate roles and potential mechanisms of MSC-Exo in mitigating the inflammatory response following ischemic stroke, aiming to pave the way for innovative clinical strategies in managing ischemic stroke.

The role of MSC-derived exosomes in neuroinflammation

MSC-Exo are small vesicles characterized by a lipid bilayer membrane structure, originating from MSCs, and typically ranging in size from 30 to 150 nm. These exosomes harbor a rich assortment of biologically active components such as lipids, proteins, RNA, and cytokines, endowing them with the capability to modulate signaling pathways within nearby or distant cells.[10–11] A notable feature of MSC-Exo is their remarkable ability to traverse the

blood–brain barrier with ease, coupled with their resilience to the processes of freezing and thawing, thereby facilitating convenient storage. Leveraging the multi-lineage differentiation potential inherent to MSCs, MSC-Exo also exhibit self-renewal properties, bolstering their therapeutic potential.[12] Moreover, MSC-Exo can be harvested from a diverse array of tissues in the body, encompassing adipose tissue, bone marrow, embryonic stem cells, dental pulp, placenta, umbilical cord, Wharton's jelly, and more. This broad tissue availability ensures a plentiful supply of MSC-Exo resources for various therapeutic applications.

Exosomes derived from MSCs exhibit diverse biological functions depending on their source.[13] For instance, in a murine model of cerebral artery ischemia, exosomes sourced from human umbilical cord blood stem cells have demonstrated the ability to reduce infarct size. Exosomes obtained from human bone marrow stem cells have demonstrated potential in enhancing recovery from neural damage and offering long-term protection to the nervous system. While there is considerable research elucidating the varied physiological roles of exosomes secreted by both rodent and human cells, there remains a notable gap in comparative studies assessing the therapeutic efficacy of MSC-Exo from different origins utilizing animal models of cerebral artery ischemia.[14] Consequently, the extent of disparity in therapeutic outcomes among MSC-Exo from distinct sources remains largely unexplored.

The mechanism by which MSC-exo exert their effects in the context of neuroinflammation involves intricate interactions with different cell types in the CNS, as well as the modulation of various signaling pathways and mediators.

Interaction with CNS Cell Types

- Neurons: MSC-exo can directly interact with neurons, promoting neuronal survival and neuroregeneration. They can transfer neuroprotective proteins and miRNAs that modulate neuronal gene expression, enhancing neuronal plasticity and reducing apoptosis.
- Astrocytes: MSC-exo can modulate astrocyte activity, promoting their protective roles. Exosomes can alter the secretion of neurotrophic factors and cytokines from astrocytes, contributing to an environment that supports neuronal survival and dampens inflammation.
- Microglia: Microglia are the primary immune cells in the CNS and play a critical role in neuroinflammation. MSC-exo can influence microglial activation states, shifting them from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype. This shift reduces the secretion of pro-inflammatory cytokines and enhances the production of anti-inflammatory and regenerative factors.
- Endothelial Cells: By interacting with endothelial cells of the BBB, MSC-exo can promote barrier integrity and function. They can also modulate the immune response by affecting the trafficking of immune cells across the BBB.

Mediation by Bioactive Molecules

- miRNAs: MSC-exo are rich in miRNAs that can regulate gene expression in recipient cells. Specific miRNAs have been shown to modulate inflammatory pathways, reduce oxidative stress, and enhance neuroprotection.
- Proteins: Exosomal proteins, including growth factors, cytokines, and enzymes, can directly affect the behavior of target cells. For instance, growth factors like brain-derived neurotrophic factor (BDNF) found in MSC-exo can support neuronal growth and differentiation.
- Lipids: The lipid content of MSC-exo can also play a role in modulating neuroinflammation, though this aspect is less well-understood. Lipids can influence membrane dynamics and signaling pathways in recipient cells.

Other Mediators and Mechanisms

- Modulation of the Immune Response: MSC-exo can influence the proliferation, differentiation, and function of various immune cell types, including T cells, B cells, and dendritic cells, leading to a more anti-inflammatory and tolerogenic immune environment.
- Exosomal Tetraspanins: These membrane proteins can facilitate the direct interaction between MSC-exo and recipient cells, influencing cell adhesion, fusion, and the delivery of exosomal cargo.
- Signaling Pathways: MSC-exo can modulate key signaling pathways involved in inflammation and cell survival, such as the NF-κB pathway, PI3K/Akt pathway, and MAPK pathways.

The therapeutic potential of MSC-exo in treating neuroinflammatory conditions lies in their ability to cross the BBB, their biocompatibility, and their capacity to carry and deliver a complex cargo of bioactive molecules. These characteristics allow them to modulate the CNS environment, offering a promising approach for the treatment of neuroinflammatory diseases. Ongoing research is focused on elucidating the detailed mechanisms of action, optimizing exosome isolation and characterization, and developing clinical translation strategies.

Presently, the primary focus of research revolves around the localized inflammatory response to ischemia, while there remains a significant gap in studies investigating the potential of immune modulators or anti-inflammatory agents to alleviate post-ischemic stroke inflammation. Within this context, MSC-Exo emerge as promising candidates, exerting their effects by modulating various cells and cytokines, thus potentially serving as crucial elements in the immunomodulatory mechanisms associated with ischemic stroke. Recent investigations have underscored the multifaceted potential of MSC-Exo, demonstrating their ability to suppress neuroinflammation, diminish neuronal apoptosis, and facilitate neurogenesis.[15] A study by Yang et al. delves into the functional significance of microRNA-29b-3p (miR-29b-3p), carried by EVs derived from bone marrow-derived mesenchymal stem cells (BM-MSCs), in angiogenesis during fracture healing, focusing specifically on attention to its regulatory effects through PTEN/PI3K/ACT axis.[16] The study methodology used a combination of in vitro and in vivo experiments to elucidate the underlying mechanisms and therapeutic potential. Initially, BM-MSC-EVs were isolated and characterized, and miR-29b-3p knockdown BM-MSCs were generated using lentiviral methods. Co-culture experiments with human umbilical vein endothelial cells (HUVECs) demonstrated the effects of EV-encapsulated miR-29b-3p on HUVEC proliferation, migration, and angiogenesis using various assays, including the CCK-8 assay, scratch test, and tube formation assay. Through a series of bioinformatics analyses, luciferase activity assays, and molecular analyses, the downstream target gene of miR-29b-3p was identified as PTEN, a negative regulator of the PI3K/AKT pathway. Subsequent experiments using siRNA against PTEN confirmed its involvement in mediating the effects of miR-29b-3p on HUVEC. The study further confirmed these results in a mouse model of femur fracture, where local administration of BM-MSC-EV promoted neovascularization at the fracture site and increased bone volume and mineral density. In contrast, miR-29b-3pKD-BM-MSCs-EVs exhibited reduced angiogenic effects

and impaired fracture healing ability. The findings highlight the key role of BM-MSCs-EVs in delivering miR-29b-3p to target endothelial cells, thereby modulating angiogenesis through the PTEN/PI3K/AKT axis. This study not only expands our understanding of the molecular mechanisms underlying fracture healing, but also highlights the therapeutic potential of microRNA-based approaches using BM-MSCs-EVs in promoting bone regeneration and angiogenesis.[16] Similarly, Giunti et al. conducted research in a mouse model of chronic progressive demyelination induced by brain-spinal cord virus infection, where they observed that administration of MSC-Exo led to a reduction in spinal cord infiltration and downregulation of glial fibrillary acidic protein and ionized calcium-binding adapter molecule 1 expression in the mouse brain.[17] Moreover, they found that miR-467f and miR-466q loaded in MSC-Exo could suppress the activation response of glial cells by inhibiting the p38 MAPK signaling pathway. These findings highlight the distinctive anti-inflammatory properties of MSC-Exo, primarily characterized by their ability to restrain the inflammatory activation response of glial cells, and modulate pro-inflammatory cytokines through immunomodulatory molecules, thereby mitigating the detrimental effects of inflammation.

MSC-Exo regulate neuroinflammation in ischemic stroke by influencing the activity of glial cells

Glial cells are essential components of neural tissue, fulfilling critical functions in nurturing and protecting neurons, as well as releasing various bioactive substances to regulate neuronal growth, development, and uphold brain homeostasis.[18] In response to ischemic injury, both microglial cells and astrocytes undergo considerable activation, collectively contributing to the inflammatory response at the site of damage.[19] These cells play pivotal roles in maintaining the equilibrium of the brain tissue microenvironment. Extensive research has underscored the significant impact of MSC-Exo in modulating the activity of microglial cells and astrocytes.

MSC-Exo suppress the inflammatory activation phenotype of microglial cells

The transition of microglial cell phenotypes represents one of the earliest inflammatory responses observed following ischemic stroke injury.[19–21] M1-type microglial cells are primarily involved in provoking pro-inflammatory reactions and facilitating antigen presentation, while M2-type microglial cells play a pivotal role in tissue repair by clearing necrotic tissue.[20] Consequently, promoting the transition from M1 to M2 phenotypes holds critical significance in the treatment of post-ischemic stroke injuries, as it can effectively mitigate the levels of pro-inflammatory cytokines and facilitate the repair processes within the brain tissue. Studies conducted in a mouse model of Japanese encephalitis virus infection have provided compelling evidence demonstrating the remarkable therapeutic potential of MSC-Exo. These exosomes have been shown to possess the capability to suppress inflammation, attenuate the activation of M1-type microglial cells, induce the activation of M2-type microglial cells, and alleviate neuronal damage, disruptions in the blood–brain barrier, and viral load burden.[21–25] As a result, MSC-Exo presents potential therapeutic advantages for the treatment of neuroinflammation. Both MSCs and MSC-Exo exhibit the ability to restrain the activation of microglial cells and facilitate the transition from the M1 to M2 phenotypes. Nonetheless, elucidating the precise molecular pathways through which MSC-Exo regulate inflammation remains a critical area of investigation.

miRNAs play a pivotal role in modulating microglial cell activity through MSC-Exo. Extensive research indicates that miRNAs can be packaged and delivered to specific target cells within exosomes and extracellular vesicles.[21–25] MSC-Exo containing miRNAs demonstrate the capacity to regulate microglial cell activation and possess anti-inflammatory properties. For example, miR-223-3p carried by MSC-Exo can alleviate inflammation triggered by M1-type polarization of microglial cells, thereby mitigating ischemic stroke-induced damage, and promoting the transition of microglial cells towards the M2 phenotype, ultimately facilitating functional recovery post-ischemic stroke.[21] Similarly, miR-216b-5p facilitates the shift of microglial cells from M1 to M2 phenotype by inhibiting the TLR4/NF-κB pathway and activating the PI3K/AKT signaling pathway.[22] Exosomes containing miRNA-126 hinder the activation of microglial cells in stroke-afflicted mice, thereby diminishing inflammation and fostering neurogenesis and angiogenesis.[23] Furthermore, miR-30d-5p carried by MSC-Exo suppresses neuronal damage by impeding autophagy-mediated polarization of M1-type microglial cells.[24]

MSC-Exo is involved in mediating the signaling pathways that activate microglial cells. Research has shown that various cellular signaling cascades are associated with the activation of M2-type microglial cells, with cyclic adenosine monophosphate response element-binding protein and NF-κB being two major transcription factors.[25–26]

In another study, researchers shed light on the role of wall shear stress (WSS), which mimics the fluid frictional force found in the arterial vasculature, in enhancing the immunoregulatory function of human bone marrow derived MSCs.[27] The study shows that WSS exposure significantly increases the expression of four key genes involved in MSC-mediated immune regulation: PTGS2, HMOX1, IL1RN and TNFAIP6. Mechanistically, WSS stimulates multiple mechanotransduction pathways, including calcium ion ($Ca2+$) flux and activation of Akt, MAPK, and focal adhesion kinase (FAK). Interestingly, inhibition of PI3K-Akt or $Ca2+$ signaling failed to reduce WSS-induced COX2 expression, whereas FAK inhibition effectively blocked COX2 induction, indicating a critical involvement of focal adhesions in this process. Additionally, co-culture assays indicate that WSS preconditioning enhances the anti-inflammatory activity of MSCs, resulting in a more potent suppression of TNF-α production by activated immune cells. Importantly, this enhanced efficacy is dependent on FAK-mediated COX2 induction, highlighting the key role of the FAK signaling cascade in enhancing the immunomodulatory function of MSCs under the influence of biomechanical forces. Overall, our results highlight the potential of biomechanical force-based approaches to enhance the reparative and regenerative properties of MSCs in regenerative medicine. By elucidating the signaling cascade involving FAK in mediating the effects of WSS on MSC immunomodulation, this study opens the possibility for innovative strategies aimed at enhancing the therapeutic efficacy of MSCs through modulation of biomechanical force.[27]

Apart from the signaling cascades, MSC-Exo can also engage in other pathways such as TLR4, AKT, and MAPK to facilitate M2-type polarization.[28] MSC-Exo modulate microglial cell activation through the secretion of cytokines and neurotrophic factors. These exosomes contain a diverse array of cytokines, neurotrophic factors, and anti-inflammatory molecules, resulting in notable enhancements in the extracellular environment by influencing microglial cell behavior.[29] Research indicates that MSC-Exo can generate neurotrophic factors while simultaneously reducing the production of pro-inflammatory factors.[30] Moreover, investigations by Hao et al. propose that MSC-Exo may hinder microglial cell activation by suppressing the release of IL-1β, TNF-α, and IL-6, and

by inhibiting cholesterol-25-hydroxylase, an enzyme implicated in exacerbating brain inflammation and activating microglial cells.[31] These findings further underscore the role of MSC-Exo in regulating microglial cell activity and modulating neuroinflammation (Fig. 2a, b).

MSC-Exo decrease the inflammatory potential of astrocytes

In contrast to microglial cells, astrocytes respond to ischemic stroke stimuli in larger quantities and with prolonged inflammatory responses, underscoring their significance as a pivotal target for effectively managing central inflammation.[6] Astrocytes predominantly demonstrate two distinct responses to focal ischemia: a steady-state/neuroprotective reaction and reactive astrogliosis. Reactive astrogliosis induced by neuroinflammation typically presents as A1-type, whereas ischemia-induced reactive astrogliosis appears as A2-type.[32] A1-type astrocytes release neurotoxic factors that swiftly damage neurons and oligodendrocytes, while A2-type astrocytes offer neuroprotective benefits through the upregulation of neurotrophic factors. Thus, promoting A2-type activity and suppressing A1-type activity are crucial steps in advancing astrocyte-mediated therapy for ischemic stroke.

MSC-Exo exert a dual effect of diminishing the activation of A1-type cells while enhancing the activity of A2-type cells.[33] This modulation occurs through the regulation of the Nrf2-NF-κB signaling pathway, facilitating the restoration of A1-type astrocyte activation and alleviating inflammation both internally and externally. Further exploration is needed to fully comprehend the intricate signaling pathways through which MSC-Exo influence astrocytes.

MSC-Exo play a significant role in controlling local inflammation through the regulation of cytokines, a process crucial for their impact on astrocytes.[34] By decreasing the levels of pro-inflammatory cytokines like TNF-α and IL-1β, MSC-Exo boost the activity of A2-type astrocytes.[35] In addition to directly influencing astrocyte activity, MSCs also enhance neurofunction by upregulating the expression of various growth factors produced by astrocytes, including insulin-like growth factor 1, epidermal growth factor, vascular endothelial growth factor, and basic fibroblast growth factor.[36] These findings indicate that MSC-Exo can suppress the activation of A1-type astrocytes, reduce the secretion of pro-inflammatory cytokines, stimulate the production of A2-type astrocytes, with the released neurotrophic factors playing a pivotal role in this process.

MSC-Exo modulate neuroinflammation in ischemic stroke through the regulation of immune cells and inflammatory mediators

MSC-Exo modulate immune cells to inhibit inflammation in the nervous system

In the context of brain tissue damage caused by ischemic stroke, MSC-Exo are involved in regulating the activity of immune cells.[37–38] This includes inhibiting the function of antigen-presenting cells and decreasing the presence of B lymphocytes, natural killer cells, T lymphocytes, and macrophages at the site of ischemic stroke.[39–40] They play a crucial role in modulating the immune response, effectively dampening inflammation by stimulating the proliferation, activation, and secretion of immune cells. This, in turn, helps shield against neuronal damage and create an environment conducive to neurovascular regeneration. T lymphocytes, particularly Th and regulatory T cells, are pivotal in exerting anti-inflammatory effects. Exosomes facilitate the transition of Th1 to Th2 cells, enhance the population of regulatory T cells, and induce apoptosis in peripheral blood mononuclear cells and CD3 + T cells, thus alleviating inflammation.[39–41] While mounting evidence sheds light on Th phenotype switching induced by various factors, the intricate molecular mechanisms involved necessitate further investigation and consolidation. Although the influence of MSC-Exo on regulatory B cells has been extensively studied in the context of tumor immune evasion, its role in ischemic stroke warrants further exploration.[42]

MSC-Exo modulate inflammatory mediators to inhibit inflammation in the nervous system

Inflammatory mediators such as IL-1, IL-6, TGF-β, and IL-10 play crucial roles in the immune response after ischemic stroke.[34,37] IL-1 levels rise shortly after the stroke, triggering the release of other cytokines, chemokines, and cell adhesion molecules, which ultimately disrupt the blood–brain barrier.[43] Studies have demonstrated that transplanting MSCs reduces IL-1 production in microglial cells and encourages regulatory T cells to adopt an anti-inflammatory phenotype.[44–45] IL-6, another key cytokine in the post-stroke inflammatory response, is significantly decreased in the brains of rats treated with MSC-Exo.[46] MSC-Exo also promotes the production of the anti-inflammatory cytokine TGF-β and activates microglial cells through the TGF-β/Smad2/3 pathway, slowing down microglial cell activation.[47–48] However, research on the ability of MSC-Exo to increase IL-10 levels is limited. Inflammatory mediators represent important targets for MSC-Exo at inflammatory sites, but the precise mechanisms by which they create an immune-suppressed microenvironment in the injured area remain unclear. Therefore, further investigation into the effects of MSC-Exo on inflammatory mediators and the development of targeted therapeutic agents for neuroinflammatory responses following ischemic stroke, including the isolation and purification of effective molecules from exosomes, is essential.

Mechanisms of action of MSC-exo on neuroinflammation and neurological function after stroke

MSC-exo present a sophisticated therapeutic approach to neuroinflammation and neurological rehabilitation following a stroke, employing mechanisms that transcend simple immunosuppression.

MSC-exo possess a remarkable capability to intricately regulate immune responses within the milieu of post-stroke recovery. Far from simply dampening immune activities, these exosomes skillfully orchestrate the activities of various immune cells. One of their notable functions includes guiding macrophages and microglia away from an inflammatory (M1) disposition towards a reparative (M2) stance. This transition is pivotal, as M2 cells are known for their secretion of cytokines and growth factors that are pivotal for the repair of damaged tissues and the stimulation of neural regeneration. This sophisticated immunomodulatory strategy not only mitigates the damage inflicted by early post-stroke inflammation but also sets the stage for the body's natural repair and regeneration processes. Within the sphere of neurovascular remodeling, MSC-exo undertake a critical dual function by creating an environment that is simultaneously supportive of angiogenesis and neurogenesis. Through the delivery of a carefully curated selection of growth factors and microRNAs, MSC-exo energize endothelial and neural stem cells. This not only aids in the repair of the blood–brain barrier but also in the seamless integration of emerging neurons into the damaged brain tissue, a process that is vital for both structural and functional neural recovery. This process relies heavily on a balanced inflammatory environment to ensure successful outcomes. Astrocytes exhibit a wide range of functions following a stroke, which extend well beyond their traditional role in scar formation. Triggered by MSC-exo, astrocytes can adopt a

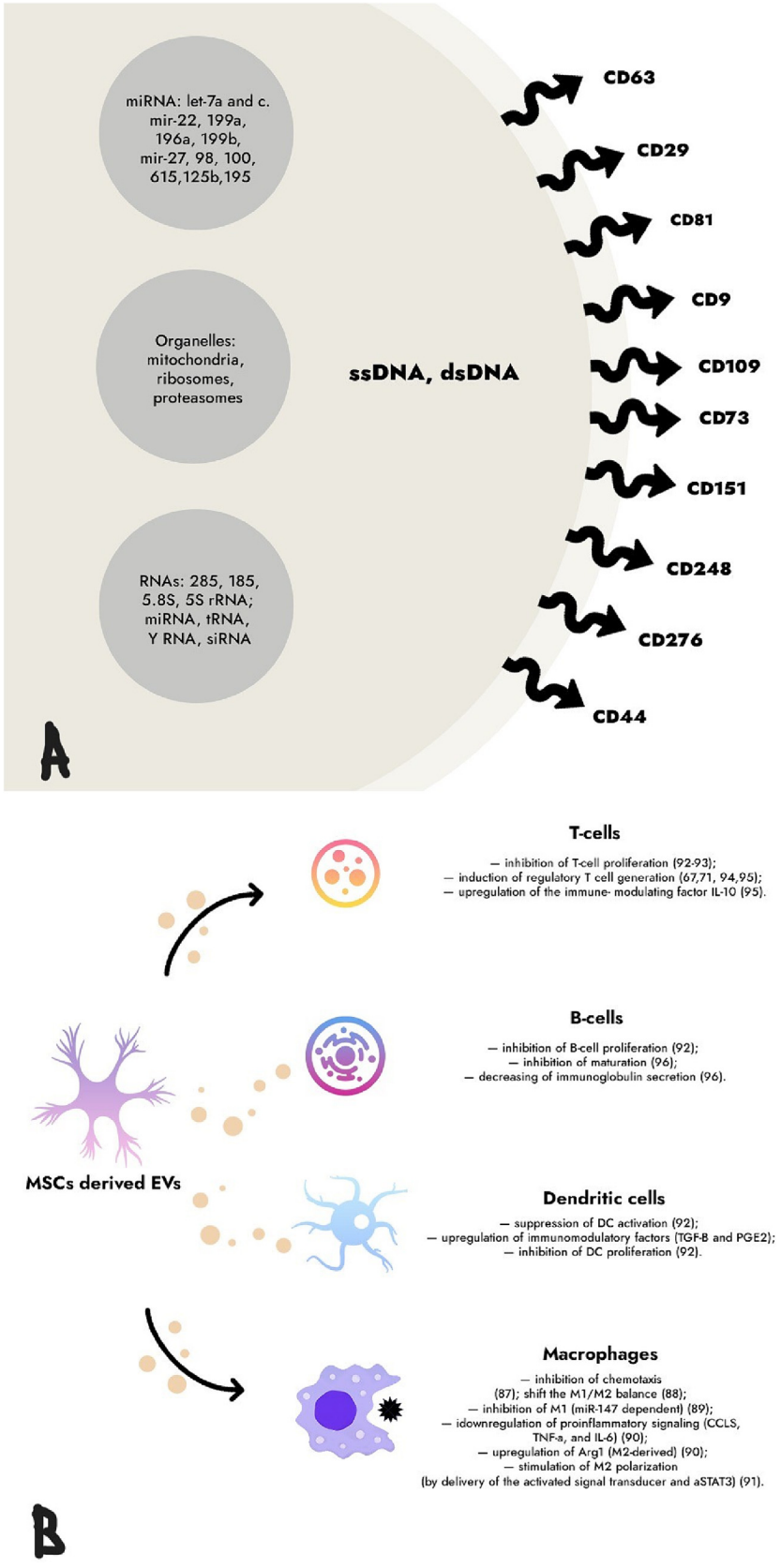

Fig. 2. a. The composition of molecules and the immune-regulating impacts of extracellular vesicles derived from mesenchymal stem cells. EVs are characterized by distinct membrane markers and encompass a diverse array of proteins, lipids, nucleic acids, and cellular organelles. 2b. EVs derived from MSCs can elicit various immunosuppressive responses and play a role in promoting immune tolerance.

phenotype that significantly promotes brain repair while suppressing inflammatory responses. This enhances neuronal survival and aids in the recuperation of synaptic functionalities, highlighting MSC-exo's pivotal role in fostering an anti-inflammatory environment conducive to brain recovery. The extracellular matrix (ECM) is integral for synaptic plasticity and the restructuring of neural circuits in the stroke recovery phase. MSC-exo transport enzymes and regulatory substances capable of modifying the ECM structure, which in turn influences neuronal plasticity and supports the reformation of functional neural connections within an inflammation-regulated setting. This aspect is crucial for the cognitive and functional rehabilitation of individuals who have suffered a stroke. Furthermore, the MSC-exo's cargo, abundant in miRNAs, plays an essential role in modulating gene expression related to inflammation, cell demise, and survival within areas of the brain affected by stroke. By altering the landscape of miRNAs, MSC-exo cultivate an environment that is primed for repair, regeneration, and functional restoration, moving beyond the mere suppression of inflammation to support a constructive recovery phase. An often-underappreciated function of MSC-exo is their capacity to transfer healthy mitochondria or mitochondrial elements to cells that have been compromised by stroke. This process enhances energy production and reduces oxidative stress, which are critical for maintaining cell viability and functionality during the recovery period. This mitochondrial support is key to debris clearance and the advancement of repair mechanisms, all within a neuroinflammatory response that is carefully balanced.

In summary, the impact of MSC-exo on the recovery process following a stroke is extensive and multi-dimensional, going far beyond simple immunosuppression. They are instrumental in creating an optimal recovery environment through the modulation of immune cell activities, the promotion of neurovascular remodeling, the regulation of astrocyte functionality, the reconstitution of the ECM, the adjustment of miRNA profiles, and the enhancement of mitochondrial function. Together, these multifaceted actions orchestrate a delicately balanced approach to neuroinflammation management, underlining the therapeutic potential of MSC-exo in harnessing the beneficial aspects of neuroinflammation for neurologic recovery while minimizing the detrimental impacts of post-stroke inflammation.

Mechanism of MSC-Exo in the treatment of hemorrhagic stroke

The initial management of hemorrhagic stroke (HS) predominantly revolves around the evacuation of the hematoma and shielding the neighboring tissue from further harm inflicted by the hematoma. Subsequent treatment phases are primarily directed towards the restoration of impaired nerves.[49] Revolutionary biological interventions such as EVs or exosomes have emerged as pivotal advancements across diverse domains of neural repair. While a plethora of studies have delved into the correlation between MSC-Exo and HS, the precise mechanisms governing neural recuperation remain subject to debate. Nerve regeneration entails a labyrinthine sequence of responses and interactions between cells and EVs/exosomes. These microscopic vesicles ferry bioactive substances to target cells through mechanisms such as diffusion, endocytosis, and receptor-mediated transport. A wealth of scientific inquiry has underscored the pivotal role of EVs/exosomes in contributing to HS therapy via two principal avenues: (1) Direct intervention entails the surface proteins of exosomes recognizing receptors on target cells, thereby instigating signal transduction, and fostering intercellular communication. (2) Indirect impacts transpire when exosomes convey therapeutic agents for HS treatment. They possess the capability to fuse with target cells, thereby delivering bioactive factors to act upon receptors on the cellular surface and facilitate the transmission of information. Nerve repair typically encompasses the mitigation of inflammation, stimulation of angiogenesis, and facilitation of nerve restructuring. Presently, the focus of MSC-Exo in nerve repair predominantly revolves around these three domains. The therapeutic mechanisms attributed to MSC-Exo may encompass the stimulation of cell proliferation, promotion of angiogenesis, inhibition of apoptosis, release of active factors, and facilitation of nerve regeneration.

Neuroinflammation stands as a pivotal contributor to the pathogenesis of HS. Upon the early activation of microglia in HS, both cellular demise and the breakdown of products set off an inflammatory cascade, culminating in heightened neuronal loss. The onset of HS triggers an extensive neuroinflammatory reaction, implicated in exacerbating brain damage.[50–53] Consequently, mitigating neuroinflammation emerges as a promising target for therapeutic intervention. MSC-Exo possess the capacity to convey microRNAs, proteins, or other contents, endowing them with anti-inflammatory effects.[54] A recent study highlighted that bone marrow-derived MSC exosomes (BMSC-exos) alleviate neuroinflammation and exhibit neuroprotective properties in brain tissues post-subarachnoid hemorrhage (SAH) by inhibiting NF-κB and activating AMPK pathways. BMSC-exos additionally modulate the polarization of microglia towards the M2 phenotype, downregulating IL-1β, CD16, CD11b, INOS, while upregulating CD 206 expression.[55] Another investigation revealed that BMSC-EVs transport miR-183-5p to the HS site in rat brain tissues, suppressing the NLRP3 pathway by targeting PDCD4, thereby mitigating neuroinflammation post-diabetic HS.[56] Intriguingly, systemic administration of exosomal/miR-193b-3p attenuates the inflammatory response through NF-κB p65 acetylation by suppressing HDAC3 expression and activity. These effects ameliorated neurobehavioral deficits and neuroinflammation after SAH.[57] Hence, MSC-Exo exhibit potential anti-inflammatory effects in the pathological progression of cerebral hemorrhage by inhibiting inflammatory factors and curtailing M2 microglia activation.

Angiogenesis, the process of forming new capillaries from existing blood vessels, plays a pivotal role in various physiological and pathological contexts. In the aftermath of cerebral hemorrhage, the vascular endothelium undergoes destruction, while adjacent tissues and blood vessels experience ischemic stress, exacerbating brain damage. Recent findings underscore the critical involvement of HIF-1α in HS-induced angiogenesis.[58] Facilitating cerebral angiogenesis and neurogenesis emerges as a crucial strategy for mitigating functional brain damage following HS. Bone marrow-derived mesenchymal stem cell-derived extracellular vesicles (BMSC-EVs) have demonstrated efficacy in promoting angiogenesis.[59] Transplantation of BMSC-exo into mice with ischemic stroke exhibited notable enhancements in angiogenesis and neurogenesis, thereby fostering neurological function recovery.[60] Additionally, research has revealed that exosomes derived from MSCs pre-treated with extract from ischemic rat hearts facilitate angiogenesis by delivering DMBT1 to repair brain injuries.[61] Notably, exosomes derived from mouse brain endothelial cells (EC-Exo) have been shown to significantly enhance primary cortical neuron axonal growth and facilitate endothelial capillary formation, resulting in increased axonal density, myelin density, blood vessel density, arterial diameter, and improved neurological cognitive function.[62]

Multiple studies suggest that mitigating and regulating immune responses hold promise as an approach for treating HS.[63–64] In acute stroke scenarios, the byproducts of microglial activation and cellular demise instigate an inflammatory cascade that inflicts damage upon vessels and parenchyma within minutes to hours post-ischemia or hemorrhage. Swift implementation of immune interventions aimed at curbing brain inflammation, vascular permeability, and tissue edema is imperative to attenuate the destruc-

tive effects of acute immune reactions and avert subsequent immunosuppression. Several recent findings propose that achieving these objectives may be feasible in ischemic and hemorrhagic strokes through the utilization of disease-modifying drugs for multiple sclerosis, indicating that effective immune interventions could potentially decelerate and reverse brain damage after HS. Hence, exosomes may potentially enhance the prognosis of HS through immune intervention.

Conclusion

Based on the immunomodulatory and regenerative properties of MSC-Exo, they hold great promise as a groundbreaking therapeutic strategy for addressing neuroinflammatory conditions. The small size of MSC-Exo enables them to breach the blood–brain barrier, making them excellent carriers for delivering medications to sites of inflammation. By engaging various regulatory pathways, MSC-Exo have the capacity to modulate the activities of microglial cells, astrocytes, immune cells, and inflammatory molecules, thereby exerting protective effects on the integrity of the blood–brain barrier and the overall health of the central nervous system and peripheral tissues. As a cell-free treatment option for stroke, MSC-Exo therapy boasts numerous advantages, including low risk of immune rejection, enhanced stability, remarkable ability to traverse the blood–brain barrier, and reduced likelihood of thrombosis and microvascular complications during administration. In recent years, there has been a growing focus on MSC-Exo, with encouraging therapeutic outcomes documented across various medical conditions. Nevertheless, several hurdles must be surmounted before MSC-Exo can be integrated into clinical practice for treating stroke. Currently, the precise mechanisms through which MSC-Exo modulate inflammation following stroke remain obscure, and the exact composition of MSC-Exo remains uncertain, posing challenges in enhancing their therapeutic efficacy with precision.[48,65] Moreover, MSC-Exo display heterogeneity and are produced at relatively low rates, necessitating the exploration of innovative technologies to scale up production. Extensive exploration in both fundamental and clinical realms is imperative to unravel the mechanisms underlying MSC-Exo's actions and establish essential standards for their production and quality control, thereby facilitating their widespread clinical adoption.

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CRediT authorship contribution statement

Ozal Beylerli: Writing – review & editing, Conceptualization. **Ilgiz Gareev:** Writing – original draft. **Huaizhang Shi:** Supervision. **Tatiana Ilyasova:** Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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