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Review

Exosomes in stroke management: A promising paradigm shift in stroke therapy

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From the Contents

Abstract

Effective treatment methods for stroke, a common cerebrovascular disease with a high mortality rate, are still being sought. Exosome therapy, a form of acellular therapy, has demonstrated promising efficacy in various diseases in animal models; however, there is currently insufficient evidence to guide the clinical application of exosome in patients with stroke. This article reviews the progress of exosome applications in stroke treatment. It aims to elucidate the significant potential value of exosomes in stroke therapy and provide a reference for their clinical translation. At present, many studies on exosome-based therapies for stroke are actively underway. Regarding preclinical research, exosomes, as bioactive substances with diverse sources, currently favor stem cells as their origin. Due to their high plasticity, exosomes can be effectively modified through various physical, chemical, and genetic engineering methods to enhance their efficacy. In animal models of stroke, exosome therapy can reduce neuroinflammatory responses, alleviate oxidative stress damage, and inhibit programmed cell death. Additionally, exosomes can promote angiogenesis, repair and regenerate damaged white matter fiber bundles, and facilitate the migration and differentiation of neural stem cells, aiding the repair process. We also summarize new directions for the application of exosomes, specifically the exosome intervention through the ventricular–meningeal lymphatic system. The review findings suggest that the treatment paradigm for stroke is poised for transformation.

Key Words: angiogenesis; animal model; cerebrovascular disorder; extracellular vesicle; mortality rates; neural stem cell; neuroinflammation; oxidative stress; programmed cell death; therapy

Introduction

Stroke, a cerebrovascular disorder, is characterized by high morbidity and mortality rates; therefore, it poses significant medical and scientific challenges globally (Guzik and Bushnell, 2017; Aguirre et al., 2023). This cerebrovascular disorder can be primarily divided into two types: ischemic stroke (IS), constituting approximately 60% of all stroke cases, and hemorrhagic stroke, including intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). IS occurs due to cerebral blood vessel obstruction, leading to disrupted blood flow and hypoxic damage to the brain tissue. Conversely, hemorrhagic stroke transpires when cerebral vessels rupture, leading to the extravasation of blood components into either the brain parenchymal or subarachnoid space. Furthermore, stroke survivors frequently endure long-term consequences, including paralysis, anesthesia, and locomotion dysfunction, contributing to unfavorable prognoses (Hewer, 1990). Current stroke interventions exhibit limited efficacy in reducing mortality and enhancing long-term outcomes (Richards and Cramer, 2023; Maryenko, 2024). Furthermore, there is a lack of consistent evidence regarding the effectiveness of drug treatments and the long-term prognosis for patients with stroke following hospital discharge.

Extracellular vesicles, which originate from cellular endosomal systems, are cell-derived structures that are categorized by size into exosomes (30–150 nm), microvesicles (500–1000 nm), and apoptotic bodies (1000–5000 nm). The study of extracellular vesicles dates back to 1967, when Peter Wolf first observed structures resembling extracellular vesicles under a transmission electron microscope (Wolf, 1967). Subsequent research has gradually elucidated the biological effects of exosomes (**Figure 1**). More recently, exosomes were found to encapsulate various active substances, including RNA, and in 2013, the Nobel Prize in Physiology or Medicine was awarded for the discovery and research of exosomes. As a result, exosomes are emerging as a promising cell-free treatment for various diseases due to their remarkable biological characteristics. For example, as they are derived from cells, exosomes exhibit lower immunogenicity and toxicity; therefore, they do not trigger immune responses or induce adverse side effects when used as a treatment (Liang et al., 2021). Additionally, exosomes can specifically bind to target cells, enabling precise therapeutic interventions. For instance, introducing exosomes laden with tumor antigens can stimulate the immune system to eliminate tumor cells selectively (Shi et al., 2021c). Exosomes comprise a diverse array of bioactive molecules, including long-chain non-coding RNAs (lncRNAs), microRNAs (miRs), lipids, and proteins. These constituents endow exosomes with potential therapeutic effects and enable them to significantly influence intercellular communication (Isaac et al., 2021). In recent decades, exosomes have been acknowledged as critical mediators of intercellular communication and effective cargo delivery tools, exhibiting a high penetration rate across the blood–brain barrier (BBB) (Wang et al., 2021; Liu et al., 2023a). Furthermore, exosomes have been identified in various cell types and have demonstrated considerable potential in modulating intracellular signaling in neurons and in treating diseases of the central nervous system (CNS) (Fan et al., 2022; Wang and Yang, 2023). Chen et al. (2020d) reported that exosomes derived from human adipose-derived mesenchymal stem cells (AD-MSCs) mitigated traumatic brain injury by

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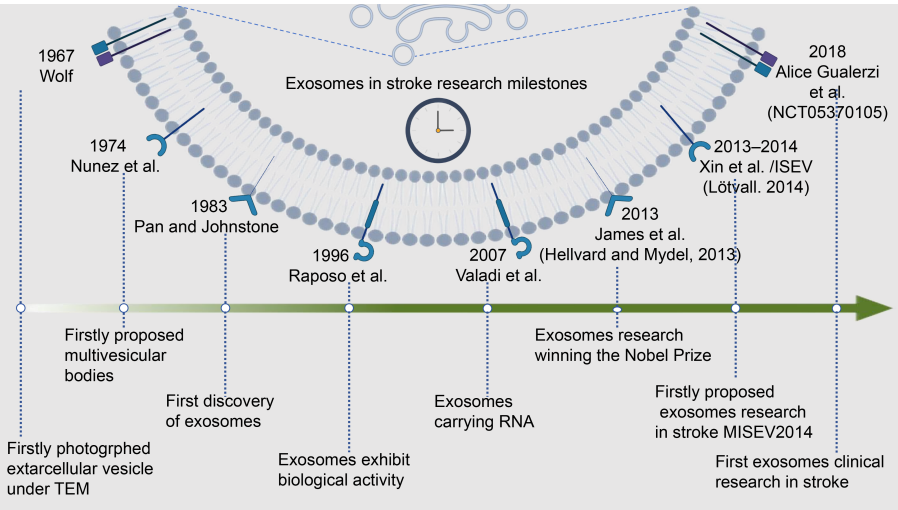

Figure 1 | **Milestone of exosomes researches in stroke.**
ISEV: International society for extracellular vesicles; MISEV: minimal information for studies of extracellular vesicles; TEM: transmission electron microscope.

inhibiting neuroinflammatory signals and reducing premature microglial activation in a rat model, suggesting the anti-inflammatory functions of stem cell-derived exosomes. Similarly, Deng et al. (2021) discovered that exosomes derived from astrocyte cell lines can alleviate amyloid-β-induced neurotoxicity both *in vivo* and *in vitro*. Milad Riazifar et al. (2019) introduced exosomes derived from human MSCs for use in a murine model of multiple sclerosis, emphasizing their potential therapeutic effects in mitigating autoimmune responses and neurodegeneration. These studies collectively indicate the significant therapeutic efficacy of exosomes, which are increasingly emerging as a viable cell-free therapeutic modality for various CNS disorders. However, the precise role of exosomes in stroke management—a condition marked by a high incidence, mortality, and poor prognosis—remains to be fully understood. Consequently, further research is imperative to explore the application of exosomes in stroke therapy and to uncover their substantial therapeutic potential.

Recently, there has been a significant increase in the use of exosomes for the diagnosis and treatment of stroke, particularly concerning injury repair and neural regeneration (Larson et al., 2024; Wang et al., 2024). This development has garnered considerable attention (Yang et al., 2024). Exosomes can serve as biomarkers for stroke diagnosis; specific exosomes have been identified in the blood of patients with stroke, which can be used to indicate the severity and prognosis (Wang et al., 2018; Lee et al., 2022). Additionally, exosomes can be utilized in stroke therapy, especially in preclinical studies. In a rat model of middle cerebral artery occlusion (MCAO), Zhao et al. (2020) discovered that exosomes derived from mesenchymal stem cells facilitated microglial polarization following cerebral ischemia through intracellular signaling transduction. Furthermore, we previously revealed that exosomes enriched with microRNA 25-3p, harvested from the blood of young, healthy male adults, could curtail ferroptosis in mouse neurocytes by modulating the p53-mediated depletion of glutathione peroxidase 4 (GPX4), ultimately improving behavioral outcomes in mice following ICH (Yang et al., 2023c).

The potential of exosomes for stroke treatment appears significant, warranting further exploration of their therapeutic value. However, despite substantial advancements in stroke-related research on exosomes, comprehensive reviews remain scarce, and there is a notable gap in translating these findings into clinical practice. To deepen our understanding of exosomes and their underlying mechanisms, this review aims to provide a comprehensive reference for the prospective use of exosomes in stroke therapy. We present an organized overview of the preclinical applications of exosomes in research on IS and hemorrhagic stroke, including insights into various pathological mechanisms and the regenerative and repair processes associated with stroke. These areas have received less attention in prior summaries. Consequently, this review aims to consolidate the current knowledge on the use of exosomes in stroke treatment, facilitating the advancement of exosomes applications in stroke therapy in future studies.

Search Strategy

For the retrieval of clinical research on exosomes, the PubMed search engine (ClinicalTrials.gov) was utilized with the following search terms: "Condition/Disease" column: stroke OR ischemic stroke OR intracerebral hemorrhage OR subarachnoid hemorrhage; "Intervention/treatment" column: exosomes OR extracellular vesicles; other filter: All. A total of eight registered clinical trials were recorded. Upon evaluation, seven trials were included in this review (**Table 1**), while one was excluded due to irrelevance in the utilization of exosomes.

For the retrieval of preclinical studies related to exosomes in stroke therapy, we utilized PubMed, Embase, and Web of Science search engines. To maximize our search results, we employed free-text searches using the following terms: (stroke OR ischemic stroke OR intracerebral hemorrhage OR intracerebral hemorrhage OR subarachnoid hemorrhage OR subarachnoid hemorrhage) AND (extracellular vesicles OR exosomes). The inclusion and exclusion processes are illustrated in **Figure 2**.

Clinical Research on Exosomes in Stroke Management

With research advancements on exosomes in stroke studies, a significant number of clinical investigations have emerged. To clarify the role and status of exosomes in stroke diagnosis and treatment, we identified relevant clinical studies (**Table 1**). We found that exosomes have garnered considerable attention from researchers worldwide in stroke-related clinical research, further affirming their potential application and value in stroke treatment. Notably, most clinical studies on exosomes in stroke are ongoing and require additional research centers' involvement and support.

Significant progress has been achieved in exosome research related to stroke diagnosis, rehabilitation, and prognostic indicators (Pratiwi et al., 2024; Tang et al., 2024). Some research teams have effectively utilized exosomes and their contents as important indicators of post-stroke rehabilitation outcomes and prognosis (Xu et al., 2021; Shi et al., 2023b). However, challenges remain in research on stroke treatment, including the limited number of projects, insufficient sample sizes, and a lack of studies focused on hemorrhagic stroke (Zhou et al., 2022a; Markowska et al., 2023). Thus, the scale and scope of the related research requires further expansion. These challenges may be attributed to the complexity of exosome therapy, ethical considerations, and existing standard guidelines for stroke diagnosis and treatment (Zhou et al., 2022a; Markowska et al., 2023; SelvaM et al., 2023). Innovating exosome interventions based on current, relatively standardized diagnostic and treatment practices also presents certain difficulties. Consequently, the application of exosomes in the field of stroke requires extensive preclinical research to establish their safety and efficacy, providing a solid foundation for multi-center, large-sample clinical application studies and translation.

Main Pathophysiology in the Sub-Acute Phase of Stroke

To comprehensively investigate the significance and potential of exosomes in preclinical research on stroke, it is essential first to understand the mechanisms underlying pathological damage following stroke. Stroke triggers a cascade of pathological and physiological responses within the brain, including neuroinflammation, oxidative stress, programmed cell death (PCD), and repair mechanisms. These processes will subsequently be discussed in detail (**Figure 3**).

Neuroinflammation

Neuroinflammation is an inevitable pathological event in IS and hemorrhagic stroke (Candelario-Jalil et al., 2022). Extensive research has underscored the profound influence of neuroinflammation on stroke progression, highlighting the crucial role of mitigating inflammation to improve stroke outcomes. Damage to the BBB following a stroke triggers inflammatory responses and activates the polarization of macrophages and microglia

Table 1 | **Clinical researches on exosome utilization in stroke**

Time and country	Registration ID	Current status	Subject (sample size)	Exosome source/ modification	Intervention and frequency	Follow-up time	Outcome/expectation
2018-Italy	NCT05370105	In progress	IS and ICH *n* = 100 (35–75 years)	Patient's blood	Blood collection	Undescribed	Serum exosomes as stroke rehabilitation biomarker
2024-Switzerland	NCT06319742	Recruiting	Cerebral ischemia *n* = 200 (≥ 18 years)	Patient's blood	Blood collection	3 d, 3 and 12 mon	Using exosomes to identify different types of cerebral ischemia
2021-Britain	NCT05645081	Recruiting	TIA *n* = 360 (≥ 18 years)	Patient's vascular endothelial cells	Undescribed	Over 12 mon	Exosomes as a predictor from TIA to stroke
2019-Iran	NCT03384433	Undescribed	IS *n* = 5 (40–80 years)	Patient's mesenchymal stromal cells (miR-124 enriched)	Stereotaxis/ intracranial injection, once	12 mon	Exosomes enriched by miR-124 improve patient's neurological function
2024-China	NCT06138210	Recruiting	IS *n* = 20 (18–70 years)	Human induced pluripotent stem cell	i.v. q.d. 7 times serum collection	7, 14, 90 d	Exosomes improve neurological function, blood cytokine marker, and infarct volume
2022-China	NCT05326724	Recruiting	IS *n* = 30 (50–75 years)	Acupuncture induced exosomes	Acupuncture once a week	3 mon	Exosomes improve post-stroke recognition
2021-Denmark	NCT06257823	Recruiting	IS and TIA *n* = 140 (≥ 60 years)	Patient's blood	Blood collection	24 h, 12 mon	Exosomes profile is a predictor of post-stroke cognitive impairment

i.v.: Intravenous; ICH: intracerebral hemorrhage; IS: ischemic stroke; q.d.: once a day; TIA: transient ischemic attack.

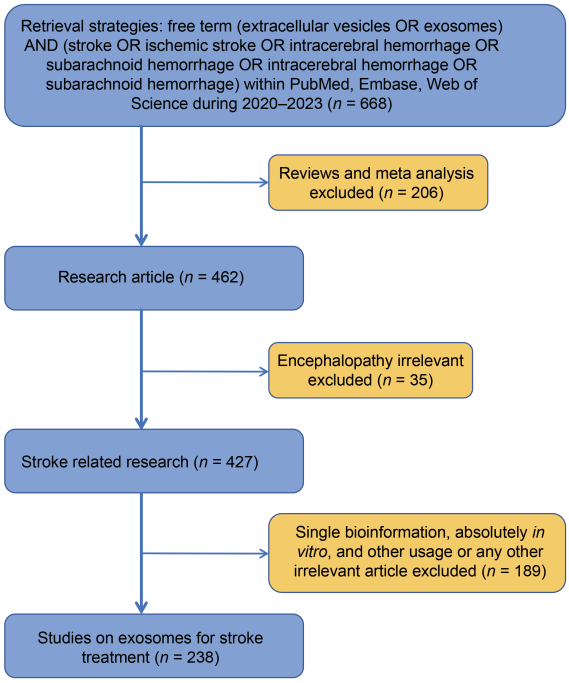

Figure 2 | **Flowchart of data retrieval and inclusion and exclusion strategies.**

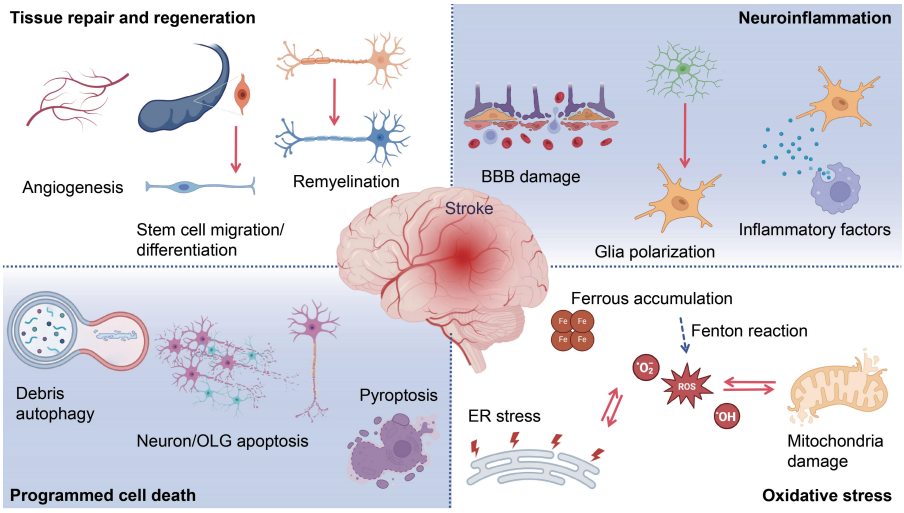

Figure 3 | **Main post-stroke pathophysiology.**
ER: Endoplasmic reticulum.

(Jayaraj et al., 2019; **Figure 3**). Consequently, damage to the BBB becomes a primary catalyst for neuroinflammation, engaging polarized microglia and other immune cells derived from the periphery, thereby shaping the secondary axis of neuroinflammation (Lu et al., 2024). The activation of astrocytes and microglia leads to the release of inflammatory factors that exacerbate cellular damage, ultimately resulting in white matter and neuronal injury (Li et al., 2022b; Guan et al., 2024). Furthermore, neuroinflammation compromises the integrity of the surviving BBB, leading to vascular spasms, obstruction of cerebrospinal fluid (CSF), and increased neural cell permeability (Candelario-Jalil et al., 2022; Hu et al., 2023a). These changes culminate in post-stroke edema and elevated intracranial pressure, which exerts mechanical strain on local brain tissue and vasculature, further exacerbating tissue necrosis (Delgardo et al., 2023).

Oxidative stress

Oxidative stress is a significant pathophysiological process that occurs during the progression of stroke (Butterfield and Halliwell, 2019; Zhang et al., 2025). A notable contradiction exists between the high oxygen demand of neurons and the substantial reduction in oxygen supply following a stroke (Du et al., 2020). This disparity leads to the accumulation of free radicals and the depletion of normal antioxidant systems, such as glutathione and superoxide dismutase (Chouchani et al., 2014; Zeng et al., 2022; **Figure 3**). Concurrently, hemoglobin degradation increases ferrous iron accumulation, further amplifying free radical production (Shi et al., 2021a). Excessive free radicals, if not promptly cleared, can mediate lipid damage to cell membranes and organelles, ultimately leading to ferroptosis of neurons and oligodendrocytes (Li et al., 2023d). The management of free radical accumulation and the consequent mitochondrial damage presents a significant challenge following a stroke (Ni et al., 2022; Zhang et al., 2023f). The growing understanding of oxidative stress, coupled with the emergence of ferroptosis, underscores that it is imperative to address oxidative stress-induced damage after a stroke. Consequently, the exploration of effective antioxidant strategies in the post-stroke context has garnered considerable interest and necessitates further investigation.

Programmed cell death

Programmed cell death, a regulated form of cell death, can be classified into apoptosis, autophagy, and other types (**Figure 3**). Neuronal apoptosis plays a crucial role in post-stroke pathology (Zhi et al., 2021; Yang et al., 2023a). Stroke-induced focal ischemia and hypoxia activate apoptotic signaling pathways, leading to neuronal apoptosis and the formation of apoptotic microbodies (Mao et al., 2022; Tuo et al., 2022). Extensive research has focused on mitigating neuronal apoptosis after stroke; the results have shown that targeting this process can improve both molecular and behavioral outcomes. Another form of PCD is autophagy, which helps clear damaged organelles within cells by merging them with lysosomes to form autophagosomes (Shi et al., 2023a). Autophagy facilitates cellular repair and contributes to recovery following brain injury (Xiaoqing et al., 2023; Zheng et al., 2023). Recently, numerous studies have revealed the pivotal role of autophagy in stroke recovery (Beccari et al., 2023; Liu et al., 2023b; Tedeschi et al., 2023). Pyroptosis, another form of PCD, is characterized by a progressive reduction in cell size, deformation, and the formation of small membrane vesicles that can be engulfed by adjacent cells. The underlying mechanisms of post-stroke pyroptosis are complex and not fully understood. Current research implicates various factors, including oxidative stress, inflammatory responses, and mitochondrial dysfunction, as contributors to pyroptosis. Strategies for treating post-stroke pyroptosis remain under investigation; however, preliminary findings suggest that inhibiting the expression of genes associated with pyroptosis or administering antioxidants may help to mitigate neurological impairments following a stroke (Long et al., 2023). Despite extensive research on PCD across various neuronal system models, further studies are imperative to comprehensively understand, categorize, and treat PCD following stroke.

Damage repair and neural regeneration

Damage repair and neural regeneration are critical pathophysiological processes following a stroke (Sheikh et al., 2024). During the initial stages of stroke, a decrease in the blood supply activates the hypoxia signaling pathway, implicating angiogenesis as an essential mechanism for early damage repair (Sun et al., 2020; Yang and Torbey, 2020; **Figure 3**). In response to signaling molecules, endothelial cells undergo proliferation and differentiation, forming new structures that resemble blood vessels, which serve as vital conduits for delivering nutrients and oxygen to the damaged tissue, promoting the regeneration and repair of neurons (Yang and Torbey, 2020).

In addition, research has confirmed damage to white matter fiber bundles and the death of oligodendrocytes, as well as their subsequent repair, occurring days after a stroke (Zheng et al., 2021). Our previous investigations identified a variety of potential techniques and underlying processes that could mitigate the deterioration of white matter fiber tracts, offering valuable insights for their clinical application (Xia et al., 2019; Chen et al., 2020c; Li et al., 2020). Furthermore, the role of neural stem cells in post-stroke repair has garnered significant attention. Neural stem cells possess the ability for self-renewal and multi-directional differentiation, enabling them to differentiate into neurons, oligodendrocytes, and astrocytes (Tuazon et al., 2019). Typically, neural stem cells remain dormant; however, upon injury or stimulation, they become activated and begin to proliferate and differentiate (Urbán et al., 2019). Following a stroke, the compromised brain tissue releases signaling molecules, such as growth factors and cytokines, which attract neural stem cells from the surrounding region to migrate toward the damaged site (Barkho and Zhao, 2011). Once they reach the affected area, these neural stem cells proliferate and differentiate, forming new neurons and glial cells, thereby aiding in the restoration of damaged neural tissue.

Biological Characteristics of Exosomes

In addition to studying the pathology of stroke, understanding the attributes and origins of exosomes can provide valuable insights for innovative research and intervention strategies related to stroke.

Exosomes, a class of extracellular vesicles, were initially identified using transmission electron microscopy (TEM) (Johnstone et al., 1989). Under microscopic examination, exosomes show a distinctive double-layer membrane structure derived from the cell membrane, forming a closed vesicle. Nanoparticle tracking analysis and TEM are commonly employed to identify exosomes and directly measure their particle diameter. Additionally, exosomal membrane-bound peptides and proteins can be used to identify exosomes qualitatively.

Exosomes carry various membrane-specific markers, including tetraspanin-integrin transmembrane proteins such as CD9, CD63, and CD81. Furthermore, specific cell-type membrane-bound molecules, including type II major histocompatibility complex (MHC-II), C–X–C chemokine ligand (CXCL), and CD86, can also be detected on exosomal membranes. Other exosomal membrane proteins, which include adhesion-related peptides like integrins, intercellular adhesion molecule-1, and cadherins, facilitate cell adhesion with exosomes (Zhang et al., 2022b).

The phospholipid bilayer membrane of exosomes can be targeted for labeling and tracking techniques. Recent studies have utilized the PKH67 fluorescent dye to demonstrate the uptake process of exosomes and their localization within or outside of cells (Hu et al., 2022b; Wang et al., 2023d). Various methods are currently available to observe exosomes at different levels.

Exosomes are recognized for their significant cargo-carrying capacity. According to the Exosome Database (http://exocarta.org/), they have been reported to contain over 9769 proteins, 3408 mRNAs, and 2838 miRs. Additionally, exosomes include other molecules, such as lipids, circular RNAs (circRNAs), lncRNAs, and mitochondrial components (Li and Fang, 2023). Their robust packaging and transport capabilities render exosomes a promising tool for future scientific research and stroke drug therapy; however, further investigations are needed to uncover the additional cargo carried by exosomes. Understanding their origin, biogenesis, and release mechanisms is crucial and constitutes an essential first step toward elucidating their roles in the physiology of the central nervous system and the pathophysiology of stroke.

Derivation of exosomes

Almost every cell type has the theoretical capacity to export exosomes for cellular communication and molecular delivery (Hade et al., 2021; Ni et al., 2023). Exosomes, derived from the cells themselves, can be generated by any cell type. Investigating the origins of exosomes used in stroke treatment facilitates the advancement of subsequent research and provides valuable insights into the exosomes with potential therapeutic benefits for stroke. Additionally, this research serves as a reference for the large-scale production of exosomes for stroke treatment *in vitro*. We summarize the origins or donors of exosomes relevant to stroke therapy based on studies conducted over the last 4 years (2020–2023) (**Figure 4**).

Statistically, stem cells are the primary source of exosomes (**Figure 4**) and have become the most popular origin for exosomes used in stroke treatment. Stem cells have a high capacity for exosomes biogenesis and promising potential for research and clinical applications (Volpe, 2020). Stem cells exhibit robust proliferation and differentiation abilities (Yamanaka, 2020; Aboul-Soud et al., 2021), which facilitate rapid *in vitro* expansion, cultivation, and the efficient production and extraction of a multitude of exosomes (Hade et al., 2021; Lotfy et al., 2023). Consequently, stem cells are an ideal source of exosomes and a valuable asset for scientific research in this field (Hade et al., 2021). Additionally, stem cells encompass a broad spectrum of bioactive molecules, including proteins, nucleic acids, and carbohydrates (Matsuzaka and Yashiro, 2022). These molecules play vital roles in regulating immune responses, promoting tissue repair and regeneration, and other physiological processes (Matsuzaka and Yashiro, 2022; Malekpour et al., 2023). Furthermore, stem cells exhibit a certain degree of stability and tolerance, allowing them to exist and function over extended periods (Poetsch et al., 2022; Zhao et al., 2023).

While stem cells hold potential for disease treatment and damage repair, exosomes derived from stem cells serve as crucial communication and cargo transportation tools in response to stroke-induced damage (Lee et al., 2022). Therefore, exosomes derived from stem cells may contain essential substances and signaling molecules necessary for repair and regeneration, positioning them as potential therapeutic carriers in stroke pathology. It is important to note that as stem cells differentiate, their exosome production activity decreases (Patel et al., 2017). Additionally, the cost of obtaining primary stem cells and culture conditions present challenges for their clinical translation.

Fluid tissues, such as plasma, are the second highest source of exosomes for stroke treatment; thus, plasma has become a prominent source for exosome extraction. Plasma offers several advantages: it is readily available, easy to store,

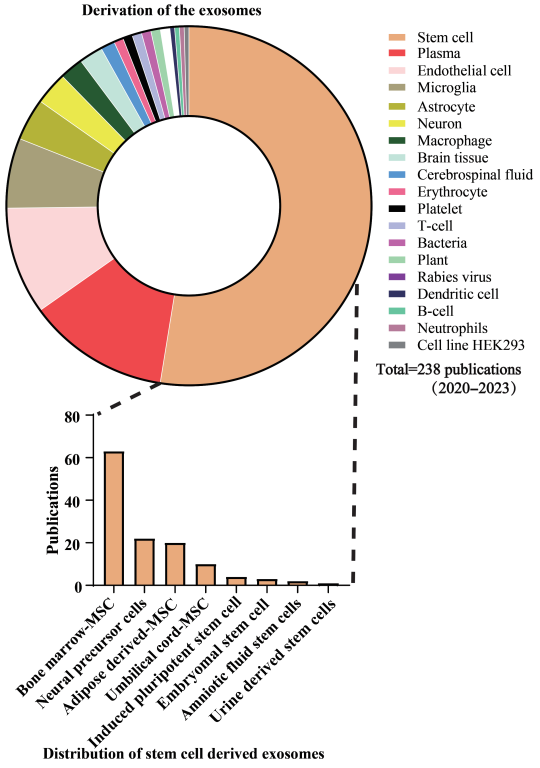

Figure 4 | Frequency distribution of exosome sources for stroke treatment.
HEK: Human embryonic kidney.

and has low extraction-related costs. Additionally, the methods for isolating and purifying exosomes from plasma are well-established, enhancing the scalability and positioning plasma as a viable source for future exosome-based therapies (Zhang et al., 2022c). Exosomes from other cellular sources have also been documented, including glial cells. These cells, which are abundant in the brains of mammals and rodents, play a pivotal role in the pathological processes following a stroke, with astrocytes and microglia implicated in neuroinflammation and oligodendrocytes involved in white matter damage. Therefore, investigating exosomes derived from glial cells could represent a crucial step in understanding stroke onset, progression, and prognosis. Moreover, CSF, which originates from the epithelial cells of the choroid plexus within the ventricles, is also recognized as an important source of exosomes for stroke treatment. The significance of these exosomes should not be underestimated. Investigating their role before and after the onset of a stroke could be vital in unraveling disease progression. The CSF is an essential reference for neurological diagnostics and therapeutics in clinical settings (Garcia et al., 2021). With advancements in exosomes research, subtle alterations in CSF-derived exosomes may facilitate the early detection and diagnosis of stroke by identifying initial micropathological changes in the vessels involved. Consequently, exosomes derived from the CSF could be an indispensable tool for the future diagnosis and treatment of stroke.

Biogenesis and release of exosomes

Understanding the mechanisms underlying the biogenesis and assembly of exosomes is crucial in harnessing their production and modification. The process of exosome biogenesis shares similarities with intracellular protein synthesis and processing. Exosomes originate from the cellular endosomal system, specifically the endoplasmic reticulum and Golgi apparatus (van Niel et al., 2018). The process of exosome biogenesis initiates with the invagination of the endosomal membrane, leading to the creation of intraluminal vesicles (ILVs) (Kalluri and LeBleu, 2020; Krylova and Feng, 2023). These ILVs, referred to as primary exosomes, are responsible for encapsulating specific proteins, nucleic acids, and other molecules and are subsequently transported to the Golgi apparatus via vesicular transport vessels (Gurunathan et al., 2021). Within the Golgi apparatus, primary exosomes undergo membrane fusion and remodeling, which leads to the formation of multivesicular bodies (mVBs) (Arya et al., 2024). mVBs can either fuse with lysosomes for degradation and subsequent reutilization or interact with the plasma membrane, thereby releasing ILVs as exosomes into the extracellular space (van Niel et al., 2018). The biogenesis of exosomes involves several subcellular mechanisms, including the Endosomal Sorting Complexes Required for Transport (ESCRT) system, which consists of multiple protein subunits and plays a crucial role in exosome formation. ESCRT assists in sorting cargo into ILVs by recognizing specific motifs or ubiquitinated proteins, promoting their recruitment into ILVs. The primary exosomes are formed in the endoplasmic reticulum through ESCRT binding to its proteins and lipids, leading to the formation of endoplasmic reticulum-derived vesicles. The ESCRT-I and ESCRT-II complexes facilitate the identification and clustering of these vesicles during their transit to the Golgi apparatus, ultimately leading to the creation of mVBs. The ESCRT machinery also plays a crucial role in mVB biogenesis; ESCRT-0 and ESCRT-I complexes are responsible for vesicle formation and recognition of ILVs. Furthermore, the ESCRT-III complex promotes the fusion of the inner lumen of the internal vesicles with the outer vesicles, resulting in the finalization of mVBs. This streamlined explanation provides a clear and concise overview of the intricate process of exosome biogenesis. In addition to ESCRT-dependent mechanisms, there are also ESCRT-independent mechanisms, which involve tetraspanins, lipids, and other proteins that contribute to the sorting of exosomal cargo (van Niel et al., 2018).

Exosome biogenesis is a controllable biological process. Exosomes can be distinguished by their unique lipid composition, encompassing phosphatidylserine, cholesterol, sphingomyelin, and glycosphingolipids. These lipids are vital for the biogenesis and stability of exosomes as they participate in processes such as membrane curvature, protein recruitment, and cargo sorting (Skotland et al., 2019). Modulating the lipid composition of exosomes can significantly impact their therapeutic applications, such as targeting specific cell types or enhancing stability. Both intrinsic cellular factors and external environmental conditions influence exosome synthesis. Genetic factors and cell maturation levels are pivotal in the biogenesis of exosomes. As mentioned above, stem cells, particularly those at lower maturity levels, exhibit an enhanced capacity for exosome production (Patel et al., 2017); however, this capability diminishes as the stem cells progress through maturation and differentiation (Patel et al., 2017).

Various environmental factors also influence the synthesis and secretion of exosomes. In hypoxic conditions, stem cells increase the release of exosomes, which likely function as a distress signal (Li et al., 2022a; Yang et al., 2023b). In summary, the biogenesis of exosomes depends on factors such as the cell type, microenvironment, and the physiological or pathophysiological state of the donor cell.

The assembly of exosomes involves the fusion of mVBs with the plasma membrane. This process is regulated by various proteins, lipids, and intracellular calcium concentration (van Niel et al., 2018). During the fusion of mVBs with cellular membranes, the ESCRT-III complex orchestrates the bending and scission of cell membranes to facilitate exosome secretion. Hypoxic environments can also enhance the release of exosomes, potentially through mechanisms that depend on intracellular calcium levels (Savina et al., 2003). The Rab GTPase family and SNARE proteins are essential for exosome release; they ensure precise docking and fusion at the plasma membrane (Song et al., 2019). Additionally, environmental cues and cellular stress conditions influence both the quantity and quality of exosome release (van Niel et al., 2018).

In summary, the generation and assembly of exosomes are crucial for their biological functions and therapeutic potential. Understanding these molecular mechanisms will pave the way for manipulating the composition, cargo, and release of exosomes. Further research in this area will enable us to fully harness the potential of exosomes in various biomedical applications, including targeted drug delivery, regenerative medicine, and disease diagnostics.

Modification of exosomes *in vitro*

After their production, exosomes may undergo a series of artificial engineering modifications to enhance their targeting capabilities and therapeutic effectiveness. These modifications can be categorized as cargo loading or surface modifications based on the intended location and objective of the alteration (**Figure 5**).

Cargo loading

Cargo loading modifications enable exosomes to selectively and efficiently load specific drugs and molecules, enhancing their therapeutic efficacy. Various methods can be employed for cargo loading, including physical, genetic engineering, and chemical engineering approaches. Physical methods typically involve co-culturing target drugs or molecules with exosome donor cells. For example, Tian et al. (2018) cultured exosomes with curcumin to produce curcumin-rich exosomes for treating IS in mice. Other physical cargo loading methods, such as electroporation and ultrasound, increase the permeability of exosomes, enhancing the ability of drugs to enter their interior (Lai et al., 2020).

Genetic engineering can also be employed for cargo loading by transfecting donor cells with nucleic acid fragments that contain the target molecule's nucleic acid sequence. This can be achieved using transfection tools such as plasmids or lentiviruses. For example, Yang et al. (2020b) used plasmid transfection to introduce neurotrophic factors into exosomes, promoting neural regeneration and aiding recovery following IS in mice. Similarly, Guo et al. (2023) generated exosomes enriched with miR-124 to treat ICH in mice through lentivirus-mediated transfection of donor cells.

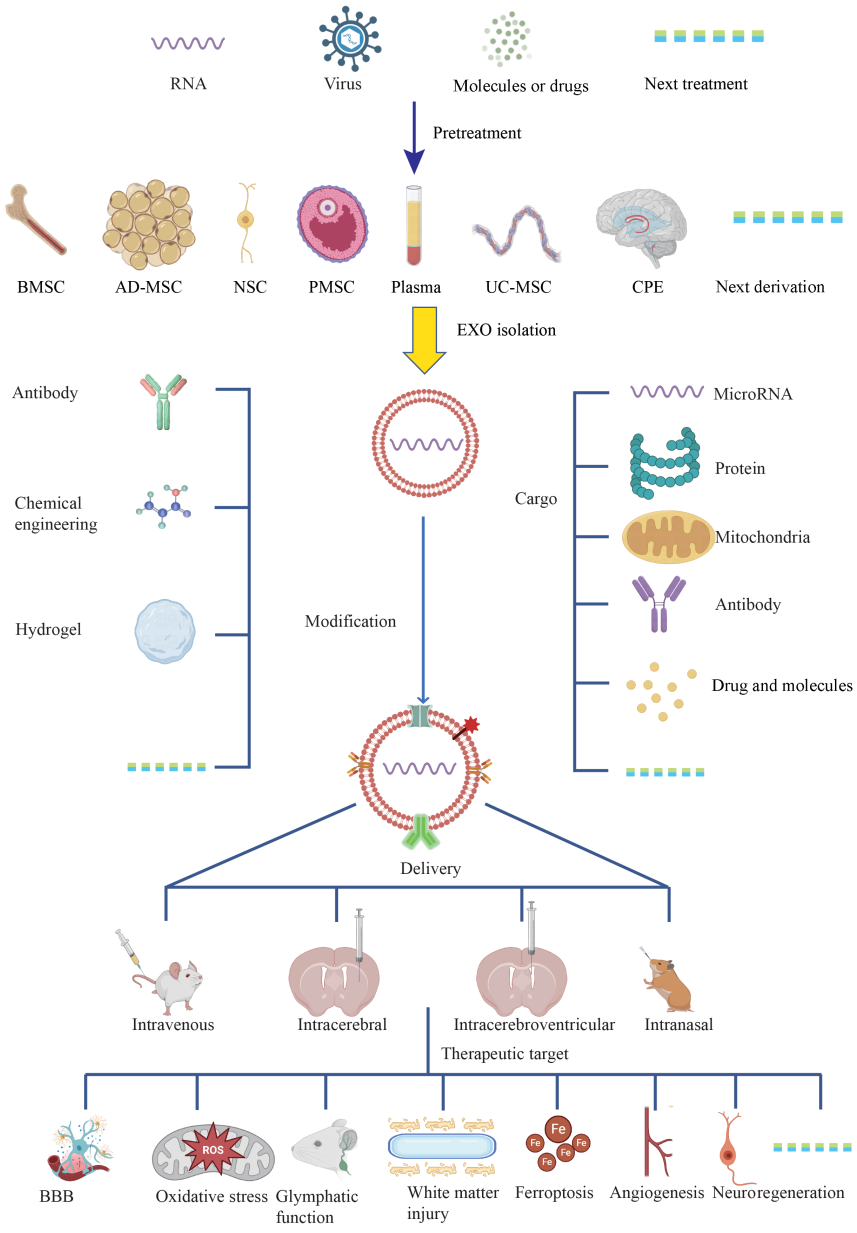

Figure 5 | Exosome source, loading, modification, and delivery.

AD-MSC: Human adipose-derived mesenchymal stem cell; BMSC: bone marrow stem cell; EXO: exosome; NSC: neural stem cells; UC-MSC: umbilical cord-derived mesenchymal stem cell.

Chemical engineering techniques can facilitate cargo loading into exosomes. These methods can be broadly classified into liposome-dependent and non-liposome-based. Non-liposome-based methods often utilize chemical reagents. For example, Zhu et al. (2023) used commercial transfection kits to introduce brain-derived neurotrophic factor into exosomes for the therapeutic management of IS in a rat model. In contrast, liposomes are artificial small vesicles characterized by their bilayer membrane structure, facilitating exosomal cargo encapsulation. For example, Zhou et al. (2022b) encapsulated miR-145-5p in nano-liposomes and transfected BV2 cell lines; they subsequently isolated exosomes derived from the BV2 cells, which were found to improve ischemia–reperfusion injury in rats.

Surface modification

Compared with cargo loading, the surface modification of exosomes involves artificial alteration of their surface membrane proteins or lipids to facilitate targeted delivery to specific organs or tissues. This can be accomplished through either genetic engineering or chemical engineering modifications. Genetic engineering techniques frequently incorporate surface modifications, often involving the conjugation of transmembrane proteins with targeting ligands. For example, Yang et al. (2017) utilized plasmid-transfected donor cells to engineer exosomes with neuron-targeting capabilities in a mouse model of IS. This was achieved by conjugating the rabies virus glycoprotein (RVG), a neurophilic molecule, to the lysosome-associated membrane protein 2B (Lamp-2B), a component of the exosomal membrane, thus ensuring anchoring of RVG to the exosomes surface.

In a similar approach, Ran et al. (2020) improved exosome targeting and therapeutic efficacy by linking the transmembrane protein CD63 on the exosome surface with a specific ligand designed for targeted delivery. Additional examples of genetic engineering modifications include the use of lactadherin and platelet-derived growth factor receptor-mediated connections (Morishita et al., 2015; Liang et al., 2021; Tian et al., 2021).

Chemical engineering surface modification involves the direct alteration of exosome surfaces using organic or inorganic chemical methods, including the copper-catalyzed azide-alkyne cycloaddition reaction, commonly referred to as "click chemistry." For example, Jia et al. (2018) conjugated a neuropilin-1-targeted peptide to the surface of exosomes using click chemistry, endowing them with glioma-targeting properties. Additionally, the polyethylene glycol-grafted 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) method has been used to directly link ligand molecules to the exosome surface through the polyethylene glycol raft of the exosomes, facilitating targeted delivery. For example, Cao et al. (2019) utilized DSPE-PEG to attach the tumor-targeting peptide RGD (CRGDKGPDC) to exosomes, enabling effective targeting of tumor tissues. Furthermore, cholesterol and long-chain lipids on the exosomal membrane can also serve as carriers for targeting ligands. Ye et al. (2018) enhanced the targetability of exosomes in glioblastoma by connecting membrane lipids to low-density lipoprotein cholesterol.

Taken together, these findings demonstrate that engineered exosomes have the capacity to become a focal point in future research endeavors.

Post-Stroke Injury Mechanism and Exosomal Intervention

Upon understanding the characteristics of exosomes, the forthcoming sections will identify potential intervention targets for exosomes following a stroke. This section aims to clarify the role of exosomes in the various phases of stroke pathology.

Amelioration of neuroinflammation

Targeting damage to the BBB has emerged as an effective strategy to mitigate neuroinflammation. The unique membrane architecture of exosomes facilitates their transmigration across the BBB, enabling the delivery of therapeutic agents to ischemic regions following a stroke (**Table 2**). A substantial body of research has focused on the role of exosomes in protecting against BBB damage. Li et al. (2023e) demonstrated that exosomes inhibit autophagy of BBB-related endocytic connection proteins induced by Cav-1, resulting in a protective effect on the BBB. Similarly, Li et al. (2023c) showed that intravenous injection of exosome rejuvenates the BBB in a mouse model of IS. Comparable findings were reported by Zhang et al. (2021c), who revealed the nuclear factor kappa B (NF-κB) inhibitory effect of neural progenitor cell–derived exosomes. Additionally, an SAH model suggested a potential protective role of exosomes in the BBB (Wang et al., 2023e).

After a stroke, BBB function is significantly impaired, leading to increased permeability. Peripheral blood leukocytes are among the first cells to infiltrate the brain parenchyma through the compromised BBB, exacerbating inflammation (Candelario-Jalil et al., 2022). A study on IS confirmed that exosomes can mitigate leukocyte leakage across the BBB post-stroke (Wang et al., 2022). Similarly, a mouse model of ICH yielded comparable results, demonstrating that exosomes administered via nasal intervention can effectively reduce peripheral leukocyte infiltration (Guo et al., 2023). In addition to leukocytes from the peripheral blood, glial cells within the brain parenchyma, including astrocytes and microglia, contribute to the inflammatory response. Astrocytes are integral to the formation of the BBB, and upon receiving inflammatory signals, they undergo polarization. Traditionally, polarized astrocytes are classified into two subtypes: A1 (pro-inflammatory) and A2 (anti-inflammatory) (Fan and Huo, 2021). These polarized astrocytes further activate microglia, promoting their polarization. Similarly, based on cell surface markers and functions, polarized microglia are classified into M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes (Guo et al., 2022).

Exosomes have the potential to inhibit glial cell polarization. Li et al. (2021) confirmed that exosomal miR-124 can counteract STAT signaling, inhibiting astrocyte polarization. Zhou et al. (2022b) demonstrated a transition effect between M1 and M2 microglial subtypes following IS. Yang et al. (2020b) modified the surface of exosomes using plasmid transfection with RVG and loaded neurotrophic factors into the exosomes, resulting in an increase in M2 microglia around the ischemic region. Xia et al. (2021) found that embryonic stem cell-derived exosomes regulate neuroinflammation by expanding T-regulatory cells through the delivery of exosomal TGF-β. In an ICH model, Gao et al. (2022) showed that SIRP variant-enriched exosomes promote microglial polarization toward the M2 subtype and activate T-regulatory cells, exerting anti-inflammatory effects after ICH. In an SAH model, exosomes have been reported to inhibit the polarization of microglia into pro-inflammatory types. Han et al. (2021) demonstrated that exosomes improve M1 microglial polarization in a rat model of SAH by inhibiting the NF-κB pathway and promoting the AMPK pathway. Additionally, Qian et al. (2022) and Wang et al. (2023b) found that exosomal miR-140-5p inhibits M1 microglial polarization by counteracting inflammation-related signaling pathways downstream of the mRNA.

Inflammatory factors are the primary executors of neuroinflammation; upon binding to their cognate receptors, they cause cellular structural damage. Increasing research has demonstrated that exosomes can mitigate the production of these inflammatory agents, thereby alleviating the inflammatory response. Tian et al. (2021) utilized exosomes derived from engineered neuroprogenitor cell lines to treat mice with IS. The results indicated that the intervention significantly reduced the inflammatory response and diminished the concentrations of inflammatory markers. Similarly, Xie et al. (2023) reported that exosomes administration had a long-term inhibitory effect on inflammation following IS. Ding et al. (2021) confirmed this in a diabetic rat model of ICH, demonstrating that exosomal miR-183-5p can inhibit the nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome, effectively suppressing the inflammatory response. This anti-inflammatory effect persisted for 28 days after ICH in rats. Laso-García et al. (2023) collected plasma from patients who recovered well after ICH. They found that the extracted exosomes could reduce the levels of inflammatory factors and alleviate damage to white matter fiber bundles when administered to rats after ICH. This protective effect also lasted for 28 days post-hemorrhage. In another mouse model of ICH, Sun and Xu (2023) reported that exosomes-derived miR-150-3p exerted an anti-inflammatory effect by inhibiting the TNF receptor-associated factor 6/NF-κB pathway. Previous studies have confirmed

Table 2 | **Exosomes reduce neuroinflammation in animal models of ischemic stroke**

Animal	Model	Exosomes source/modification	Intervention and frequency	From modeling to observation	Outcome measure	Mechanism	Reference
SD rats	IS	BMSC	i.v., once	24 h	BBB damage↓	Exosomes ⊙ Cav-1 induced ZO-1, Claudin-5 endocytosis	Li et al., 2023e
C57BL/6J mice	ICH	Microglia cell line BV2/mir-124 (cargo) lentivirus transfection	Intranasal 3 times s.i.d.	1 or 3 d	BBB damage↓	Exosomal mir-124 ⊙ Gr-1+ Cell infiltration	Guo et al., 2023
SD rats	SAH	Dendritic cell	i.v., once	N/A	BBB damage↓	Exosomal mir-3064-5p ⊙ SIRT6/PCSK9	Wang et al., 2023e
SD rats	IS	BMSC/Mir-145 (cargo) liposome transfection	i.v., once	28 d	Microglia M1/m2	Exosomal mir-145 ⊙ FOXO1	Zhou et al., 2022b
C57BL/6 mice	ICH	BMSC/SIRPα-v (cargo) genetic engineering	i.v., 14 times s.i.d	1, 3, 7, 14, 35 d	Microglia M1/m2	Exosomal SIRPα-v ⊕ T-reg/m2 microglia SIRPα-v ⊙ RBC-CD47 induced anti-engulfed	Gao et al., 2022
SD rats	SAH	BMSC	i.v., once	24 or 48 h	Microglia M1/m2	Exosomes ⊕ ampk ⊙ NF-κB	Han et al., 2021
C57BL/6 mice	IS	Neural progenitor cell/RGD-4C (surface) genetic engineering	i.v., once	24 or 36 h	⊙ Inflammation factors	Exosomal mirna ⊙ mapk	Tian et al., 2021
SD-diabetic rats	ICH	BMSC	i.v., once or once every 5 d	1, 2, 7, 28 d	⊙ Inflammatory markers	Exosomal mir-183-5p ⊙ PDCD4/NLRP3	Ding et al., 2021
C57BL/6 mice	SAH	BMSC/RVG-lamp2b (surface) genetic engineering/mir-193b-5p (cargo) electroporate	i.v., once	24 h	⊙ Inflammatory markers	Exosomal mir-193b-5p ⊙ HDAC3 ⊕ p65 acetylation	Lai et al., 2020

⊕ : Activate; ⊙ : inhibit; ampk: adenosine monophosphate activated protein kinase; BBB: brain–blood barrier; BMSC: bone marrow stem cell; Cav-1: caveolin-1; FOXO1: forkhead box protein O1; h: hour(s); HDAC3: histone deacetylase 3; lamp-2b: lysosomal-associated membrane protein 2B; i.v.: intravenous; ICH: intracerebral hemorrhage; IS: ischemic stroke; NF-κB: nuclear factor kappa light chain enhancer of activated B cells; PCSK9: proprotein convertase subtilisin/kexin type 9; PDCD4: programmed cell death 4; RVG: rabies virus glycoprotein; s.i.d.: once a day; SD: Sprague–Dawley; SIRPα-v: signal-regulatory protein alpha-variant; SIRT6: sirtuin 6; ZO-1: zonula occludens-1.

that the phosphorylation of NF-κB subunits p50 and p65 is critical for the activation of NF-κB signaling (Giridharan and Srinivasan, 2018; Khan et al., 2024). In a SAH mouse study, Lai et al. (2020) used bone marrow-derived stem cells (BMSCs) transfected with the miR-193b-3p virus to extract exosomes rich in miR-193b-3p for intravenous injection in mice. They found that miR-193b-3p promotes the acetylation of p65, thereby reducing NF-κB activation and the inflammatory response.

In summary, research on exosomes in the context of neuroinflammation has gained significant traction and may lead to substantial transformative impacts.

Prevention of oxidative injury to neurocytes

The potential of exosomes to aid in post-stroke antioxidation merits further investigation. The mitochondria play a significant role in oxidative injury. As a result, there is increasing interest in utilizing exosomes for mitochondrial protection. Chamberlain et al. (2021) discovered a specific type of exosomes derived from oligodendrocyte progenitor cells that can carry sirtuin 2 (SIRT2), a silent information regulator that aids in the deacetylation of mitochondrial-related proteins within axons. This process helps maintain equilibrium in axonal mitochondrial energy metabolism and enhances the production of adenosine triphosphate (ATP). The targeted delivery of mitochondria-related molecules via exosomes holds promise for achieving balanced energy metabolism. Additionally, mitochondrial DNA (mtDNA) encodes a range of proteins and enzymes essential for the cellular respiratory chain (Byappanahalli et al., 2023). The concentration of mtDNA within exosomes varies based on factors such as age, nutritional status, and overall health (Lazo et al., 2021). Therefore, exosomal mtDNA may become a key focus in understanding the occurrence and progression of stroke among the elderly (Dave et al., 2023). Concurrently, further investigation into exosome-derived mitochondrial subunits and functional proteins, particularly those related to the respiratory chain, is warranted. Xia et al. (2022) revealed that exosomes originating from adipose-derived mesenchymal stem cells (AD-MSCs) can transport mitochondrial-specific components, facilitating the repair of damaged mitochondria and reducing the macrophage-mediated inflammatory response. Collectively, these findings underscore the potential importance of exosome-mediated mitochondrial protection. However, the role of exosomes in delivering mitochondrial-related content has not yet been thoroughly investigated in the context of stroke research.

In the field of stroke antioxidant therapy, a study conducted by Wang et al. (2023c) indicated that exosomes containing Krüppel-like factor 4 (KLF4) can modulate mitochondrial energy metabolism via the lncRNA ZFAS1 pathway, thereby mitigating oxidative stress following a stroke (**Table 3**). Similarly, Ma et al. (2022) found that exosomes derived from the brain tissue of healthy mice reduced the production of reactive oxygen species and alleviated endothelial oxidative stress in MCAO mice. Furthermore, Yang and Chen (2022) confirmed that exosomes containing lncRNA ZFAS1 (zinc finger nuclear transcription factor antisense RNA 1) from BMSCs could counteract oxidative stress-related microRNAs in mice, achieving therapeutic effects. Other studies have reported similar findings. For example, Wang et al. (2023a) suggested that exosomal miR-210 could stimulate the vascular endothelial growth factor receptor 2/phosphatidylinositol 3-kinase (PI3K) pathway, leading to a reduction in the levels of oxidative stress. Guo et al. (2021) engineered exosomes with the traditional Chinese medicine compound quercetin to intervene in rats with IS, discovering that it reduced reactive oxygen species production by activating the nuclear factor (erythroid-derived 2)-like 2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway. In a rat model of ICH, Yi and Tang (2021) found that exosomal miR-19b could inhibit iron-regulatory protein 2, thereby reducing neuronal ferroptosis. In another ICH study, Hu et al. (2023b) reported that exosomal miRs could suppress the PTEN signaling pathway, leading to decreased reactive oxygen species production. They indicated that inhibition of the PTEN signaling pathway could reduce neuronal necrosis and apoptosis, which was mediated by exosomal miR-23b. Exosomes have also been shown to reduce oxidative stress at the lesion site in the SAH model. For example, Zhang et al. (2023e) discovered that exosomal miR-18a-5p could antagonize the ectodermal-neural cortex 1/sequestosome-1 (ENC1/P62) signaling pathway, alleviating endoplasmic reticulum stress following SAH.

These studies demonstrate the potent antioxidant effects of exosomes, establishing a critical foundation and providing significant translational value for antioxidant therapies aimed at mitigating oxidative damage following a stroke.

Alleviation of programmed cell death

Recently, studies have focused on using exosomes to prevent neuronal PCD, highlighting the advantages of exosomes in regulating PCD (Chen et al., 2020a; Liu et al., 2021a). Mechanistically, exosomes can transfer their contents to target cells via cellular signal transduction, thereby regulating PCD and determining cell fate. Exosomes derived from vascular endothelial progenitor cells, rich in angiotensin-converting enzyme 2 (ACE2), effectively reduce apoptosis and oxidative stress levels in cerebral vascular endothelial cells of aged C57BL/6 mice with IS (Pan et al., 2023; **Table 4**). The underlying mechanism could be attributed to the suppression of the PTEN signaling pathway by exosomal miR-17-5p, which subsequently activated the related signaling pathways. Similarly, another study has corroborated that exosomes originating from astrocytes mitigate neuronal apoptosis and promote autophagy in damaged neurons within a C57BL6 mouse model of IS (Chen et al., 2020b). Mechanistically, exosomal circSHOC2 (a circRNA associated with splicing homology and cysteine-rich domain-containing protein 2) suppresses miR-7670-3p, thereby alleviating its inhibitory influence on SIRT1. Additionally, investigations have corroborated that exosomes originating from astrocytes subjected to oxygen–

Table 3 | **Exosome utilization on reducing oxidative stress after stroke**

Animal	Model	Exosomes source/ modification	Intervention and frequency	From modeling to observation	Outcome measure	Mechanism	Reference
C57BL/6 mice	IS	Brain tissue	i.v., three times s.i.d.	2 d	Endothelial oxidative stress↓	Exosomes-induced ROS↓mDA↓	Ma et al., 2022
C57BL/6J mice	IS	BMSC	i.v., once	24 h	Oxidative stress↓ Neuroinflammation↓	Exosomal lncRNA ZFAS1 ⊙ miR-15a-5p	Yang and Chen, 2022
C57BL/6J mice	IS	BMSC	i.v., once	3 d	Oxidative stress↓ Balanced mitochondrial dynamic	Exosomal KLF4 ⊙ lncRNA ZFAS1/Drp1	Wang et al., 2023c
SD rats	IS	Plasma	i.v., once	2 h	Oxidative stress↓	modified GAP43- exosomal Que ⊕ Nrf2/HO-1/NQO1 ROS↓	Guo et al., 2021
C57BL/6J mice	IS	EPC/miR-210 (cargo) liposome transfection	i.v., once	2 d	Oxidative stress↓ Apoptosis↓	Exosomal miR-210 ⊕ VEGFR2/ PI3K and TrkB/PI3K	Wang et al., 2023a
Wistar rats	ICH	BMSC	i.v., once	1, 3, 7 d	Oxidative stress↓ Pyroptosis↓ Apoptosis↓	Exosomal miR-23b ⊙ PTEN ROS↓	Hu et al., 2023b
C57BL/6 mice	ICH	ADSC/miR-19b-3p (cargo) liposome transfection	i.v., once	2 d	Ferroptosis↓	Exosomal miR-19b ⊙ IRP2	Yi and Tang, 2021
SD rats	SAH	BMSC	i.c.v., once	3 d	ER stress↓	Exosomal miR-18a-5p ⊙ ENC1/ P62	Zhang et al., 2023e

⊕ : Activate; ⊙ : inhibit;.BMSC: bone marrow stem cell; ER: endoplasmic reticulum; HO-1: heme oxygenase 1; i.c.v.: intracerebroventricular; i.v.: intravenous; ICH: intracerebral hemorrhage; IRP2: iron regulatory protein 2; IS: ischemic stroke; mDA: malondialdehyde; NQO1: NAD(P)H quinone dehydrogenase 1; PI3K: phosphatidylinositol 3-kinase; PTEN: phosphatase and tensin homolog; ROS: reactive oxygen species; ROS: reactive oxygen species; s.i.d.: once a day; SAH: subarachnoid hemorrhage; SD: Sprague–Dawley; TrkB: tyrosine receptor kinase B; VEGFR2: vascular endothelial growth factor receptor 2; ZFAS1: zinc finger antisense 1.

Table 4 | **Exosome utilization on regulating programmed cell death after stroke**

Animal	Model	Exosomes source/modification	Intervention and frequency	From modeling to observation	Outcome measure	Mechanism	Reference
Aged mice C57BL/6 mice	IS	Endothelial progenitor cells/ACE2 (cargo) lentivirus transfection	i.v., once	2 d	Endothelial cell apoptosis↓ Oxidative stress↓	Exosomal miR-17-5p ⊙ PTEN Exosomal miR-17-5p ⊕ PI3K/Akt	Pan et al., 2023
C57BL/6J mice	IS	Astrocyte	i.v., 9 times t.i.d.	3 d	Neurocyte apoptosis↓ Autophagy↑	Exosomal circSHOC2 ⊙ miR-7670-3p/SIRT1	Chen et al., 2020b
C57BL/6 mice	IS	OGD/R astrocyte	i.v., once	1, 2, 3, 7, 14 d	Autophagy↑	Exosomal Nampt ⊙ AmPK/mTOR	Deng et al., 2022
C57BL/6 mice	IS	m2 microglia BV2	i.v., 3 times s.i.d.	3 d	Neurocyte apoptosis↓	Exosomal miRNA-137 ⊙ Notch1	Zhang et al., 2021a
C57BL/6 mice	IS	BMSC	i.v., once	1 d	Neurocyte apoptosis↓ Oxidative stress↓	Exosomes-induced ROS↓SOD↑	Zhang et al., 2023a
SD rats	ICH	BMSC/miR-146a-5p (cargo) lentivirus transfection	i.v., once	2 d	Neuron apoptosis↓ Microgliam1 polarization↓	Exosomal miR-146a-5p ⊙ IRAK1/NFAT5	Duan et al., 2020
SD rats	ICH	BMSC/miR-133b (cargo) liposome transfection	i.v., once	4 d	Neurocyte apoptosis↓	Exosomal miR-133b ⊙ RhoA/ERK/IREB	Shen et al., 2018
SD rats	SAH	BMSC	i.v., once	2 d	Neuron apoptosis↓	Exosomal miR-17-5p ⊙ PTEN Exosomal miR-17-5p ⊕ PI3K/Akt	Gao et al., 2020
SD rats	SAH	Human umbilical cord-mSC/miR-26b-5p inhibitor (cargo) liposome transfection	i.v., once	24 h	Neuron apoptosis↓ Neuroinflammation↓	Exosomal miR-26b-5p ⊙ p38mAPK/STAT	Liu et al., 2021b
SD rats	SAH	BMSC	i.v., once	24 h	Neurocyte apoptosis↓ Neuroinflammation↓	Exosomal Nampt ⊕ AmPK/mTOR	Xiong et al., 2020

⊕ : Activate; ⊙ : inhibit; ACE2: Angiotensin-converting enzyme 2; AmPK: adenosine monophosphate activated protein kinase; BMSC: bone marrow stem cell;circSHOC2: circular RNA related to SH2 domain containing protein 2; d: day(s); ERK: extracellular signal-regulated kinase; i.v.: intravenous; ICH: intracerebral hemorrhage; IRAK1: interleukin-1 receptor associated kinase 1; IREB: iron responsive element binding protein;IS: ischemic stroke; mAPK: mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; Nampt: nicotinamide phosphoribosyltransferase; NFAT5: nuclear factor of activated T cells 5; OGD/R: oxygen glucose deprivation/re-oxygenation; PI3K: phosphatidylinositol 3-kinase; PTEN: phosphatase and tensin homolog; RhoA: Ras homolog familymember A; SIRT1: sirtuin 1; ROS: reactive oxygen species;s.i.d.: once a day; SD: Sprague–Dawley; SOD: superoxide dismutase; STAT: signal transducer and activator of transcription; t.i.d.: three times a day.

glucose deprivation (OGD) facilitate autophagy in damaged cells within a C57BL6 mouse model of IS (Deng et al., 2022). Furthermore, in mice with IS, exosomes derived from the microglia cell line BV2 could inhibit Notch1 signaling and reduce neuronal apoptosis (Zhang et al., 2021a). Similarly, Zhang et al. (2023a) extracted exosomes from BMSCs and reported analogous outcomes in mice, demonstrating a reduction in neuronal cell apoptosis and oxidative stress levels. In a rat ICH model, Duan et al. (2020) found that exosomes enriched with miR-146-5p derived from BMSCs reduced neurocyte apoptosis and decreased the number of M1 microglia around the hematoma. Another study by Shen et al. (2018) also used BMSCs as a donor of exosomes in a rat model of ICH and found a significant reduction in neurocyte apoptosis compared to the control group after 4 days of intervention. Further analysis revealed that exosomal miR-133 inhibits the Ras homolog gene family member A/extracellular regulated protein kinases/immediate-early response gene product B (RhoA/ERK/IREB) signaling pathway. In SAH, exosomes also displayed a powerful anti-apoptotic effect. In a rat model of SAH, exosomes derived from BMSCs alleviated neurocyte apoptosis, which was related to miR-17-5p contained in the exosomes (Gao et al., 2020). The mechanism was similar to the study mentioned above: miR-17-5p inhibited the PTEN signaling pathway and activated PI3K /protein kinase B (AKT) signaling (Pan et al., 2023). In addition, Liu et al. (2021b) used exosomes derived from human umbilical cord MSCs enriched with miR-26b-5p for intravenous injection in a rat model of SAH, demonstrating that exosome-derived miR-26b-5p inhibited the p38 mitogen-activated protein kinase/signal transducer and activator of transcription (p38 MAPK/STAT) signaling pathway, thus reducing neurocyte apoptosis and neuroinflammation. Similarly, another rat model of SAH revealed that exosomes derived from BMSCs containing nicotinamide phosphoribosyltransferase (Nampt) inhibited the adenosine 5′-monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) pathway and alleviated neurocyte apoptosis and neuroinflammation (Xiong et al., 2020).

In summary, current investigations are exploring the role of exosomes in enhancing PCD within neuronal networks. Several notable studies have provided innovative approaches for the treatment of PCD associated with stroke.

Injury repair and neuroregeneration

The contents of exosomes can stimulate blood vessel regeneration in the vicinity of the lesion following a stroke (Hu et al., 2023c; Liu et al., 2023c; **Table 5**). For example, Hu et al. (2022a) established that exosomes originating from BMSCs facilitate the formation of blood vessels around the infarcted area in a rat model of IS. The underlying mechanism involves exosomal miR-21-5p, which activates the VEGF signaling pathway, ultimately leading to angiogenesis. Additionally, this study employed human umbilical vein endothelial cells as an *in vitro* validation system. These exosomes, which promoted vascular regeneration in the infarcted area of rats, also stimulated the proliferation, migration, and differentiation of endothelial progenitor cells *in vitro*. Similarly, Zhang et al. (2021b) used C57BL6 mice and discovered that exosomal TGF-β enhanced angiogenesis and protected against neuronal apoptosis via the Smad2/3 pathway. Furthermore, Han et al. (2018) revealed that exosomes derived from BMSCs promoted blood vessel regeneration and restored white matter fiber bundles in a Wistar rat model of ICH, with the effects lasting up to 28 days post-ICH onset.

The efficacy of exosomes in mitigating damage to white matter fiber bundles has also been documented. Otero-Ortega et al. (2017) suggested that exosomes derived from AD-MSCs effectively promote the repair of white matter fiber bundles in rats following IS, with effects lasting up to 28 days after the infarction. Another study by Xin et al. (2021) found that exosomes rich in miR-17-92 promoted neuronal axon regeneration and remyelination in Wistar rats following IS. Mechanistically, miR-17-92 exerts its effects by activating the PI3K/AKT/mTOR signaling pathway, highlighting the long-term impact of exosomes. Zhu et al. (2023) used exosomes derived from neural stem cells enriched with BDNF and injected them into the basal ganglia of rats after IS. They demonstrated that these exosomes promoted the migration and differentiation of neural stem cells over an extended period while simultaneously reducing neural stem cell apoptosis. Similarly, Zhang et al. (2020) used interferon-gamma (IFN-γ) stimulated human neural stem cells as the source of exosomes, which were found to improve behavioral outcomes and reduce the infarct volume in rats following injection into the basal ganglia. *In vitro* experiments also confirmed their ability to reduce neural stem cell apoptosis. Mechanistically, these effects are linked to the miRs contained within the exosomes.

In summary, exosomes may influence angiogenesis, white matter repair, and the migration and differentiation of neural stem cells. This phenomenon comprehensively demonstrates the sophisticated nature of exosomes and their potential as a cell-free therapeutic approach.

Intraventricular Administration of Exosomes Following Stroke

In addition to exosome modification, the delivery of exosomes post-stroke is an important issue. Efficient drug delivery routes are crucial for alleviating neuronal damage, promoting axon and myelin sheath self-repair, and improving the overall

Table 5 | **Exosome utilization for promoting tissue repair and regeneration after stroke**

Animal	Model	Exosomesource/modification	Intervention and frequency	From modeling to observation	Outcome measure	Mechanism
SD rats	IS	Human-NSC/BDNF (cargo) non-liposome transfection	Striatum injected once	1, 3, 7, 14, 28 d	NSC migration and differentiation↑ NSC apoptosis↓	Exosomal BDNF
SD rats	IS	Human-NSC/IFN-γ stimulation	Striatum injected once	1, 3, 7, 14, 28 d	Better behavioral and structural outcomes hNSC survival↑	Exosomal micro-RNA profile
SD rats	IS	ADmSC	i.v., once	2, 7, 28 d	White matter repair	Exosomal cargo
SD rats	IS	BMSC	i.v., once	1, 3, 7, 14 d	Angiogenesis↑	Exosomal miR-21-5p ⊕ VEGF, VEGFR2, Ang-1, Tie-2↑
Wistar rats	IS	BMSC/miR-17-92 (cargo) lentivirus transfection	i.v., once	1, 3, 7, 14, 21, 28 d	Axonal extension, myelination↑	Exosomal miR-17-92 ⊕ PI3K/Akt/mTOR
C57BL/6J mice	IS	OGD preconditioned microglia	i.v., once	2, 5, 7 d	Angiogenesis↑ Neuron apoptosis↓	Exosomal TGF-β ⊕ Smad2/3
Wistar rats	ICH	BMSC	i.v., once	1, 7, 14, 21, 24–28 d	Angiogenesis White matter remodeling	Exosomal cargo

⊕ : Activate; ⊙ : inhibit; ADmSC: adipose-derived mesenchymal stem cell; Ang-1: angiopoietin-1; BDNF: brain-derived neurotrophic factor; BMSC: bone marrow stem cell; hNSC: human neural stem cell; i.v.: intravenous; ICH: intracerebral hemorrhage; IFN-γ: interferon-γ; IS: ischemic stroke; mTOR: mammalian target of rapamycin; NSC: neural stem cell; OGD: oxygen-glucose deprivation; PI3K: phosphatidylinositol 3-kinase; SD: Sprague–Dawley; TGF-β: transforming growth factor-beta; Tie-2: tyrosine kinase with immunoglobulin-like and EGF homology domains 2; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2.

prognosis after a stroke (Han and Jiang, 2021). Furthermore, exploring the various intervention strategies for exosomes therapy following a stroke is a critical topic.

Exosomes are most often administered via intravenous injection (**Figure 5**). This method offers a convenient and straightforward approach to administering exosomes and has significant potential to become the preferred route in future clinical use. However, following a stroke, the affected arteries may experience vasospasm or occlusion, and dysfunction of the BBB can hinder the effective administration of drugs and agents via intravenous injection (Nguyen et al., 2021). Other studies have also shown that post-stroke vascular complications, such as vasospasm, obstruction, and rupture, can lead to hemodynamic and vascular dysfunctions, impeding drug delivery (Hu et al., 2012; Anttila et al., 2018).

A limited number of studies have employed stereotactic injections into the brain parenchyma for exosomes delivery (Zhang et al., 2020; Zhu et al., 2023). This method offers high precision due to stereotactic technology (Zhang et al., 2020; Nance et al., 2022), which helps avoid medication effects in other areas, thereby enhancing the treatment outcomes. Since the medication is administered directly into the brain parenchyma, it can act more rapidly, reducing the treatment duration and minimizing the impact on other tissues and organs (Tuma et al., 2023). Consequently, the likelihood of side effects is significantly diminished. However, stereotactic technology requires advanced expertise and sophisticated equipment, making it a complex procedure. Complications, such as bleeding and infection, further restrict its clinical application (Yue et al., 2023).

Recently, intraventricular (ICV) administration of drugs and agents has gained considerable attention beyond the conventional delivery routes described above, leading to a surge in research related to CNS diseases and advancements in related technologies (Atkinson, 2017). ICV injection presents a viable alternative, bypassing BBB impediments and significantly enhancing drug concentrations within the cranium. Therefore, this method facilitates the efficient and convenient delivery of therapeutic agents. Furthermore, ICV administration circumvents drug metabolism by the liver and absorption through the intestines, thereby diminishing adverse reactions and side effects, as well as reducing drug accumulation in the body, which lowers the risk of drug overdose (Oberrauch et al., 2022). Notably, the ICV administration of exosomes is increasingly recognized as a front-line therapeutic strategy for neurological diseases (Liu et al., 2022).

We have compiled a summary of research related to ICV exosome therapy post-stroke, highlighting the significant efficacy and intervention effects (**Table 6**). For example, Zhang et al. (2023d) utilized C57BL/6 mice to establish an IS model and harvested exosomes from human-induced pluripotent stem cell-derived neural stem cells. These exosomes were subsequently administered ICV. The beneficial effects of the exosomal intervention persisted for up to 56 days post-infarction. Mechanistically, the exosomal microRNAs enhanced the survival of neural stem cells while concomitantly reducing neuronal apoptosis and oxidative stress levels, thereby corroborating the robust therapeutic potential of ICV exosome intervention following IS. A comparable study was conducted by Heras-Romero et al. (2022), who used a Wistar rat model of IS. They utilized primary cortical astrocytes as the source of exosomes, which were then administered through ICV injection to promote axonal regrowth and protect white matter fiber bundles. The underlying mechanism may be attributed to the cargo contained within the exosomes. Pathipati et al. (2021) concurrently extracted BMSCs for intraventricular and intranasal exosome interventions in neonatal C57BL/6 mice. Three days post-cerebral infarction, the exosome intervention group (both ICV and intranasal) exhibited reduced glial cell inflammatory responses and fewer neuronal apoptotic cells than the control group. Similarly, in the Wistar rat model of IS, Mahdavipour et al. (2020) discovered that exosomes derived from neural stem cells enhanced the cell survival rate by promoting axonal growth and reducing the glial inflammatory response after IS. The efficacy of ICV exosome intervention was further substantiated in an animal model of ICH. Yang et al. (2023c) utilized C57BL/6J mice to establish a model of cerebral parenchymal hemorrhage and subsequently administered ICV injections of exosomes obtained from the plasma of healthy adult humans for 3 consecutive days. Subsequent RNA sequencing analysis indicated that miR-25-3p, present within these exosomes, was highly abundant in the plasma of healthy adults. Functionally, miR-25-3p exerted its neuroprotective effects by inhibiting p53, thereby mitigating p53-mediated iron-induced neuronal cell death following ICH. Another study by Ahn et al. (2021) used newborn Sprague–Dawley (SD) rats to establish a model of ICV hemorrhage. They administered exosomes derived from human umbilical cord stem cells through ICV injection. This intervention mitigated neuroinflammation and neuronal cell death while concurrently facilitating the remyelination of damaged white matter. In the context of a SAH model, Zhang et al. (2023e) used exosomes obtained from BMSCs for ICV intervention. Observations at 3 days post-modeling revealed that miR-18a-5p, derived from the exosomes, suppressed the ENC1/p62 signaling pathways, thereby mitigating neuronal apoptosis, neuroinflammation, and endoplasmic reticulum stress. Additionally, Cheng et al. (2022) used exosomes from BMSCs to perform ICV injections in rats experiencing SAH. They confirmed that the exosomal lncRNA KLF3-AS1 (Krüppel-like factor 3 antisense RNA 1) could suppress miR-83-5p, consequently elevating the expression of transcription factor 7-like 2 (TCF7L2) and mitigating cerebral vascular endothelial damage. These investigations highlight the promising therapeutic potential of ICV exosomeinterventions and offer valuable insights for future research on exosome applications via different routes. However, it is important to acknowledge that the ICV administration of exosomes also has limitations. The procedure is complex and requires specialized skills and equipment. Administration via the ventricular route poses risks, including bleeding and infection. To address these issues, future research and technological innovation are crucial.

In recent years, the nasal-meningeal lymphatic-ventricular pathway has emerged as a novel route for drug administration, capturing widespread attention among scientists. Through this route, medications can directly enter the meningeal lymphatic system via the nasal mucosa, subsequently reaching the ventricles. This offers a non-invasive new method for treating brain

Table 6 | **Intraventricular administration of exosomes after stroke**

Animal	Model	Exosomes source/ modification	Intervention and frequency	From modeling to observation	Outcome measure	Mechanism	Reference
C57BL/6 mice	IS	Human iPS-derived NSC	i.c.v. once	0, 3, 7, 14, 21, 28, 35, 42, 49, 56 d	NSC-survival↑/apoptosis↓ Oxidative stress↓ Neuroinflammation↓	Exosomal micro-RNA	Zhang et al., 2023d
Wistar rats	IS	Astrocyte	i.c.v., once	1, 7, 14, 21 d	White matter preservation	Exosomal cargo	Heras-Romero et al., 2022
Postnatal day 9–10 C57BL6 mice	IS	BMSC	i.c.v. or intranasal, once	3 d	Microglia response↓ Neurocyte apoptosis↓	Exosomal cargo	Pathipati et al., 2021
Wistar rats	IS	NSC	i.c.v., once	1 or 7 d	Microglia response↓ Neuroblast proliferation↑	Exosomal cargo	Mahdavipour et al., 2020
C57BL6 mice	ICH	Human plasma	i.c.v., three times s.i.d.	1, 3, 7, 14, 28 d	Neurocyte ferroptosis↓	Exosomal miR-25-3p ⊕ p53 induced ferroptosis	Yang et al., 2023c
Newborn SD rats	IVH	Human umbilical cord blood-derived mSC	i.c.v., once	1, 7, 28 d	Neurocyte death↓ Neuroinflammation↓ Myelination↑ Nerve regeneration↑	Exosomal BDNF	Ahn et al., 2021
SD rats	SAH	BMSC	i.c.v., once	3 d	Neuron apoptosis↓ Inflammatory response↓ ER stress↓	Exosomal miR-18a-5p ⊙ ENC1/P62	Zhang et al., 2023e
SD rats	SAH	BMSC	i.c.v. once	2 d	Brain microvascular endothelium injury↓	Exosomal KLF3-AS1 ⊙ TCF7L2↑	Cheng et al., 2022

⊕ : Activate; ⊙ : inhibit; BDNF: brain-derived neurotrophic factor; BMSC: bone marrow stem cell; d: day; i.c.v.: intracerebroventricular; ICH: intracerebral hemorrhage; iPS: induced pluripotent stem cell; IS: ischemic stroke; IVH: intraventricular hemorrhage; KLF3-AS1: Krüppel-like factor 3-antisense RNA 1; NSC: neural stem cell; SAH: subarachnoid hemorrhage; SD: Sprague–Dawley.

diseases (Hoang et al., 2023; Spera et al., 2023). As a result, this approach is expected to overcome the limitations of traditional methods of ventricular administration, thereby enhancing the efficiency and safety of medication treatments. Therefore, in-depth research and exploration of this pathway's potential will bring new hope not only for exosome administration but also for the treatment of brain diseases (Semyachkina-Glushkovskaya et al., 2022). The nasal cavity communicates with the ventricular-meningeal lymphatic system, which is closely related to post-stroke damage repair and the response to hematoma absorption (Tsai et al., 2022). Additionally, the glymphatic system, also referred to as the glia-lymphatic system, which was first named in 2012 by Iliff et al. (2021), serves as an important material transport channel that connects the perivascular space of the subarachnoid space with the brain parenchyma. The glymphatic system is a vital mechanism for clearing metabolic waste in the central nervous system, performing a function analogous to that of ventricular-meningeal lymphatic drainage (Liu et al., 2023d). This system operates through aquaporin-4 (AQP4), located on the end feet of astrocytes, which facilitates the rapid exchange of fluid between the CSF and brain interstitial fluid, thereby eliminating metabolites and abnormal proteins from the brain interstitial fluid. Evidence suggests that exosome treatments may upregulate the mRNA expression of AQP4 in astrocyte end feet, subsequently enhancing the cleansing function of the glymphatic system (Esmati et al., 2023). Another review reported that exosomes originating from peripheral sources might target AQP4 through specific microRNAs, resulting in the depolarization of astrocyte end feet AQP4 and disrupting the functionality of the glymphatic system, ultimately influencing the onset and progression of diabetic cognitive impairment (Zhang et al., 2023b). However, the application of exosomes in this system for stroke treatment remains unclear. The removal of damaged fragments, hematomas, and metabolic waste is essential for a positive prognosis after a stroke, highlighting the need for a deeper understanding of the role of the glymphatic system post-stroke (Ye et al., 2023; Zhu et al., 2024).

Future research should investigate the potential impact of exosomal applications, both within and external to the ventricles, on the functionality of the glymphatic system.

Challenges and Prospects of Exosome Application in Patients with Stroke

Exosomes possess significant potential for biomedical applications; however, their clinical use remains challenging due to factors related to the exosomes themselves, such as their efficacy and safety within the human body, in addition to external factors, including policy regulations, ethical constraints, and economic investments. A detailed analysis is provided below.

Safety and personalization considerations

The safety profile of exosomes remains incompletely understood. As research continues to elucidate the complex composition of exosomes, it has become clear that they can harbor various cellular components, potentially exerting diverse effects on recipient cells (Tan et al., 2024). Additionally, the propensity of different tissues and organs to absorb exosomes varies, which could lead to unforeseen accumulation and subsequent side effects or toxicity (Lu et al., 2023).

While genetically engineered exosomes can traverse the BBB and target specific neural cells, the biochemical safety implications of such modifications cannot be underestimated. This concern is particularly relevant when considering their use in patients with stroke, necessitating heightened safety evaluations (**Figure 6**). The study of personalized exosome therapy emerges as a pivotal avenue for future investigations (Zhan et al., 2022; Li et al., 2023b). Immortalized cell lines engineered for this purpose, in addition to autologous MSCs from patients, present promising sources for exosome-based interventions (Sengupta et al., 2020; Zhang et al., 2022a; Zhou et al., 2022c). Exosomes derived from patient plasma showcase significant clinical potential (Yu et al., 2021; Cai et al., 2022). Harvesting exosomes from plasma offers convenience and mitigates the risks associated with invasive procedures (Muller et al., 2014). Methodologically, plasma exosome therapy aligns closely with component transfusion and regenerative serum therapies, in which exosomes are isolated through ultracentrifugation and other techniques before being reintroduced into the patient (N V Lakshmi Kavya et al., 2022). These methodologies are relatively advanced and provide ethical and moral precedents for their use.

In summary, despite the promising role of exosomes in stroke intervention, a thorough understanding of their safety is imperative for clinical translation (Giovannelli et al., 2023). Ongoing studies should rigorously assess the safety of exosomes, explore the feasibility of tailored exosome treatments, and address pertinent challenges to advance the application of exosomes in stroke management.

Ethical and policy considerations

Ethical and policy considerations are integral to exosome research and its clinical applications. Exosomes possess unique attributes, which include their natural cellular production, ultra-micro volume, and ability to be administered via injection or infusion, which minimizes patient harm during treatments. However, ethical concerns exist regarding exosome sourcing, modification, and safety efficacy (Nestor et al., 2021; Tan et al., 2024). As previously noted, exosome safety, side effects, and toxicity require further elucidation. Whether exosomes provide long-term benefits to patients with stroke while potentially causing harm necessitates extensive experimental investigation.

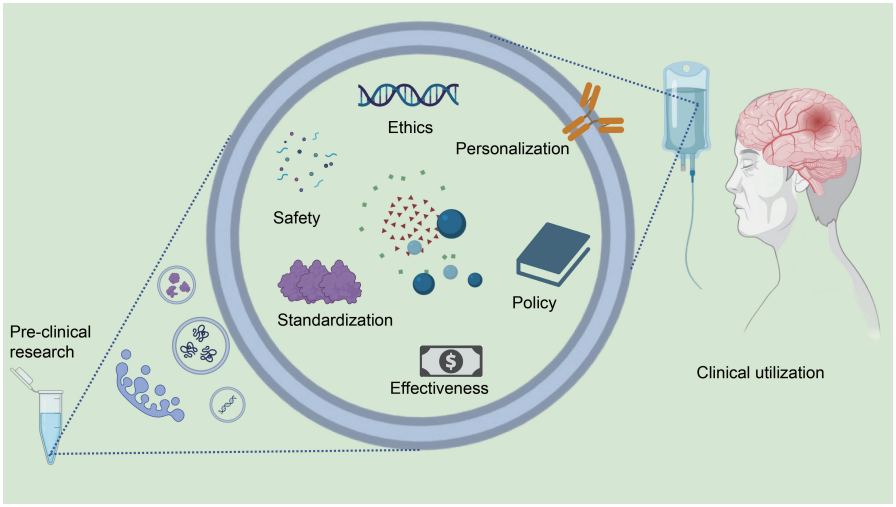

Figure 6 | **Barriers and challenges of clinical translation of exosomes.**

Moreover, determining the overall benefit of long-term exosome therapy for patients with stroke requires substantial clinical research. The sources of exosomes also raise ethical questions (Esfandyari et al., 2020; Zhu and He, 2023). While personalized sources of exosomes are ideal for stroke treatment, scalability and efficiency demands may lead to the use of allogeneic tissue MSCs or plasma (Sengupta et al., 2020; Zhou et al., 2022c; Li et al., 2023a). Consequently, ensuring informed consent, protecting donor privacy, and adhering to relevant legislation are imperative for advancing exosome-based stroke interventions. Given the current state of exosome research, addressing ethical issues in exosome-related clinical trials is crucial (He et al., 2020). This includes conducting ethical reviews during the initiation of research and implementing policy regulations pertinent to the study of exosomes (Delgado-Peraza et al., 2023; Johnson et al., 2023). Clarifying the effectiveness and safety of exosomes in preclinical studies is essential for public, ethical, and legal acceptance (Shi et al., 2021b). Such measures will facilitate policy support for research institutions and companies specializing in stroke treatments, thereby enhancing the proliferation and advancement of exosome-based interventions and ultimately benefiting a greater number of patients with stroke.

Standardization and effectiveness considerations

When applying exosomes to stroke treatment, a focus on standardization and the overall therapeutic benefit is necessary (Doyle and Wang, 2019; Lai et al., 2022). This involves the extraction, modification, and application of exosomes. The extraction and modification process must be standardized to ensure a high yield and quality of clinically used exosomes. Strict quality control standards should incorporate measures such as exosome count, content stability, toxicity, and purity detection methods (Yang et al., 2020a). These parameters must be clearly defined before clinical application. At the technical level, cost considerations are also crucial, as scale and efficiency pose challenges for the clinical use of exosomes. Efforts should focus on maximizing exosome output at a lower cost, thereby enhancing their accessibility for patients with stroke. Exosomes derived from the patient's own cells are ideal; however, engineered immortal cell lines present the most promising approach for large-scale production. This method not only improves yield and efficiency but also reduces costs, making exosome therapy more attainable for patients with stroke. Furthermore, the standardization of dosage and administration is equally important. Although some guidelines exist for exosome separation and identification methods, more comprehensive protocols are needed for clinical treatment applications (Darragh et al., 2023; Davidson et al., 2023). Currently, guidelines for dosage and administration are lacking, necessitating the further exploration of effective dosages to achieve therapeutic goals while minimizing the frequency and amount of administration, particularly for intracranial stroke interventions.

In summary, exosomes represent an emerging therapeutic approach with promising potential for a wide range of applications (Lee et al., 2022; Mondal et al., 2023). However, to facilitate their routine clinical use, it is essential to address existing challenges and engage in comprehensive research and discussions regarding efficacy, safety, ethical considerations, and regulatory frameworks (Shi et al., 2021b; Zhang et al., 2023c). The clinical implementation of exosome-based therapies requires a collaborative effort from scientific institutions, governmental bodies, regulatory agencies, and legal departments, which will subsequently advance the application of exosomes in stroke treatment, ultimately benefiting patients with stroke.

Limitations

This review has some limitations. First, due to time constraints, we limited our review to studies on exosomes related to stroke published in the past 3 years. This approach introduces a bias in our search and may have resulted in the omission of relevant literature. Second, we did not perform a comprehensive quality assessment and classification of all collected literature, which may hinder the clinical translation of our conclusions. For instance, the sources of exosomes in the included research articles vary; some are derived from cells cultured *in vitro*, while others originate from animal or human blood or body fluids. This variation limits the external applicability of the results. Additionally, exosome extraction methods differ, potentially leading to biased experimental outcomes. Regarding animal models, pre-clinical studies on exosomal interventions have predominantly utilized rodents, with a scarcity of experiments involving other species, affecting the universality of the conclusions. Concurrently, given the broad and potent biological effects of exosomes, existing studies lack comprehensive research on exosomes with identical backgrounds and sources; they are typically confined to specific biological effects and signaling pathways. Furthermore, effects that defy reasonable explanation based on current exosomes understanding may remain unreported or prove challenging to report. These limitations necessitate gradual resolution in future exploration and research.

Conclusion

The primary aim of this review was to explore the utilization and mechanisms of exosomes in stroke therapy, highlighting their significant therapeutic impact and providing a reference for clinical application. This review systematically summarizes the sources, production processes, modifications, and intervention methods of exosomes in stroke treatment, in addition to their applications under different stroke pathophysiological mechanisms.

We found that the number of clinical studies on exosomes is limited, with most still in progress. This highlights the urgent need for research in this area, particularly regarding exosomes and stroke. In preclinical studies, exosomes can originate from various tissues and cells; however, most of the current research focuses on exosomes derived primarily from stem cells and blood sources. This suggests that these sources provide the most efficient and straightforward methods for acquiring exosomes. We have compiled a comprehensive summary of techniques for exosome identification, which can be categorized into direct observation and indirect molecular labeling. Regarding exosome modifications, we have consolidated the methods used in prior studies, including cargo loading and surface modifications. Exosomes utilized in stroke treatment can be modified through various physical, chemical, and biological methods to enhance their drug-loading capacity and concentration, while facilitating specific neuronal targeting. These methodologies serve as valuable references for future analogous studies. Regarding the therapeutic effects, exosomes have been shown to possess potent biological effects in alleviating post-stroke inflammatory responses by modulating inflammatory cells and cytokines. They also mitigate oxidative stress following a stroke by reducing the production of reactive oxygen species and stabilizing mitochondria. Furthermore, exosomes have demonstrated significant efficacy in protecting neurons from PCD after a stroke. In the field of neural regeneration, exosomes can protect neural stem cells against apoptosis following a stroke, while also enhancing their migration and facilitating tissue regeneration,

particularly angiogenesis and white matter restoration. Currently, the primary method for administering exosomes in experimental animal studies is through intravenous injections; however, there has been growing interest in delivering exosomes via the ventricular lymphatic system, which has demonstrated significant therapeutic potential. Our findings suggest that administering exosomes through the cerebroventricles can serve multiple functions, including mitigating neuronal damage, reducing PCD, and promoting neural regeneration following a stroke. Collectively, these insights may serve as a valuable reference for the future clinical translation of exosome applications in stroke therapy and provide a foundation for subsequent basic and clinical research.

We have also identified challenges associated with the clinical application of exosomes, including concerns regarding safety, standardization, and ethical considerations. Personalized exosome therapy, which poses fewer ethical concerns and offers increased safety, may emerge as a promising treatment option for patients with stroke. We anticipate that these barriers and challenges will be addressed in future research, facilitating the application of exosomes in stroke treatment.

In conclusion, this review consolidates the current state of exosomes and their therapeutic utilization for stroke, with a primary focus on preclinical studies. By delineating the various mechanisms involved in stroke, in addition to the derivation, identification, modification, and administration of exosomes, we aimed to demonstrate the significant potential, translational value, and therapeutic efficacy of these cell-derived structures for clinical applications. This review also suggests potential avenues for future innovation by summarizing the research and advancements related to exosome utilization in animal models of stroke to date. We hope that ongoing research on exosomes will continue to yield significant outcomes and that more randomized controlled trials will be conducted to facilitate the clinical application of exosomes, ultimately benefiting a wide range of patients with stroke.

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