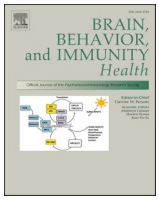
Brain, Behavior, & Immunity - Health 40 (2024) 100835

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx



Promise of mesenchymal stem cell-derived extracellular vesicles for alleviating subarachnoid hemorrhage-induced brain dysfunction by neuroprotective and antiinflammatory effects

Kiran Sankarappan, Ashok K. Shetty *

Institute for Regenerative Medicine, Department of Cell Biology and Genetics, Texas A&M University Health Science Center School of Medicine, College Station, TX, USA

ARTICLE INFO

Keywords:
Anti-apoptotic effects
Antiinflammatory effects
Extracellular vesicles
Mesenchymal stem cells
Neuroprotective effects
Stem cell derived EVs
Subarachnoid hemorrhage

ABSTRACT

Subarachnoid hemorrhage (SAH), accounting for ~5% of all strokes, represents a catastrophic subtype of cerebrovascular accident. SAH predominantly results from intracranial aneurysm ruptures and affects ~30,000 individuals annually in the United States and ~6 individuals per 100,000 people worldwide. Recent studies have implicated that administering mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) may be beneficial in inducing neuroprotective and antiinflammatory effects following SAH. EVs are nanosized particles bound by a lipid bilayer. MSC-EVs comprise a therapeutic cargo of nucleic acids, lipids, and proteins, having the promise to ease SAH-induced long-term brain impairments. This review evaluated the findings of published studies on the therapeutic efficacy of MSC-EVs in the context of SAH. A growing body of evidence points out the therapeutic potential of MSC-EVs for improving brain function in animal models of SAH. Specifically, studies demonstrated their ability to reduce neuronal apoptosis and neuroinflammation and enhance neurological recovery through neuroprotective and antiinflammatory mechanisms. Such outcomes reported in various studies suggest that MSC-EVs hold great potential as a novel and minimally invasive approach to ameliorate SAH-induced neurological damage and improve patient outcomes. The review also discusses the limitations of EV therapy and the required future research efforts toward harnessing the full potential of MSC-EVs in treating SAH.

1. Introduction

Subarachnoid hemorrhage (SAH) represents a catastrophic subtype of cerebrovascular accident, predominantly resulting from intracranial aneurysm ruptures [Welty and Horner, 1990; Brisman et al., 2006; Claassen and Park, 2022]. SAH affects ~6 individuals per 100,000 people worldwide. In the United States, an estimated 30,000 individuals suffer from SAH annually, with a 14% mortality rate before accessing medical care [Sobey and Faraci, 1998; Palade et al., 2013; Vivancos et al., 2014; Tawk et al., 2021]. For survivors, the statistics remain grim, with 50–70% enduring severe disability or succumbing to complications of SAH [Sobey and Faraci, 1998; Palade et al., 2013; Vivancos et al., 2014]. Even with innovations in medical and surgical management over the past decades, SAH remains a considerable public health burden, accounting for ~27% of all stroke-related potential years of life lost among individuals under 65 [Vermeulen, 1996; Claassen and Park, 2022]. While risk factors such as hypertension, smoking, and trauma serve as priming elements for SAH, early brain injury (EBI) is the crux of post-SAH morbidity [Van Gijn and Rinkel, 2001; Rabinstein, 2013; Sriram et al., 2022; Bonita, 1986]. EBI is typified by acute neurological deficits that occur within 72 h post-hemorrhage. A primary mechanistic role is attributed to apoptosis triggered by caspase activation, which leads to significant neurodegeneration [Fujii et al., 2013; Palade et al., 2013; Gu et al., 2021].

SAH can precipitate various complications, including cerebral vasospasm, a pathological condition characterized by the constriction of cerebral blood vessels [Welty and Horner, 1990]. While preventing cerebral vasospasm after SAH helps reduce severe complications, it cannot entirely prevent SAH-induced EBI or neurological deficits. Therefore, effective alternative therapeutic strategies are necessary to combat SAH-induced EBI and neurological deficits [Xu et al., 2019; Claassen and Park, 2022]. Recent studies suggest that administering extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) could be beneficial in inducing neuroprotective effects following SAH. MSCs can

* Corresponding author. Institute for Regenerative Medicine, Department of Cell Biology and Genetics, Texas A&M University School of Medicine, 1114 TAMU, 206 Olsen Boulevard, College Station, TX, 77843, USA.
E-mail address: ash.shetty@tamu.edu (A.K. Shetty).

https://doi.org/10.1016/j.bbih.2024.100835
Received 18 May 2024; Received in revised form 22 July 2024; Accepted 1 August 2024

improve outcomes by acting primarily through paracrine mechanisms after intracerebral grafting in models of ischemic stroke and intracerebral hemorrhage (Myers et al., 2023; Wang et al., 2023). However, directly administering MSCs into the brain is an invasive procedure that can cause further damage to the brain and may form tumors if they undergo unlimited proliferation. On the other hand, intravenous or intranasal administration of nanosized EVs secreted by MSCs can mediate the therapeutic effects of MSCs without the above risks as they exhibit either no or very low immunogenicity and cannot replicate (Zhu et al., 2017). Since the bioactive molecules in the MSC-EV cargo can mediate tissue repair processes through antioxidant, antiinflammatory, and neuroprotective effects, studies on the administration of MSC-EVs after SAH have received significant attention.

This review critically discusses the potential of MSC-EVs for treating brain dysfunction resulting from SAH. The review confers specific microRNAs (miRNAs) within MSC-EVs and their significant impact on gene modulation, inhibition of cell death, and neuroprotection following SAH. MSC-EVs contain a wide range of miRNAs, some of which may be beneficial, while others may be detrimental to brain repair after SAH. The article discusses the net effect of MSC-EVs in animal models of SAH and their potential to alleviate long-term brain dysfunction after SAH. The limitations of MSC-EV therapy and the need for further research in this area are also deliberated.

2. Pathophysiology of SAH

SAH commonly results from ruptured intracranial aneurysms, primarily located at arterial bifurcations [Vivancos et al., 2014; Claassen and Park., 2022; Palade et al., 2013; Sriram et al., 2022]. The most common causes of SAH include arteriovenous malformations, a history of smoking, chronic hypertension, traumatic brain injury, and cardiac abnormalities [Bonita, 1986; Brisman et al., 2006; Claassen and Park, 2022; Van Gijn and Rinkel, 2001] (Fig. 1). SAH leads to mortality in ~20–30% of cases, with survivors facing risks of fatal rebleeding, seizures, or other complications [Szabo and Momen-Heravi, 2017] (Fig. 1). Cerebral vasospasm, characterized by the constriction of cerebral arteries, is a well-documented complication post-SAH [Li et al., 2019; Wanderer, 2020; Osgood, 2021; Neifert et al., 2021]. Nimodipine, a dihydropyridine calcium channel blocker, is often administered to prevent delayed cerebral ischemia [Raya and Diringer, 2014]. Furthermore, accessing the aneurysm via craniotomy and securing it through either coiling or clipping are the two primary surgical strategies for acute SAH [Colby et al., 2010; Marcolini and Hine, 2019] (Fig. 1).

EBI, seen within 72 h post-SAH [Caner et al., 2012], involves the activation of neuroinflammatory pathways, including substantial microglial activation, astrocyte reactivity, recruitment of peripheral leukocytes into the brain parenchyma, disruption of the blood-brain barrier (BBB) and intracranial edema [Fujii et al., 2013; Geraghty et al., 2019; Solár et al., 2022]. A central role in the EBI pathogenesis is attributed to the activation of pattern recognition receptors like toll-like receptors (TLRs), which trigger the NF-κB signaling pathway, fostering the expression of proinflammatory genes and cytokines [Geraghty et al., 2019; Solár et al., 2022]. The activation of apoptotic pathways is a hallmark of EBI, involving both caspase-dependent and caspase-independent mechanisms [Kaur et al., 2018; Solár et al., 2022]. Intrinsic apoptosis is mainly driven by mitochondrial cytochrome *c* releasing into the cytoplasm, activating caspase 9, while the extrinsic pathway involves the Fas ligand/receptor system. Concomitantly, necroptosis and autophagy contribute to neurodegeneration, adding layers of complexity to the EBI pathogenesis [Dhuriya et al., 2018; Gu et al., 2021].

The cerebral microenvironment also undergoes marked biochemical alterations after SAH. Excitotoxicity, driven by a surge in extracellular glutamate, incites neuronal damage by activating n-methyl d-aspartate

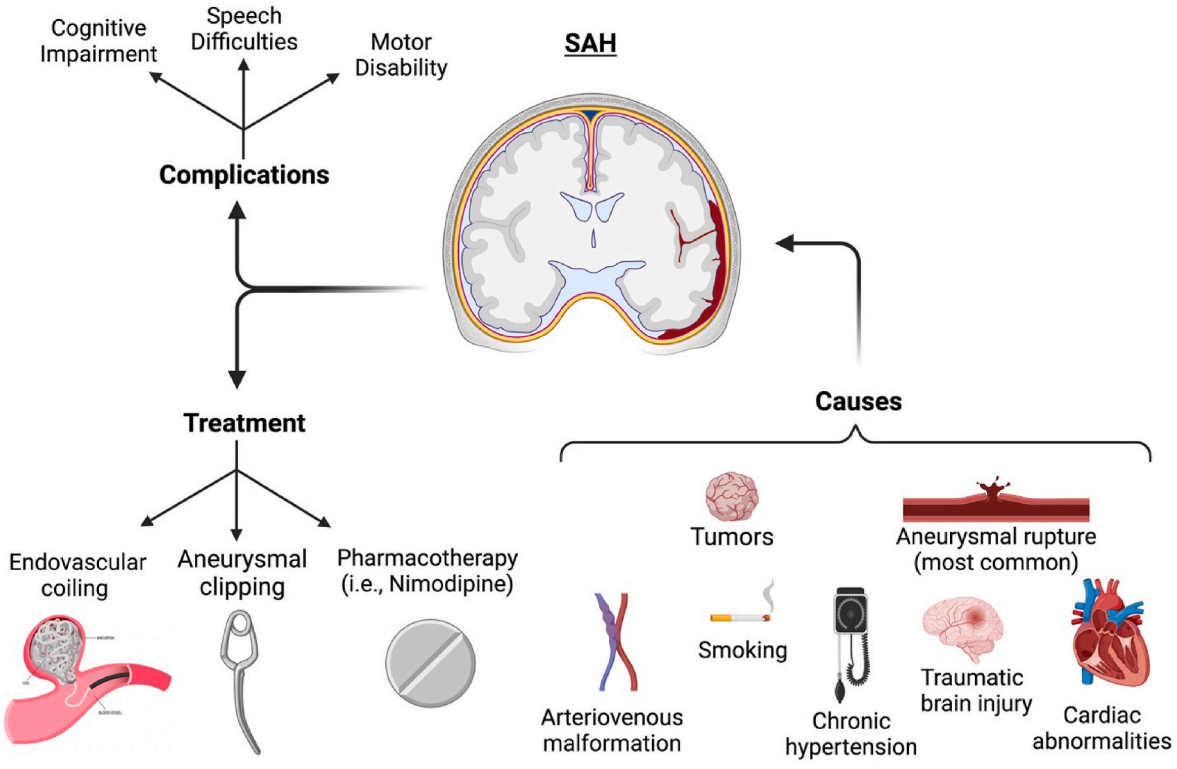


Fig. 1. A schematic representation of causes, complications, and treatment of subarachnoid hemorrhage (SAH). An initial vascular insult, such as aneurysmal rupture resulting from multiple risk factors, leads to blood extravasation into the subarachnoid space. Such an event leads to complications such as cognitive impairment, speech difficulties, and motor disability. The current treatment for SAH includes endovascular coiling, aneurysmal clipping, and pharmacotherapy with Nimodipine. Figure created with Biorender.com.

and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors [Zhang et al., 2018; Kirdajova et al., 2020]. This excitotoxicity triggers intracellular calcium overload, exacerbating oxidative stress and amplifying mitochondrial dysfunction [Kirdajova et al., 2020]. Free radicals like superoxide anions, hydroxyl radicals, and NO potentiate further damage, acting as signaling molecules in the apoptotic cascade [Kirdajova et al., 2020]. Altered ion homeostasis is another feature post-SAH, prominently illustrated by changes in sodium and potassium channel functioning [Sehba et al., 2011]. Dysregulated ion channel activity compromises neuronal membrane potentials, instigating cellular depolarization and contributing to the escalation of intracellular calcium levels, thus perpetuating the cycle of cellular damage [Zhang et al., 2018; Kirdajova et al., 2020; Harukuni and Bhardwaj., 2006; Sehba et al., 2011]. Many recent studies have pointed out that the underlying causes of many post-SAH neurological deficits are neuroinflammation and apoptosis. On the other hand, apoptosis leads to deleterious effects, including microcirculatory dysfunction, BBB disruption, and intracranial edema, cumulatively contributing to the neurological deficits observed in post-SAH patients [MacDonald., 2014].

One of the significant limitations in treating SAH is that traditional pharmacological approaches are ineffective due to their inability to cross the BBB. While nimodipine treatment can restrain cerebral vasospasm and delayed cerebral ischemia, it cannot prevent neuroinflammation, apoptosis, and endothelial dysfunction after SAH [Castanares-Zapatero et al., 2011; Viderman et al., 2021]. Thus, alternative therapies that are efficacious for restraining neuroinflammation, apoptosis, and endothelial dysfunction are needed to ease SAH-induced brain dysfunction. In this context, stem cell-derived EV therapy, particularly those derived from MSCs, appears attractive for treating SAH due to its capability to modulate inflammation, promote tissue repair, and enhance angiogenesis [Kalluri and LeBleu, 2020; Teng and Fussenegger, 2020; Bang and Kim, 2019].

3. Mesenchymal stem cell-derived extracellular EVs

EVs, nanosized particles enclosed by membranes produced by cells, carry nucleic acids, lipids, signaling molecules, and proteins [Kalluri and LeBleu, 2020; Teng and Fussenegger, 2020; Shetty and Upadhya, 2021]. EVs secreted by various cells can be found in virtually all biological fluids [Hessvik and Llorente, 2018; Teng and Fussenegger, 2020] and can also be isolated from cell culture media [Hessvik and Llorente, 2018; Kalluri and LeBleu, 2020; Teng and Fussenegger, 2020; Upadhya et al., 2020a,b]. EVs play vital biological roles in maintaining cellular homeostasis and distributing biomolecules between cells and tissues [Lasser et al., 2011; Vogel et al., 2018]. EVs comprise exosomes and microvesicles produced through distinct pathways [Teng and Fussenegger, 2020; Lasser et al., 2011]. The endosomal pathway generates exosomes, the smallest EVs with a diameter ranging from 30 to 150 nm (nm). Exosome generation sequentially involves the internalization of the plasma membrane forming early endosomes, inward invaginations into early endosomes creating intraluminal vesicles (ILVs) leading to the formation of multivesicular bodies (MVBs) [Teng and Fussenegger, 2020], and maturation and sorting of ILVs via trans-Golgi network convergence with the MVBs, facilitated by Rab GTPases and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) [Robbins and Morelli, 2014]. While some MVBs get into the lysosomal pathway, the other MVBs fuse with the plasma membrane, a process regulated by additional Rab GTPases and SNARE complexes, and release of ILVs into the extracellular space, now referred to as exosomes [Kalluri and LeBleu, 2020]. Microvesicles, ranging from 100 to 1000 nm, are generated via outward budding and fission of the plasma membrane, a process regulated by lipidic and proteinaceous factors [Hessvik and Llorente, 2018]. Both exosomes and microvesicles carry a variety of bioactive molecules and have a role in intercellular communication under physiological conditions. The guidelines of the International Society for extracellular vesicles discourage the use of terminologies such as exosomes and microvesicles in the absence of clear evidence of their production process by parental cells and recommend a general terminology of "EVs" for vesicles secreted by cells (Thery et al., 2018; Welsh et al., 2024; Upadhya and Shetty, 2024). EVs contain proteins, lipids, reparative cytokines, messenger RNAs (mRNAs), miRNAs, and long non-coding RNAs. Among these, miRNAs within EVs serve as mediators of intercellular communication, robust biomarkers for diagnostic and prognostic evaluations in disease conditions, and components of therapeutic effects mediated by stem cell-derived EVs in animal models of disease [McGeer and McGeer, 2015; Long et al., 2017; Vogel et al., 2018; Upadhya et al., 2020b; Upadhya and Shetty, 2021; Shetty and Upadhya, 2021; Upadhya et al., 2022; Ayyubova et al., 2023].

Stem cell-derived EVs have garnered significant interest due to their potential therapeutic benefits in diseases, including neurological and neurodegenerative diseases. Several characteristics render stem cell-derived EV therapy appealing for human diseases. First, because of their nano size, low immunogenicity, and therapeutic miRNA and protein cargo, stem cell-derived EVs are considered suitable for restraining the multifaceted pathophysiology in human disorders. Second, transferring miRNAs and proteins from stem cell-derived EVs to recipient cells can regulate various cellular pathways [Szabo and Bala, 2013; Szabo and Momen-Heravi, 2017]. Third, therapeutic stem cell-derived EVs are convenient for long-term storage at low temperatures and can be transported on ice without losing their biological activity (Gorgens et al., 2022). Fourth, stem cell-derived EVs can cross the BBB following intravenous administrations or be delivered quickly to various neural cells via intranasal administrations [Long et al., 2017; Kodali et al., 2019; Attaluri et al., 2023]. Fifth, stem cell-derived EVs could also be a potential vehicle for targeted drug delivery to the brain, which could enhance their therapeutic effects [Liu et al., 2021; Meng et al., 2020]. However, it is essential to characterize EVs shed by distinct stem cell types using multiple assays, including small RNA sequencing and proteomic studies (Upadhya et al., 2020b), as the miRNA and protein composition of EVs can vary depending on the age and health of donors and culture conditions and may also potentially include harmful miRNAs or proteins.

While EVs from different types of stem cells are being researched for their potential therapeutic benefits, a significant focus has been on MSC-EVs, as they contain specific bioactive molecules that can effectively mediate antiinflammatory and neuroprotective effects, showing great promise for improving treatment approaches for SAH. MSC-EV therapy is increasingly considered a promising approach for easing brain dysfunction in many neurological and neurodegenerative diseases involving acute or chronic neuroinflammation and neurodegeneration. MSC-EVs represent a cell-free therapy that harnesses mesenchymal stem cell secretome's regenerative and immunomodulatory properties [Kim et al., 2016; Long et al., 2017; Kodali et al., 2023a, b]. One key advantage of MSC-EVs is their potential for therapeutic application while circumventing limitations associated with MSC-based therapies, such as safety concerns and immunogenicity. Unlike MSCs, EVs lack the potential for uncontrolled proliferation and tumorigenicity, enhancing their safety profiles for clinical applications. They also exhibit excellent stability and shelf-life, making them suitable for storage and transportation. MSC-EVs have also been explored as delivery vehicles for therapeutic agents, ranging from small molecule drugs to vaccines and RNA-based therapies [Upadhya and Shetty, 2021]. In models of traumatic brain injury and neuroinflammation, MSC-EVs have emerged as multifunctional agents, releasing lipids, proteins, enzymes, miRNAs, and mRNAs possessing the capacity to modulate the proinflammatory M1 microglial phenotypes (i.e., classical pathway microglia) into antiinflammatory M2 phenotypes (i.e., alternative pathway microglia), to control the neuroinflammatory milieu and to prevent the progression of acute neuroinflammation into chronic neuroinflammation by inhibiting the activation of several inflammatory signaling cascades [McGeer and McGeer, 2015; Xiong et al., 2020; Kodali et al., 2023a]. In addition to

naturally enriched miRNAs, engineered MSC-EVs loaded with specific miRNAs have improved recovery in various models of human diseases, including traumatic brain injury [Zhang et al., 2022]. MSC-EVs have also exhibited neuroprotective properties by fostering neurogenesis, synaptogenesis, and angiogenesis post-injury [Elia et al., 2019; Kodali et al., 2023b].

However, the most appropriate method for separating MSC-EVs from MSC culture media and the best route of MSC-EVs administration for maximizing benefits in neurological and neurodegenerative conditions are still being worked out. Various techniques are available for isolating EVs from culture media, including precipitation, ultracentrifugation, ultrafiltration, density gradient centrifugation, and size exclusion chromatography (SEC) [Upadhya and Shetty, 2024]. Each method has its advantages and limitations in terms of the purity and yield of EVs. It is essential to choose a suitable isolation method that strikes a balance between purity and yield. For instance, ultracentrifugation and SEC are commonly used when isolating MSC-EVs [Konoshenko et al., 2018]. Ultracentrifugation requires minimal manipulation of the culture media and can yield high concentrations of EVs. However, EVs isolated through this method often contain non-EV proteins and may show membrane damage, potentially affecting their cargo delivery to target cells [Piffoux et al., 2021]. On the other hand, SEC-isolated EVs generally lack non-EV proteins and have intact membranes, but the SEC procedure leads to lower EV yields. Nonetheless, it is yet to be determined whether the specific isolation method for MSC-EVs from MSC culture media affects their therapeutic potential, as there is a lack of direct comparative studies between MSC-EVs isolated via ultracentrifugation vis-a-vis SEC. Regarding administration routes, intranasal delivery of MSC-EVs offers a non-invasive approach in which a maximal amount of EVs enter the brain and are incorporated into or come in contact with various neural cells [Long et al., 2017; Kodali et al., 2019, 2023a]. This route allows EVs to enter the brain through the olfactory pathway, bypassing the BBB [Buschmann et al., 2021]. Furthermore, recent studies have demonstrated that intranasal administration of EVs leads to the rapid targeting of EVs into neurons and microglia in virtually all brain regions [Upadhya et al., 2020b; Attaluri et al., 2023]. It has been suggested that intranasally administered EVs move through the cerebrospinal fluid in the subarachnoid space and quickly flow into the interstitial space in the brain because of communication between nasal lymphatics and the subarachnoid space [Johnston et al., 2004; Zakharov et al., 2004]. Intravenous administration, while minimally invasive and can mediate global immunomodulation, faces challenges. Most EVs get trapped in organs such as the lungs, liver, kidneys, and spleen, with only a minority entering the affected regions of the brain through the BBB [Aimaletdinov Gomzikova, 2022]. Intracerebral delivery can directly target the injury site, offering high local concentrations of EVs, but it is highly invasive and associated with significant procedural risks [Nieland et al., 2023]. Balancing these factors is crucial for optimizing the therapeutic benefits of MSC-EVs in treating SAH. Future studies need to compare the efficacy and pros and cons of various routes of MSC-EVs administration in animal models of neurological and neurodegenerative diseases.

Several preclinical studies have suggested the promise of MSC-derived EVs for curtailing neuroinflammation, neuronal apoptosis, and cerebral vasospasm in SAH models [Jafarinia et al., 2020; Khanh et al., 2020]. Hence, MSC-derived EVs have the potential to overcome the limitations of current SAH treatments to provide a versatile platform for delivering a range of therapeutic agents to affected brain regions. Since the pathophysiology of SAH is an intricate web of interconnected and concurrent events that span from vascular, neuronal, and glial alterations to disruptions in biochemical and ionic homeostasis, with each influencing and amplifying the other [Palade et al., 2013; Li et al., 2022], novel therapies capable of concurrently modulating these pathways are of great interest. MSC-derived EVs have promise due to their robust neuroprotective, antioxidant, antiinflammatory, neurogenic, and synaptogenesis effects [Kim et al., 2016, Long et al., 2017; Kodali et al., 2023a, b]. Several recent studies have reported the neuroprotective and antiinflammatory effects of MSC-EVs in experimental models of SAH, which are discussed in the following section.

4. Promise of MSC-EVs for treating SAH

Table 1 shows details of studies discussed in this section. A study by Gao and colleagues reported that intravenous administration of 100 μg rat bone marrow-derived MSC-EVs 1 h after SAH induction in a rat model significantly reduced brain edema, decreased blood-brain barrier permeability, attenuated neuronal apoptosis, and improved neurobehavioral outcomes [Gao et al., 2020]. This study employed the ultracentrifugation method to isolate EVs from MSC culture media comprising EV-depleted fetal bovine serum. Assessment of neurological deficits 48 h after SAH through measures such as spontaneous activity, reaction to side stroking, and limb symmetry revealed that SAH rats treated with MSC-EVs exhibited alleviation of neurological deficits compared to SAH rats receiving phosphate-buffered saline (PBS), implying neurological recovery promoted by MSC-EVs. Morris water maze test performed two weeks after SAH revealed improved spatial learning and memory function in SAH rats receiving MSC-EVs compared to SAH rats receiving PBS. Brain water content was also decreased in SAH rats receiving EVs, suggesting EV-mediated alleviation of brain edema. Furthermore, MSC-EV treatment reduced SAH-mediated neuronal apoptosis by upregulating the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) and downregulating the pro-apoptotic proteins bcl-2-like protein 4 (Bax) and cleaved caspase-3 [Gao et al., 2020].

Moreover, miRNA sequencing revealed that rat MSC-EVs were highly enriched with miRNA-21-5p (~23% of total miRNA content) [Gao et al., 2020]. Notably, SAH led to a transient increase in miRNA-21-5p expression, likely a mechanism of innate neuroprotective response in the injured brain in the acute phase. Consistent with this concept, intravenous administration of MSC-EVs naturally enriched with miR-21-5p after SAH led to neuroprotection via anti-apoptotic effects. However, such effects were lost when MSC-EVs with miR-21-5p knockdown were administered after SAH. As miR-21-5p inhibits the phosphatase and tensin homolog/phosphatidylinositol 3,4,5-trisphosphate-protein kinase B (PTEN/PI3K-AKT) signaling pathway involved in apoptosis, administration of MSC-EVs naturally enriched with miR-21-5p alleviated SAH-mediated upregulation of PTEN/PI3K-AKT leading to anti-apoptotic effects. The role of miR-21-5p within MSC-EVs in neuroprotective effects was also apparent from the loss of MSC-EV-mediated effects in the presence of miR-21-5p inhibitors [Gao et al., 2020]. Thus, administering EVs enriched with miR-21-5p could provide considerable neuroprotective effects after SAH. Apart from the rat MSC-EVs employed in the study, EVs shed by human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) are also enriched with miR-21-5p [Upadhya et al., 2020b, 2022]. The administration of such hiPSC-NSC-EVs could be considered for SAH patients in the future after testing their efficacy in preclinical models of SAH.

Another study also showed the efficacy of MSC-EVs in a rat model of SAH [Xiong et al., 2020]. In this study, rat bone marrow-derived MSC-EVs or PBS were intravenously administered 1 h after SAH. Such treatment improved neurological function, reduced brain edema, and enhanced BBB integrity. These improvements were associated with increased expression of miR-129-5p and reduced concentration of high-mobility group box 1 protein (HMGB1), a protein that leaks from the nucleus and drives neuroinflammation in disease conditions (Madhu et al., 2019). Furthermore, MSC-EV treatment reduced the SAH-mediated upregulation of toll-like receptor 4 (TLR4), tumor necrosis factor-alpha (TNF-α), and p53. Overall, the study implied that MSC-EVs are proficient in alleviating EBI after SAH by promoting anti-inflammatory pathways, and such effects were mediated through enhanced miR-129-5p expression in the brain [Xiong et al., 2020]. However, how MSC-EVs enhance the expression of miR-129-5p in the brain remains to be determined.

A study by Han and associates found that intravenous administration

Table 1
Summary of the effects of MSC-EVs in SAH Models.

Study and SAH Model	Source of MSCs and EV purification method	Route, timing of EV treatment, and dose	miRNA studied	Outcome/Findings
Gao et al. *(2020)*, Intravascular perforation (internal carotid artery)	Rat bone marrow, Ultracentrifugation	Caudal vein injection, 1h post-SAH, 100 μg	miR-21-5p	miR-21-5p mediated the neuroprotective effects of MSC-EVs. miR-21-5p targeted the PTEN/Akt axis in neurons to alleviate EBI apoptosis after SAH.
Xiong et al. *(2020)*, Intravascular perforation	Rat bone marrow, Ultracentrifugation	Femoral vein injection, 1h post-SAH, 200 μg	miR129-5p	MSC-EVs alleviated EBI after SAH through miR-129-5p's anti-inflammatory and antiapoptotic effects. miR-129-5p decreased the HMGB1-TLR4 pathway activity.
Han et al. *(2021)*, Intravascular perforation (common carotid and internal carotid arteries)	Rat bone marrow, Ultracentrifugation	Caudal vein injection, 10 m post-SAH, 100 μg	n/a	MSCs-EVs promoted M2 microglia polarization and suppressed SAH-induced neuroinflammation. MSCs-EVs promoted the phosphorylation of AMPK post-SAH. MSCs-EVs decreased the activation of NF-κB post-SAH.
Zhao et al. *(2019)*, Blood injection (femoral artery to cisterna magna)	Human umbilical cord (hUC), Ultracentrifugation	Femoral vein injection, 1h post-SAH, 400 μg	miR-206	Administration of hUC-MSC-EVs with miR-206-knockdown attenuated early brain injury after SAH by targeting BDNF.
Liu et al. *(2021)*, Blood injection (occipital cistern)	Human umbilical cord, Ultracentrifugation	Tail vein injection, 100 μg/mL	miR-26b-5p	miR-26b-5p containing EVs inhibited p38 MAPK/STAT3 signaling pathway to alleviate oxyhemoglobin-induced cell injury.
Lai et al. *(2020)*, Intravascular perforation (suprachiasmatic cistern)	Mouse bone marrow, Ultracentrifugation	Peripheral injection, 20 μg exosome protein mixed with 20 μL miR-193-b-3p	miR-193b-3p	EVs loaded with miR-193b-3p weakened neuroinflammation by suppressing the expression and the activity of HDAC3 and increasing NF-κB p65 acetylation after SAH.
Qian et al. *(2022)*, Intravascular perforation (anterior cerebral artery and middle cerebral artery)	Mouse bone marrow Ultracentrifugation	Femoral vein injection, 1h post-SAH, 200 μg	miR-140-5p	MSC-EVs containing miR-140-5p inhibited brain injury and microglial M1 activation in SAH mice. miR-140-5p in MSC-EVs decreased ALK5 expression to inhibit brain injury and microglial M1 activation in mice after SAH.

of 100 μg rat MSC-EVs 10 min after SAH in rats significantly decreased neuronal apoptosis and brain edema while suppressing microglial activation and production of proinflammatory cytokines interleukin-1 beta (IL-1β) and TNF-α [Han et al., 2021]. In this study, SAH rats receiving MSC-EVs displayed improved neurological scores and reduced brain water content in cerebral hemispheres, cerebellum, and brainstem 48 h post-SAH. While rats with SAH alone displayed increased numbers of activated microglia, SAH rats receiving MSC-EVs exhibited a significantly reduced number of activated microglia, elucidating the competence of MSC-EVs to suppress microglial activation after SAH. Moreover, proinflammatory genes associated with the M1 microglial phenotype, such as inducible nitric oxide synthase (iNOS), cluster of differentiation 16 (CD16), CD11b, and IL-1β, were upregulated in rats with SAH alone but reduced in SAH rats receiving MSC-EVs, suggesting the possible transformation of M1 microglia into M2 microglia [Han et al., 2021]. Indeed, the expression of genes linked to the M2 microglial phenotype, such as transforming growth factor-beta (TGF-β), arginase 1 (Arg1), and CD206, were upregulated in the parietal cortex and the hippocampus of SAH rats receiving MSC-EVs. Overall, the study demonstrated that intravenous administration of rat MSC-EVs after SAH can mediate neuroprotective, antiinflammatory, and immunomodulatory effects, suggesting their potential for translation as a novel cell-free therapy.

In summary, preclinical evidence indicates that naïve bone marrow derived MSC-EVs exhibit significant therapeutic efficacy in rodent models of SAH through various mechanisms, including suppression of neuroinflammation, inhibition of apoptosis, promotion of angiogenesis and neuroregeneration, and polarization of microglia towards a neuroprotective phenotype. However, the studies discussed above lack diversity, especially concerning the source of MSCs, the isolation method of MSC-EVs, the route of administration, and the animal subjects. All three studies tested the intravenous administration of EVs from rat bone marrow-derived MSCs isolated through the ultracentrifugation method in a rat model of SAH [Gao et al., 2020; Xiong et al., 2020; Han et al., 2021]. Additional studies in standardized SAH models using various doses of EVs isolated via ultracentrifugation and SEC methods from diverse sources of naïve MSCs (e.g., rat, mouse, and human bone marrow and adipose tissue-derived MSCs) comparing the efficacy of various routes of administration (e.g., intranasal, intravenous, and intracerebral) are critical. Furthermore, appropriate methods that optimize the generation of clinical grade MSC-EVs with consistent cargo in batch-to-batch EV preparations need to be developed, and the most appropriate post-SAH interval at which MSC-EVs administration leads to maximal therapeutic benefits needs to be identified. Achieving a consensus on these methods will establish a standardized framework to enhance reproducibility and reliability for future clinical applications of MSC-EVs to SAH patients.

5. Mechanisms by which MSC-derived EVs ease SAH-induced brain dysfunction

Recent studies have suggested several molecular mechanisms underlying the antiapoptotic and antiinflammatory effects of MSC-derived EVs in experimental SAH. These include inhibition of apoptosis through activation of brain-derived neurotrophic factor (BDNF), cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), and PTEN/AKT signaling pathways in neurons [Gao et al., 2020; Zhao et al., 2019] (Fig. 2). MSC-EVs also suppress microglial proinflammatory responses by downregulating HMGB1-TLR4 signaling and modulating AMP-activated protein kinase/nuclear factor kappa B (AMPK/NF-kB), p38 mitogen-activated protein kinase and signal transducer and activator of transcription 3 (p38-MAPK/STAT3), and activin receptor-like kinase 5/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (ALK5/NOX2) pathways to induce an antiinflammatory M2 microglial phenotype [Han et al., 2021; Liu et al., 2021; Xiong et al., 2020] (Fig. 3). Epigenetic modulation through miR-193b-3p mediated suppression of histone deacetylase 3 (HDAC3) and increased NF-kB p65 acetylation has also been demonstrated as an anti-inflammatory mechanism mediated by mouse MSC-EVs loaded with miR-193b-3p [Lai et al., 2020] (Fig. 3). The following sections provide a detailed discussion of pathways involved in antiapoptotic and anti-inflammatory effects mediated by MSC-EVs in rat models of SAH.

5.1. Antiapoptotic effects of MSC-EVs in models of SAH

Apoptosis of neurons and other brain cells is a central pathological process that contributes to early neurodegeneration following SAH.

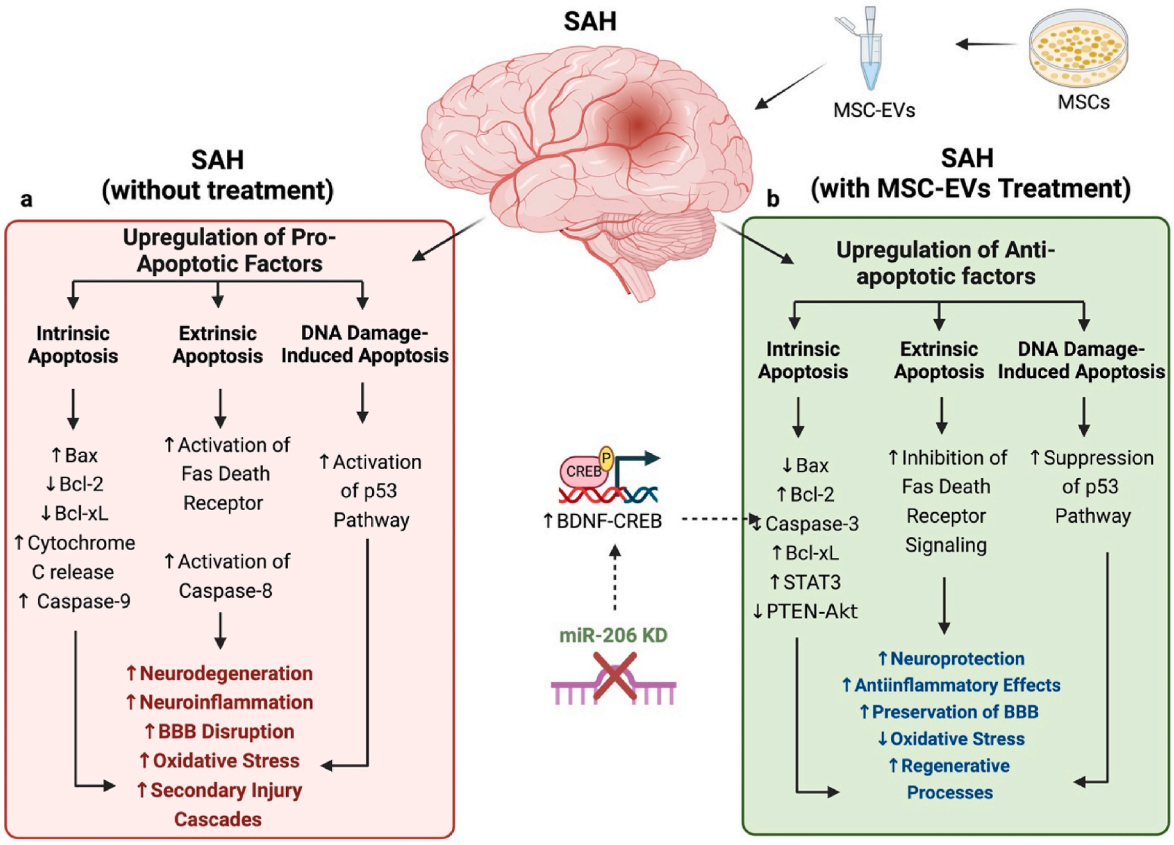


Fig. 2. A schematic representation of antiapoptotic effects of mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) in subarachnoid hemorrhage (SAH) models. The box on the left shows that SAH-induces upregulation of several pro-apoptotic factors, including bcl-2-like protein 4 (Bax), cytochrome C, caspases 8 and 9, Fas death receptor, and p53 pathway concomitant with the downregulation of antiapoptotic factors B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra-large (Bcl-xL). The box on the right shows that MSC-EVs treatment after SAH leads to the upregulation of anti-apoptotic factors including inhibition of Fas death receptor signaling and p53 pathways, leading to neuroprotection associated with antioxidant and anti-inflammatory effects and protection of the blood-brain barrier. The figure also shows the role of miRNA-206 in promoting pro-apoptotic effects, as its knockdown in MSC-EVs amplified BDNF expression and antiapoptotic effects. Figure created with Biorender.com.

Therapeutic targeting of apoptotic pathways has shown promise for neuroprotection after SAH. Zhao and collaborators quantified the anti-apoptotic effects at 48 h following intravenous administration of 400 μg EV derived from human umbilical cord MSCs (hUC-MSC-EVs) 1 h after SAH induced by double blood injection [Zhao et al., 2019]. The procedure involved the collection of 0.2 ml of autologous blood from the femoral artery, followed by its injection into the cisterna magna over 2 min. In comparison, the sham group received 0.2 ml of saline. Additionally, 0.2 ml of fresh blood was withdrawn from the opposite side's femoral artery and injected into the cisterna magna again [Zhao et al., 2019]. Notably, administration of hUC-MSC-EVs with knockdown of miR-206 significantly reduced neuronal apoptosis in the brain after SAH with downregulation of pro-apoptotic genes Bax and caspase-8 and upregulation of the anti-apoptotic gene Bcl-2 (Fig. 2). hUC-MSC-EVs with knockdown of miR-206 alleviated neurological deficits and brain edema after SAH by targeting BDNF [Zhao et al., 2019]. Additional investigation suggested the role of BDNF-CREB signaling activation in anti-apoptotic effects mediated by hUC-MSC-EVs with knockdown of miR-206, as reduced miR-206 can increase BDNF concentration and the phosphorylation of its receptor, tropomyosin receptor kinase B [Shi et al., 2022]. Such signaling activated the transcription factor CREB via phosphorylation at Ser133, which induced genes with cAMP response elements, including the anti-apoptotic genes Bcl-2 and B-cell lymphoma-extra-large (Bcl-xL). Upregulation of Bcl-2 and Bcl-xL and downregulation of the pro-apoptotic genes Bax, caspase-3, and caspase-9 in neurons reduced apoptosis in rats receiving hUC-MSC-EVs with knockdown of miR-206 after SAH [Zhao et al., 2019]. Thus, BDNF-CREB signaling is likely one of the primary mechanisms mediating the anti-apoptotic effects of hUC-MSC-EVs with knockdown of miR-206 when administered early after SAH. Transferring other cargo by hUC-MSC-EVs likely also contributed to the neurotrophic signaling cascade to upregulate neuronal anti-apoptotic gene expression. These findings also suggest that EVs enriched with neuroprotective factors like BDNF could be employed to reduce apoptosis after SAH.

The PI3K/AKT signaling is another pathway that regulates neuronal survival and apoptosis. Activation of AKT leads to phosphorylation of downstream targets that inhibit apoptosis and promote cell survival. Gao and associates demonstrated that rat bone marrow-derived MSC-EVs activate the PTEN/AKT pathway in cortical neurons to elicit anti-apoptotic effects in a rat model of SAH [Gao et al., 2020]. MSC-EVs naturally enriched with miR-21-5p incorporated into neurons following intravenous administration, which decreased the expression of PTEN, a phosphatase that can negatively regulate AKT signaling by catalyzing PI3K dephosphorylation (Fig. 3). Thus, by mediating PTEN suppression, MSC-EVs enhanced AKT activation, resulting in reduced expression of the downstream pro-apoptotic proteins Bax and cleaved caspase-3. To confirm the functional relevance of AKT activation, the authors showed that a selective AKT inhibitor MK2206 could abrogate the antiapoptotic effects of MSC-EVs [Gao et al., 2020]. To further demonstrate the role of miR-21-5p in antiapoptotic effects, the authors showed that miR-21-5p inhibition blocked the neuroprotective effects of MSC-EVs [Gao et al., 2020]. Thus, the two available studies on the neuroprotective effects of MSC-EVs in SAH indicate that MSC-EVs containing miR-206 could be harmful as they may promote apoptosis.

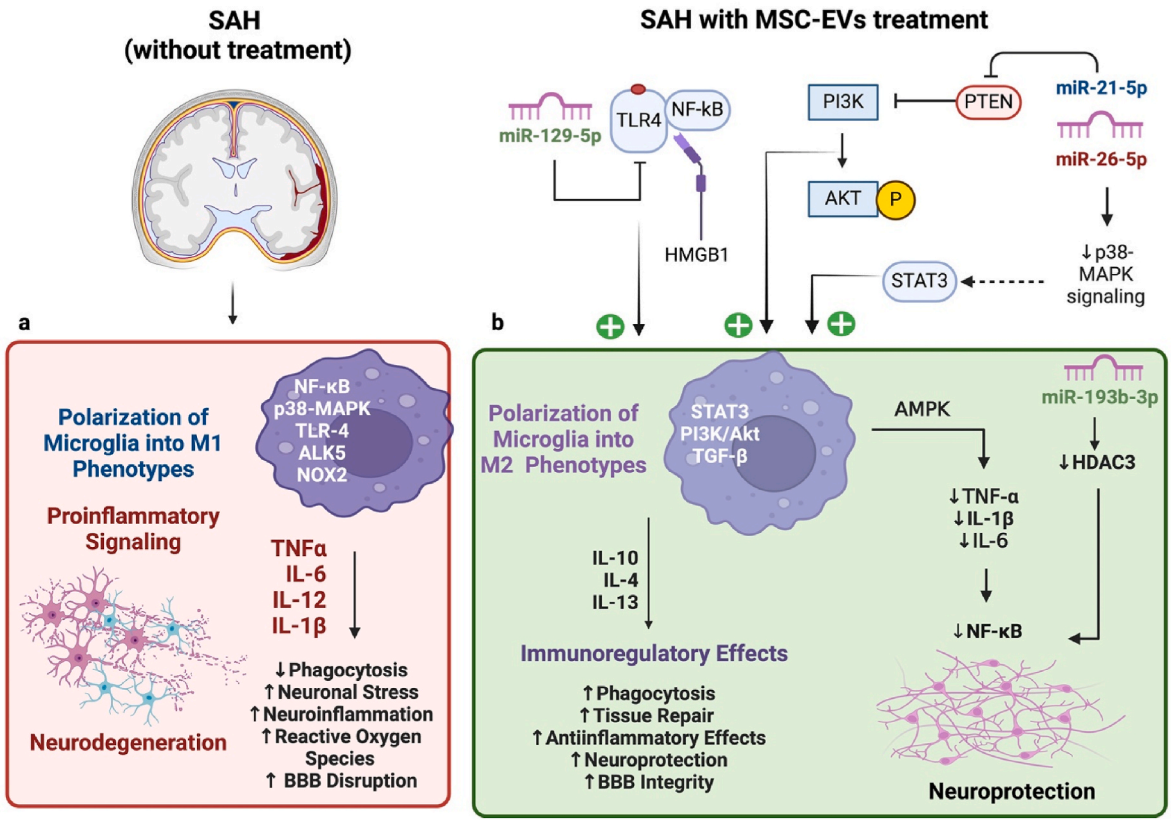


Fig. 3. A schematic representation of the response of microglia to subarachnoid hemorrhage (SAH) and the therapeutic effects of MSC-EVs. The box on the left shows that SAH-induces polarization of microglia into M1 phenotypes with upregulation of nuclear factor-Kappa B (NF-kB), p38-mitogen activated protein kinase (p38-MAPK), toll-like receptor-4 (TLR-4), activin receptor-like kinase 5/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (ALK5/NOX2) signaling with increased release of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1β, and IL-12, leading to decreased phagocytosis, increased oxidative stress, blood-brain barrier (BBB) disruption and neurodegeneration. The box on the right shows that MSC-EVs treatment after SAH induces polarization of microglia into M2 phenotype with modulation of signal transducer and activator of transcription 3 (STAT3), the phosphatase and tensin homolog (PTEN)/protein Kinase B (AKT), and transforming growth factor-beta (TGF-β) beta pathways and increased release of antiinflammatory cytokines IL-4, IL-10, and IL-13, which leads to increased phagocytosis, antiinflammatory effects, preservation of BBB integrity and neuroprotection. The top of the figure also highlights the roles of miR-129-5p, miR-21-5p, and miR-26-5p in MSC-EVs in mediating these effects by inhibiting TLR4, PTEN, and p38-MAPK signaling. Figure created with Biorender .com.

Conversely, MSC-EVs naturally enriched with miR-21-5p are highly beneficial for antiapoptotic effects (Zhao et al., 2019; Gao et al., 2020). Therefore, verifying the miRNA composition of EVs isolated from different MSCs is crucial to ensure they do not contain miRNAs that could cause adverse effects.

5.2. *Antiinflammatory effects of MSC-EVs in models of SAH*

Neuroinflammation elicited by microglial activation significantly contributes to EBI following SAH [Sriram et al., 2022; Fujii et al., 2013]. Increased secretion of proinflammatory proteins such as cytokines, chemokines, and danger-associated molecular patterns (DAMPs) may exacerbate neuronal apoptosis, blood-brain barrier disruption, and cerebral edema. Xiong and associates reported that rat bone marrow-derived MSC-EVs mitigated neuroinflammation following injection into the cisterna magna in a rat model of SAH by suppressing the HMGB1 signaling axis in microglia [Xiong et al., 2020]. HMGB1, a nuclear protein passively released by necrotic cells or actively secreted by stressed and activated cells, leaks into the extracellular space as a DAMP to elicit inflammation [Xiong et al., 2020; Magna and Pisetsky, 2014]. Through toll-like receptors 4 (TLR4), HMGB1 stimulates classical (M1) microglial activation and the release of deleterious proinflammatory mediators [Magna and Pisetsky, 2014]. The authors reported that incorporating 200 μg of MSC-EVs containing miR-129-5p into microglia reduced HMGB1 secretion [Xiong et al., 2020]. Such inhibition of HMGB1 resulted in downstream attenuation of TLR4-myeloid differentiation primary response gene 88 (MyD88)-NF-kB signaling, with reduced nuclear translocation of NF-kB p65. Consequently, the release of TNF-α, IL-1β, and IL-6 was diminished. The antiinflammatory effects were also associated with the polarization of proinflammatory M1 microglia into antiinflammatory M2 phenotypes. An additional experiment showed that miR-129-5p knockdown in MSC-EVs reversed the antiinflammatory effects [Xiong et al., 2020]. Thus, suppression of the HMGB1-TLR4-NF-κB neuroinflammatory axis in microglia by MSC-EVs protected the brain after SAH.

Another study has shown that rat bone marrow-derived MSC-EVs induce protective M2 microglia polarization after SAH by increasing the phosphorylation of AMP-activated protein kinase (AMPK) and diminishing NF-kB signaling [Han et al., 2021], which was evident from the downregulation of CD11b, CD16, IL-1β, and iNOS (M1 microglia markers) and upregulation of Arg1, CD206, and TGF-β (M2 markers) following the administration of 100 μg of MSC-EVs. AMPK stimulation inhibited inflammatory signaling in microglia by decreasing the phosphorylation of NF-kB. Overall, MSC-EVs induced a protective M2 microglia phenotype after SAH by activating AMPK, which suppressed downstream NF-κB-mediated proinflammatory signaling [Han et al., 2021].

A study by Qian and colleagues has suggested that mouse bone marrow-derived MSC-EVs can modulate microglial activation and neuroinflammation after SAH by downregulating ALK5 and NOX2 [Qian

et al., 2022]. In this study, 200 μg of MSC-EVs injected through the femoral vein transferred miR-140-5p into microglia, which resulted in ALK5 and NOX2 suppression. ALK5 is a TGF-β receptor that promotes proinflammatory microglial polarization, whereas NOX2 is a catalytic subunit of NADPH oxidase that mediates microglial reactive oxygen species generation [Qian et al., 2022]. Downregulation of ALK5 and NOX2 reduced the concentration of M1 microglia markers (iNOS, IL-1β, IL-6, and TNF-α) and increased the levels of M2 microglia markers (IL-4, IL-10, and TGF-β1) (Fig. 3). Such phenotype shift was mediated by miR-140-5p transfer, as pre-inhibition of miR-140-5p mitigated the beneficial effects of MSC-EVs [Qian et al., 2022].

Furthermore, studies in a rat model have shown that hUC-MSC-EVs containing miR-26b-5p play a crucial role in reducing microglial signaling by modulating the p38-MAPK/STAT3 pathways after SAH [Liu et al., 2021]. The incorporation of 100 μg hUC-MSC-EVs containing miR-26b-5p following tail vein injection inhibited p38-MAPK phosphorylation and upregulated STAT3 phosphorylation [Liu et al., 2021]. This finding suggests that miR-26b-5p is a key player in the antiinflammatory effects of MSC-EVs. Activation of p38-MAPK signaling promotes proinflammatory microglial polarization, while STAT3 activation is associated with antiinflammatory microglial responses. Accordingly, hUC-MSC-EV treatment reduced iNOS, IL-1β, and TNF-α concentrations and increased IL-10 and TGF-β1 levels. Neutralization of miR-26b-5p abrogated the inhibitory effects of hUC-MSC-EVs on p38-MAPK signaling and M1 microglia activation [Liu et al., 2021]. This emphasizes the intriguing role of miR-26b-5p in the antiinflammatory effects of MSC-EVs, sparking further interest in its potential applications.

While acetylation of NF-kB p65 leads to antiinflammatory effects, deacetylation by HDAC3 can promote proinflammatory effects. Lai and colleagues demonstrated that mouse bone marrow-derived MSC-EVs loaded with miR-193b-3p can mitigate neuroinflammation in a mouse model of SAH by suppressing HDAC3 and increasing NF-κB p65 acetylation [Lai et al., 2020]. Peripheral intravenous injection of 20 μg MSC-EVs loaded with miR-193b-3p led to their incorporation into microglia, reducing HDAC3 expression [Lai et al., 2020]. In this study, the MSC-EVs were conjugated with rabies viral glycoprotein peptide to enable transport across the BBB to deliver miR-193b-3p into microglia. HDAC3 downregulation led to increased acetylation of NF-κB p65 in microglia, which attenuated NF-κB-mediated transcription of proinflammatory genes, including TNF-α, IL-1β, and IL-6. The antiinflammatory effects were also confirmed by reduced microglial activation and myeloperoxidase activity. Thus, MSC-EV mediated delivery of miR-193b-3p could suppress HDAC3 expression in microglia, leading to NF-κB p65 acetylation and reduced microglial activation. These findings reveal epigenetic modulation of NF-κB signaling as a mechanism by which engineered MSC-EVs can attenuate neuroinflammation and confer neuroprotection after SAH.

Overall, in models of SAH, naive rat bone marrow MSC-EVs can reduce inflammation by inhibiting HMGB1 signaling in microglia [Xiong et al., 2020], increasing AMPK phosphorylation, and decreasing NF-kB signaling [Han et al., 2021], as well as downregulating ALK5 and NOX2 [Qian et al., 2022]. Furthermore, naive hUC-MSC-EVs containing miR-26b-5p can modulate microglial signaling by affecting the p38-MAPK/STAT3 pathways [Liu et al., 2021]. Another study suggested that mouse bone marrow MSC-EVs loaded with miR-193b-3p could alleviate neuroinflammation in SAH models by increasing NF-kB p65 acetylation through HDAC3 suppression [Lai et al., 2020]. Thus, both naive and miRNA-loaded MSC-EVs show promise in significantly reducing neuroinflammation following SAH.

6. Concluding Remarks, limitations, and future directions

Many preclinical studies have supported the potential use of MSC-derived EVs as a treatment for SAH. Traditional MSC therapies face multiple challenges, including chromosome abnormalities and cell transformations in MSCs cultured long-term, thromboembolism, fibrosis, and the potential for the formation of malignant neoplasms [Baranovskii et al., 2022]. MSCs likely also do not cross the BBB when administered intravenously or intranasally. In contrast, MSC-EVs offer a promising alternative for SAH treatment due to their low or nonexistent immunogenicity and lack of tumorigenic potential [Zhu et al., 2017]. They also can quickly target injured brain regions following non-invasive intranasal administration [Long et al., 2017; Kodali et al., 2019, 2023a]. Furthermore, MSC-EVs can efficiently cross the BBB, and they can also be engineered to overexpress specific proteins or loaded with specific miRNAs to deliver therapeutic molecules of interest to enhance their antiapoptotic and neuroinflammatory effects. MSC-EVs, particularly those enriched with specific miRNAs, have been found to effectively impact vital molecular pathways associated with apoptosis and neuroinflammation, including BDNF-CREB, PTEN/AKT, HMGB1-TLR4, AMPK/NF-kB, p38-MAPK/STAT3, and ALK5-NOX2 signaling. These effects helped to reduce the negative consequences of SAH and suggest a less invasive yet highly targeted strategy for managing SAH. However, there are still significant challenges to overcome before MSC-EVs can be used clinically to treat SAH, and these challenges must be addressed to fully realize the therapeutic potential of MSC-EVs.

The pathogenesis of SAH involves oxidative stress, neuroinflammation, and neurodegeneration [Kaur et al., 2018; Geraghty et al., 2019; Kirdajova et al., 2020 Solár et al., 2022]. MSC-EVs can target these key processes and offer therapeutic benefits for SAH treatment. MSC-EVs have demonstrated abilities to reduce oxidative stress, neuroinflammation, and neuronal cell death, suggesting they could be used to alleviate the damaging effects of SAH. For example, MSC-EVs can target the BDNF-CREB and PTEN/AKT pathways to inhibit neurodegeneration after SAH. In addition, the ability of MSC-EVs to reduce neuroinflammation and oxidative stress by modulating the HMGB1-TLR4, AMPK/NF-kB, p38-MAPK/STAT3 signaling pathways, downregulating ALK5 and NOX2 and suppressing HDAC3 to acetylate NF-kB p65 could be beneficial after SAH. However, further research is needed to fully address whether MSC-EVs can rectify specific aspects of SAH, such as altered ion homeostasis, BBB disruption and cerebral vasospasm, to maximize the therapeutic potential of MSC-EVs in SAH treatment.

Although using MSC-EVs derived from MSCs to treat SAH shows promise, further studies must address several limitations. Preclinical studies, mainly using rodent MSC-EVs, have shown promising results, but many aspects still need to be investigated in this domain. There is a conspicuous paucity of studies investigating the following issues: 1) the efficiency of EVs from human MSCs derived from various sources, such as bone marrow and adipose tissue; 2) the effects of varying doses of MSC-EVs; 3) the potential immunological reactions after high dose allogeneic MSC-EV administration; 4) the miRNA and protein composition of EVs from human MSCs derived from different sources; 5) potential long-term adverse effects; 6) the effects of single versus multiple administrations; 7) the difficulties of clinical translation such as standardization of production, scalability, targeted delivery to sites within the central nervous system, regulatory approval, and commercialization; and 8) there are other signaling pathways yet to be studied and discovered. Furthermore, most preclinical studies have focused on short-term outcomes, such as neuroprotection and reduced neuroinflammation. However, SAH is associated with long-term complications such as cognitive deficits and delayed cerebral ischemia. Thus, future studies need to examine the long-term implications of MSC-EV administration on enduring cognitive deficits after SAH.

Furthermore, the data on EV bioavailability, pharmacokinetics, dosing, and potential off-target effects are yet to be discerned. The most effective timing for MSC-EV intervention post-SAH still needs to be determined. The complexity of the crosstalk between multiple signaling pathways modulated by MSC-EVs also necessitates a comprehensive understanding of their systems-level impact. Mechanistic studies employing advanced molecular techniques, such as single-cell RNA sequencing or proteomics, could provide invaluable insights into the

molecular determinants driving MSC-EV-mediated neuroprotection and antiinflammatory effects. EVs derived from MSCs possess immense therapeutic potential. However, studies on overexpression or knockdown of specific miRNAs may identify specific miRNAs mediating therapeutic effects; however, the additive effects of other molecules contained in EVs cannot be excluded. Through such multidimensional analyses, the full therapeutic potential of MSC-EVs in combating the multifaceted challenges presented by SAH could be unlocked in the coming years to consider clinical application.

The use of MSC-EVs for treating SAH is not currently being tested in clinical trials, but trials are examining their potential in other diseases. Seven published clinical studies have tested the safety of allogeneic human MSC-EVs in conditions such as chronic kidney disease, skin hyperpigmentation, acne scars, graft versus host disease, coronavirus disease-19, and osteoarthritis [Lotfy et al., 2023]. In addition, 14 ongoing clinical trials are investigating the efficacy of allogeneic MSC-EVs in a variety of conditions, including diabetes mellitus, acute ischemic stroke, coronavirus pneumonia, multiple organ dysfunction syndrome, graft versus host disease, Alzheimer's disease, acute respiratory distress syndrome, pulmonary infection, periodontitis, and osteoarthritis [Lotfy et al., 2023]. These studies will assess the safety of MSC-EVs and would likely provide the foundation for future clinical trials in SAH using MSC-EVs.

For consistent results using MSC-EVs, it is essential to establish a standardized method for preparation and administration. Analyzing and comparing different methods will help identify potential issues, such as variations in EV yield, purity, and biological activity. This approach will provide valuable information to standardize future research and improve therapeutic effectiveness. Optimizing cell culture conditions, scalable EV isolation techniques, and stringent characterization protocols are essential to ensure purity, potency, and reproducibility. Determining the best dosing and timing of human MSC-EV administration post-SAH is crucial. Evidence from rodent MSC-EV studies suggests earlier intervention may enhance neuroprotection and reduce secondary injury processes. While intravenous administration is widely used for its practicality, the intranasal route may offer better efficacy by helping EVs reach injured brain regions. Since there have been no clinical trials on SAH, current knowledge on the safety and efficacy of MSC-EVs comes from preclinical studies mainly using rodent MSC-EVs. This highlights the need for preclinical and clinical studies using clinical-grade human MSC-EVs to validate safety and efficacy in human patients.

The clinical translation of human MSC-EV therapy for SAH will require investigations focusing initially on small-scale Phase I trials in SAH patients, primarily assessing the safety of doses, timing of interventions, and the most efficient routes of administration for targeting MSC-EVs into microglia and neurons in injured brain regions. Following the confirmation of safety, large-scale, randomized controlled trials need to evaluate the efficacy of MSC-EVs in SAH populations. However, such studies will require the generation of clinical-grade, off-the-shelf MSC-EVs with a consistent naturally enriched miRNA cargo or MSC-EVs loaded with miRNAs known to modulate neuroinflammation and provide neuroprotection. Obtaining clinical-grade EVs from bone marrow or adipose tissue-derived MSCs with consistent and naturally enriched miRNA cargo is a challenging endeavor because of the inherent heterogeneity of MSCs among volunteers, influenced by factors such as age, sex, and individual health status, which can lead to variability in the miRNA and protein composition of EVs produced. Different sources of MSCs (e.g., bone marrow and adipose tissue) may have distinct characteristics. Long-term cultures of MSCs may result in cellular modifications, senescence, or differentiation into other cell types, impacting the composition and therapeutic efficacy of EVs released by them. Furthermore, ensuring standardized culture conditions for EV production without using animal-derived components such as fetal bovine serum containing bovine serum albumin (BSA) is crucial for clinical translation but can be technically demanding. BSA is commonly used as a supplement for cell culture, but it introduces concerns about potential immunogenicity and contamination of EV biologic. Addressing these issues requires a rigorous regimen of quality control, optimized culture protocols, and advanced characterization techniques to produce reliable and consistent clinical-grade MSC-EVs with naturally enriched miRNA cargo for therapeutic applications.

Additionally, investigating the effectiveness of EVs derived from sources other than bone marrow derived MSCs is of great interest. These sources may include EVs from hiPSC-derived MSCs, NSCs, astrocytes, and microglia. Interestingly, EVs from hiPSC-NSCs or hiPSC-astrocytes could be obtained in large quantities, as they are easier to expand than MSCs and likely release more EVs than MSCs. Moreover, they may offer the advantage of promoting neurogenesis, synaptogenesis, and enhancing brain repair, in addition to neuroprotective and anti-inflammatory effects [Upadhya et al., 2020b; Bonetto et al., 2023]. Several studies have shown that astrocyte-derived EVs could modulate the neuroinflammatory response and maintain neuronal homeostasis [Upadhya et al., 2020b; Pistono et al., 2021].]. Microglia-derived EVs can also regulate neuroinflammation and potentially mitigate secondary brain injury after SAH [Ceccarelli et al., 2021; Makrygianni et al., 2023]. Therefore, investigating the efficacy of EVs generated from multiple neural cell types generated from hiPSCs in parallel, alone, or in combination would likely identify the type with maximal benefits for treating SAH.

Funding

The authors are supported by grants from the National Institutes of Health (NIH-NINDS R01NS106907, NIH-NIA RF1AG074256-01, and NIH-NIA R01AG075440-01 to A.K.S.) and the Department of Defense (W81XWH-17-1-0447 and W81XWH1910548 to A.K.S.). Kiran Sankarappan is supported by the Texas A&M School of Medicine.

8. Ethics declarations

8.1. *Ethics approval and consent to participate*

Not applicable.

8.2. *Consent of publication*

Not applicable.

Competing interests

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Kiran Sankarappan: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Ashok K. Shetty:** Writing – review & editing, Writing – original draft, Resources, Methodology, Data curation, Conceptualization.

Declaration of competing interest

Declarations of interest: **None**.

Data availability

No data was used for the research described in the article.

Acknowledgments

The figures were created with BioRender.com.

Abbreviations

AKT	Protein Kinase B
ALK5	activin receptor-like kinase 5
AMPK	AMP-activated protein kinase
Arg1	Arginase 1
Bax	bcl-2-like protein 4
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
Bcl-xL:	B-cell lymphoma-extra large
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CD11b	Cluster of differentiation 11b
CD16	Cluster of differentiation 16
CD45	Cluster of differentiation 45
CD-206	Cluster of differentiation 206
CREB	cAMP response element binding protein
DAMP	Danger-associated molecular pattern
EBI	Early Brain injury
HDAC3	Histone deacetylase
hiPSC-NSCs	human induced pluripotent stem cell-derived neural stem cells
HMGB1	High-mobility group box 1 protein
hUC-MSC-EVs	Human umbilical cord mesenchymal stem cell-derived extracellular vesicles
ILVs	Intraluminal vesicles
IL-1β	Interleukin-1 beta
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-10	Interleukin 10
iNOS	Inducible nitric oxide synthase
M1	Classical pathway microglia
M2	Alternative Pathway microglia
mRNA	messenger RNA
miRNA	microRNA
MSC	Mesenchymal stem cell
MSC-EVs	Mesenchymal stem cell-derived extracellular vesicles
MVB	Multivesicular body
NADPH	nicotinamide adenine dinucleotide phosphate
NF-kB	nuclear factor kappa B
NOX2	NADPH Oxidase 2
PBS	phosphate buffered saline
PI3K	phosphatidylinositol 3,4,5-trisphosphate
p38-MAPK	p38 family of mitogen-activated protein kinases
PTEN	Phosphatase and tensin homolog
SAH	Subarachnoid Hemorrhage
SEC	Size exclusion chromatography
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptors
STAT3	signal transducer and activator of transcription 3
TGF-β:	Transforming growth factor- β
TNF-α:	Tumor necrosis factor-alpha
TLR4	Toll-like receptor 4

References

Attaluri, S., Gonzalez, J.J., Kirmani, M., Vogel, A.D., Upadhya, R., Kodali, M., et al., 2023. Intranasally administered extracellular vesicles from human induced pluripotent stem cell-derived neural stem cells quickly incorporate into neurons and microglia in 5xFAD mice. Front. Aging Neurosci. 15, 1200445.

Aimaletdinov, A.M., Gomzikova, M.O., 2022. Tracking of extracellular vesicles' biodistribution: new methods and approaches. Int. J. Mol. Sci. 23 (19), 11312.

Ayyubova, G., Kodali, M., Upadhya, R., Madhu, L.N., Attaluri, S., Somayaji, Y., et al., 2023. Extracellular vesicles from hiPSC-NSCs can prevent peripheral inflammation-induced cognitive dysfunction with inflammasome inhibition and improved neurogenesis in the hippocampus. J. Neuroinflammation 20 (1), 297.

Bang, O.Y., Kim, E.H., 2019. Mesenchymal stem cell-derived extracellular vesicle therapy for stroke: challenges and progress. Front. Neurol. 10, 211.

Baranovskii, D.S., Klabukov, I.D., Arguchinskaya, N.V., Yakimova, A.O., Kisel, A.A., Yatsenko, E.M., et al., 2022. Adverse events, side effects and complications in mesenchymal stromal cell-based therapies. Stem Cell Investig 9, 7.

Bonetto, V., Grilli, M., 2023. Neural stem cell-derived extracellular vesicles: mini players with key roles in neurogenesis, immunomodulation, neuroprotection, and aging. Front. Mol. Biosci. 10, 1187263.

Bonita, R., 1986. Cigarette smoking, hypertension, and the risk of subarachnoid hemorrhage: a population-based case-control study. Stroke 17 (5), 831–835.

Brisman, J.L., Song, J.K., Newell, D.W., 2006. Cerebral aneurysms. N. Engl. J. Med. 355 (9), 928–939.

Buschmann, D., Mussack, V., Byrd, J.B., 2021. Separation, characterization, and standardization of extracellular vesicles for drug delivery applications. Adv. Drug Deliv. Rev. 174, 348–368.

Caner, B., Hou, J., Altay, O., Fujii, M., Zhang, J.H., 2012. Transition of research focus from vasospasm to early brain injury after subarachnoid hemorrhage. J. Neurochem. 123 (Suppl. 2), 12–21.

Castanares-Zapatero, D., Hantson, P., 2011. Pharmacological treatment of delayed cerebral ischemia and vasospasm in subarachnoid hemorrhage. Ann. Intensive Care 4 (1), 12, 1.

Ceccarelli, L., Giacomelli, C., Marchetti, L., Martini, C., 2021. Microglia extracellular vesicles: focus on molecular composition and biological function. Biochem. Soc. Trans. 49 (4), 1779–1790.

Claassen, J., Park, S., 2022. Spontaneous subarachnoid hemorrhage. Lancet 400 (10355), 846–862.

Colby, G.P., Coon, A.L., Tamargo, R.J., 2010. Surgical management of aneurysmal subarachnoid hemorrhage. Neurosurg Clin N Am 21 (2), 247–261.

Dhuriya, Y.K., Sharma, D., 2018. Necroptosis: a regulated inflammatory mode of cell death. J. Neuroinflammation 15, 199.

Elia, C.A., Tamborini, M., Rasile, M., Desiato, G., Marchetti, S., Swuec, P., et al., 2019. Intracerebral injection of extracellular vesicles from mesenchymal stem cells exerts reduced Aβ plaque burden in early stages of a preclinical model of Alzheimer's disease. Cells 8 (9), 1059.

Fujii, M., Yan, J., Rolland, W.B., Soejima, Y., Caner, B., Zhang, J.H., 2013. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. Transl Stroke Res 4 (4), 432–446.

Gao, X., Xiong, Y., Li, Q., Han, M., Shan, D., Yang, G., et al., 2020. Extracellular vesicle-mediated transfer of miR-21-5p from mesenchymal stromal cells to neurons alleviates early brain injury to improve cognitive function via the PTEN/Akt pathway after subarachnoid hemorrhage. Cell Death Dis. 11 (5), 363.

Geraghty, J.R., Davis, J.L., Testai, F.D., 2019. Neuroinflammation and microvascular dysfunction after experimental subarachnoid hemorrhage: emerging components of early brain injury related to outcome. Neurocrit Care 31 (2), 373–389.

Görgens, A., Corso, G., Hagey, D.W., Jawad Wiklander, R., Gustafsson, M.O., Felldin, U., Lee, Y., Bostancioglu, R.B., Sork, H., Liang, X., Zheng, W., Mohammad, D.K., van de Wakker, S.I., Vader, P., Zickler, A.M., Mamand, D.R., Ma, L., Holme, M.N., Stevens, M.M., Wiklander, O.P.B., El Andaloussi, S., 2022. Identification of storage conditions stabilizing extracellular vesicles preparations. J. Extracell. Vesicles 11 (6), e12238.

Gu, M., Mei, X.L., Zhao, Y.N., 2021. Sepsis and cerebral dysfunction: BBB damage, neuroinflammation, oxidative Stress, apoptosis and autophagy as key mediators and the potential therapeutic approaches. Neurotox. Res. 39 (2), 489–503.

Han, M., Cao, Y., Guo, X., Chu, X., Li, T., Xue, H., et al., 2021. Mesenchymal stem cell-derived extracellular vesicles promote microglial M2 polarization after subarachnoid hemorrhage in rats and involve the AMPK/NF-κB signaling pathway. Biomed. Pharmacother. 133, 111048.

Harukuni, I., Bhardwaj, A., 2006. Mechanisms of brain injury after global cerebral ischemia. Neurol. Clin. 24 (1), 1–21.

Hessvik, N.P., Llorente, A., 2018. Current knowledge on exosome biogenesis and release. Cell. Mol. Life Sci. 75 (2), 193–208.

Jafarinia, M., Alsahebfosoul, F., Salehi, H., Eskandari, N., Ganjalikhani-Hakemi, M., 2020. Mesenchymal stem cell-derived extracellular vesicles: a novel cell-free therapy. Immunol. Invest. 49 (7), 758–780.

Johnston, M., Zakharov, A., Papaiconomou, C., Salmasi, G., Armstrong, D., 2004. Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates, and other mammalian species. Cerebrospinal Fluid Res. 1 (1), 2.

Kalluri, R., LeBleu, V.S., 2020. The biology, function, and biomedical applications of exosomes. Science 367 (6478), eaau6977.

Kaur, P., Sharma, S., 2018. Recent advances in pathophysiology of traumatic brain injury. Curr. Neuropharmacol. 16 (8), 1224–1238.

Khanh, V.C., Yamashita, T., Ohneda, K., Tokunaga, C., Kato, H., Osaka, M., et al., 2020. Rejuvenation of mesenchymal stem cells by extracellular vesicles inhibits the elevation of reactive oxygen species. Sci. Rep. 10 (1), 17315.

Kim, D.K., Nishida, H., An, S.Y., Shetty, A.K., Bartosh, T.J., Prockop, D.J., 2016. Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. Proc. Natl. Acad. Sci. U.S.A. 113 (1), 170–175.

Kirdajova, D., Kriska, J., Tureckova, J., Anderova, M., 2020. Ischemia-triggered glutamate excitotoxicity from the perspective of glial cells. Front. Cell. Neurosci. 19 (14), 51.

Kodali, M., Castro, O.W., Kim, D.K., Thomas, A., Shuai, B., Attaluri, S., et al., 2019. Intranasally administered human MSC-derived extracellular vesicles pervasively incorporate into neurons and microglia in both intact and status epilepticus injured forebrain. Int. J. Mol. Sci. 21 (1), 181.

Kodali, M., Madhu, L.N., Reger, R.L., Milutinovic, B., Upadhya, R., Gonzalez, J.J., et al., 2023a. Intranasally administered human MSC-derived extracellular vesicles inhibit NLRP3-p38/MAPK signaling after TBI and prevent chronic brain dysfunction. Brain Behav. Immun. 108, 118–134.
Kodali, M., Madhu, L.N., Reger, R.L., Milutinovic, B., Upadhya, R., Attaluri, S., et al., 2023b. A single intranasal dose of human mesenchymal stem cell-derived extracellular vesicles after traumatic brain injury eases neurogenesis decline, synapse loss, and BDNF-ERK-CREB signaling. Front. Mol. Neurosci. 16, 1185883.
Konoshenko, M.Y., Lekchnov, E.A., Vlassov, A.V., Laktionov, P.P., 2018. Isolation of extracellular vesicles: general methodologies and latest trends. BioMed Res. Int., 8545347
Lai, N., Wu, D., Liang, T., Pan, P., Yuan, G., Li, X., et al., 2020. Systemic exosomal miR-193b-3p delivery attenuates neuroinflammation in early brain injury after subarachnoid hemorrhage in mice. J. Neuroinflammation 17 (1), 74.
Lässer, C., O'Neil, S.E., Ekerljung, L., Ekström, K., Sjöstrand, M., Lötvall, J., 2011. RNA-Containing exosomes in human nasal secretions. Am J Rhinol Allergy 25 (2), 89–93.
Li, K., Barras, C.D., Chandra, R.V., Kok, H.K., Maingard, J.T., Carter, N.S., et al., 2019. A review of the management of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. World Neurosurg 126, 513–527.
Li, R., Zhao, M., Yao, D., Zhou, X., Lenahan, C., Wang, L., et al., 2022. The role of the astrocyte in subarachnoid hemorrhage and its therapeutic implications. Front. Immunol. 13, 1008795.
Liu, Z., Wang, B., Guo, Q., 2021. MiR-26b-5p-modified hUB-MSCs derived exosomes attenuate early brain injury during subarachnoid hemorrhage via MAT2A-mediated the p38 MAPK/STAT3 signaling pathway. Brain Res. Bull. 175, 107–115.
Long, Q., Upadhya, D., Hattiangady, B., Kim, D.K., An, S.Y., Shuai, B., et al., 2017. Intranasal MSC-derived A1-exosomes ease inflammation and prevent abnormal neurogenesis and memory dysfunction after status epilepticus. Proc Natl Acad Sci U S A. 114 (17), E3536–E3545.
Lotfy, A., AboQuella, N.M., Wang, H., 2023. Mesenchymal stromal/stem cell (MSC) -derived exosomes in clinical trials. Stem Cell Res. Ther. 14 (1), 66.
Macdonald, R.L., 2014. Delayed neurological deterioration after subarachnoid hemorrhage. Nat. Rev. Neurol. 10 (1), 44–58.
Madhu, L.N., Attaluri, S., Kodali, M., Shuai, B., Upadhya, R., Gitai, D., Shetty, A.K., 2019. Neuroinflammation in Gulf War Illness is linked with HMGB1 and complement activation, which can be discerned from brain-derived extracellular vesicles in the blood. Brain Behav. Immun. 81, 430–443.
Magna, M., Pisetsky, D.S., 2014. The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. Mol Med Camb Mass. 20, 138–146.
Makrygianni, E.A., Chrousos, G.P., 2023. Extracellular vesicles and the stress system. Neuroendocrinology 113 (2), 120–167.
Marcolini, E., Hine, J., 2019. Approach to the diagnosis and management of subarachnoid hemorrhage. West. J. Emerg. Med. 20 (2), 203–211.
McGeer, P.L., McGeer, E.G., 2015. Targeting microglia for the treatment of Alzheimer's disease. Expert Opin. Ther. Targets 19 (4), 497–506.
Meng, W., He, C., Hao, Y., Wang, L., Li, L., Zhu, G., 2020. Prospects, and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv. 27 (1), 585–598.
Myers, M.I., Hines, K.J., Gray, A., Spagnuolo, G., Rosenwasser, R., Iacovitti, L., 2023. Intracerebral transplantation of autologous mesenchymal stem cells improves functional recovery in a rat model of chronic ischemic stroke. Transl Stroke Res. https://doi.org/10.1007/s12975-023-01208-7. Nov 2.
Nieland, L., Mahjoum, S., Grandell, E., Breyne, K., Breakefield, X.O., 2023. Engineered EVs designed to target diseases of the CNS. J Control Release 356, 493–506.
Neifert, S.N., Chapman, E.K., Martini, M.L., Shuman, W.H., Schupper, A.J., Oermann, E. K., et al., 2021. Aneurysmal subarachnoid hemorrhage: the last decade. Transl Stroke Res 12 (3), 428–446.
Osgood, M.L., 2021. Aneurysmal subarachnoid hemorrhage: review of the pathophysiology and management strategies. Curr. Neurol. Neurosci. Rep. 21 (9), 50.
Palade, C., Ciurea, A.V., Nica, D.A., Savu, R., Moisa, H.A., 2013. Interference of apoptosis in the pathophysiology of subarachnoid hemorrhage. Asian J Neurosurg 8 (2), 106–111.
Piffoux, M., Volatron, J., Silva, A.K.A., Gazeau, F., 2021. Thinking quantitatively of RNA-based information transfer via extracellular vesicles: lessons to learn for the design of RNA-loaded EVs. Pharmaceutics 13 (11), 1931.
Pistono, C., Bister, N., Stanová, I., Malm, T., 2021. Glia-derived extracellular vesicles: role in central nervous system communication in health and disease. Front. Cell Dev. Biol. 8, 623771.
Qian, Y., Li, Q., Chen, L., Sun, J., Cao, K., Mei, Z., et al., 2022. Mesenchymal stem cell-derived extracellular vesicles alleviate M1 microglial activation in brain injury of mice with subarachnoid hemorrhage via microRNA-140-5p delivery. Int. J. Neuropsychopharmacol. 25 (4), 328–338.
Rabinstein, A.A., 2013. Subarachnoid hemorrhage. Neurology 80 (5), e56–e59.
Raya, A.K., Diringer, M.N., 2014. Treatment of subarachnoid hemorrhage. Crit. Care Clin. 30 (4), 719–733.
Robbins, P.D., Morelli, A.E., 2014. Regulation of immune responses by extracellular vesicles. Nat. Rev. Immunol. 14 (3), 195–208.
Sehba, F.A., Pluta, R.M., Zhang, J.H., 2011. Metamorphosis of subarachnoid hemorrhage research: from delayed vasospasm to early brain injury. Mol. Neurobiol. 43 (1), 27–40.
Shetty, A.K., Upadhya, R., 2021. Extracellular vesicles in health, and disease. Aging Dis 12 (6), 1358–1362.
Shi, G., Zeng, P., Zhao, Q., Zhao, J., Xie, Y., Wen, D., et al., 2022. The Regulation of miR-206 on BDNF: a motor function restoration mechanism research on cerebral ischemia rats by meridian massage. Evid Based Complement Alternat Med 2022, 8172849.
Sobey, C.G., Faraci, F.M., 1998. Subarachnoid hemorrhage: what happens to the cerebral arteries? Clin. Exp. Pharmacol. Physiol. 25 (11), 867–876.
Solár, P., Zamani, A., Lakatosová, K. Joukal M., 2022. The blood–brain barrier and the neurovascular unit in subarachnoid hemorrhage: molecular events and potential treatments. Fluids Barriers CNS 19, 29.
Sriram, S., Cutler, C., Azab, M., Reddy, R., Peart, R., Lucke-Wold, B., 2022. Neuroinflammation and subarachnoid hemorrhage: a revised look at the literature. Clin Res Commun 5 (3), 14.
Szabo, G., Bala, S., 2013. MicroRNAs in liver disease. Nat. Rev. Gastroenterol. Hepatol. 10 (9), 542–552.
Szabo, G., Momen-Heravi, F., 2017. Extracellular vesicles in liver disease and potential as biomarkers and therapeutic targets. Nat. Rev. Gastroenterol. Hepatol. 14 (8), 455–466.
Tawk, R.G., Hasan, T.F., D'Souza, C.E., Peel, J.B., Freeman, W.D., 2021. Diagnosis, and treatment of unruptured intracranial aneurysms and aneurysmal subarachnoid hemorrhage. Mayo Clin. Proc. 96 (7), 1970–2000.
Teng, F., Fussenegger, M., 2020. Shedding light on extracellular vesicle biogenesis and bioengineering. Adv. Sci. 8 (1), 2003505.
Théry, C., Witwer, K.W., Aikawa, E., et al., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell. Vesicles 7, 1535750.
Upadhya, R., Madhu, L.N., Attaluri, S., Gitaí, D.L.G., Pinson, M.R., Kodali, M., et al., 2020a. Extracellular vesicles from human iPSC-derived neural stem cells: miRNA and protein signatures, and antiinflammatory and neurogenic properties. J. Extracell. Vesicles 9 (1), 1809064.
Upadhya, R., Zingg, W., Shetty, S., Shetty, A.K., 2020b. Astrocyte-derived extracellular vesicles: neuroreparative properties and role in the pathogenesis of neurodegenerative disorders. J Control Release 323, 225–239.
Upadhya, R., Madhu, L.N., Rao, S., Shetty, A.K., 2022. Proficiency of extracellular vesicles from hiPSC-derived neural stem cells in modulating proinflammatory human microglia: role of pentraxin-3 and miRNA-21-5p. Front. Mol. Neurosci. 15, 845542.
Upadhya, R., Shetty, A.K., 2021. Extracellular vesicles for the diagnosis and treatment of Parkinson's disease. Aging Dis 12 (6), 1438–1450.
Upadhya, D., Shetty, A.K., 2024. MISEV2023 provides an updated and key reference for researchers studying the basic biology and applications of extracellular vesicles. Stem Cells Transl Med. https://doi.org/10.1093/stcltm/szae052 (in press).
van Gijn, J., Rinkel, G.J., 2001. Subarachnoid hemorrhage: diagnosis, causes and management. Brain J Neurol 124 (Pt 2), 249–278.
Vermeulen, M., 1996. Subarachnoid hemorrhage: diagnosis and treatment. J. Neurol. 243 (7), 496–501.
Viderman, D., Sarria-Santamera, A., Bilotta, F., 2021. Side effects of continuous intra-arterial infusion of nimodipine for management of resistant cerebral vasospasm in subarachnoid hemorrhage patients: a systematic review. Neurochirurgie 67 (5), 461–469.
Vivancos, J., Gilo, F., Frutos, R., Maestre, J., García-Pastor, A., Quintana, F., Roda, J.M., et al., 2014. Clinical management guidelines for subarachnoid hemorrhage. Diagnosis and treatment. Neurol Barc Spain 29 (6), 353–370.
Vogel, A.D., Upadhya, R., Shetty, A.K., 2018. Neural stem cell derived extracellular vesicles: attributes and prospects for treating neurodegenerative disorders. EBioMedicine 38, 273–282.
Wanderer, S., Grüter, B.E., Strange, F., Sivanrupan, S., Di Santo, S., Widmer, H.R., et al., 2020. The Role of sartans in the treatment of stroke and subarachnoid hemorrhage: a narrative review of preclinical and clinical studies. Brain Sci. 10 (3), 153.
Wang, Q., Piao, J., Li, Y., Tu, H., Lv, D., Hu, L., Zhang, R., Zhong, Z., 2023. Bone marrow-mesenchymal stem cells alleviate microglial Pyroptosis after intracerebral hemorrhage in rat by secreting C1q/tumor necrosis factor-related protein 3. Exp. Neurol. 364, 114387.
Welsh, J.A., Goberdhan, D.C.I., O'Driscoll, L., et al., 2024. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J. Extracell. Vesicles 13, e12404.
Welty, T.E., Horner, T.G., 1990. Pathophysiology and treatment of subarachnoid hemorrhage. Clin. Pharm. 9 (1), 35–39.
Xiong, L., Sun, L., Zhang, Y., Peng, J., Yan, J., Liu, X., 2020. Exosomes from bone marrow mesenchymal stem cells can alleviate early brain injury after subarachnoid hemorrhage through miRNA129-5p-HMGB1 pathway. Stem Cells Dev 29 (4), 212–221.
Xu, W., Li, T., Gao, L., Zheng, J., Yan, J., Zhang, J., et al., 2019. Apelin-13/APJ system attenuates early brain injury via suppression of endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation and oxidative stress in an AMPK-dependent manner after subarachnoid hemorrhage in rats. J. Neuroinflammation 16 (1), 247.
Zakharov, A., Papaiconomou, C., Johnston, M., 2004. Lymphatic vessels gain access to cerebrospinal fluid through unique association with olfactory nerves. Lymphatic Res. Biol. 2 (3), 139–146.
Zhang, Z., Liu, J., Fan, C., Mao, L., Xie, R., Wang, S., et al., 2018. The GluN1/GluN2B NMDA receptor and metabotropic glutamate receptor 1 negative allosteric modulator has enhanced neuroprotection in a rat subarachnoid hemorrhage model. Exp. Neurol. 301 (Pt A), 13–25.
Zhang, J., Li, P., Zhao, G., He, S., Xu, D., Jiang, W., Peng, Q., Li, Z., Xie, Z., Zhang, H., Xu, Y., Qi, L., 2022. Mesenchymal stem cell-derived extracellular vesicles protect

retina in a mouse model of retinitis pigmentosa by anti-inflammation through miR-146a-Nr4a3 axis. Stem Cell Res. Ther. 13 (1), 394.
Zhao, H., Li, Y., Chen, L., Shen, C., Xiao, Z., Xu, R., et al., 2019. HucMSCs-derived miR-206-knockdown exosomes contribute to neuroprotection in subarachnoid hemorrhage induced early brain injury by targeting BDNF. Neuroscience 417, 11–23.
Zhu, X., Badawi, M., Pomeroy, S., Sutaria, D.S., Xie, Z., Baek, A., et al., 2017. Comprehensive toxicity and immunogenicity studies reveal minimal effects in mice following sustained dosing of extracellular vesicles derived from HEK293T cells. J. Extracell. Vesicles 6 (1), 1324730.