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REVIEW

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# Mesenchymal stem cell exosomes therapy for the treatment of traumatic brain injury: mechanism, progress, challenges and prospects

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## Abstract

Traumatic brain injury (TBI) is a heterogeneous disease characterized by brain damage and functional impairment caused by external forces. Under the influence of multiple mechanisms, TBI can cause synaptic dysfunction, protein aggregation, mitochondrial dysfunction, oxidative stress, and neuroinflammatory cascade reactions, resulting in a high disability and mortality rate for patients and a heavy burden on families and society. Exosomes are cell-derived vesicles that encapsulate a variety of molecules, including proteins, lipids, mRNAs, and other small biomolecules. Among these, exosomes derived from mesenchymal stem cells (MSCs) have garnered significant attention owing to their therapeutic potential in the nervous system, offering broad clinical applicability. Recent studies have demonstrated that MSC-derived exosome injections in traumatic brain injury models effectively mitigate local inflammatory damage and promote nerve regeneration following injury. Owing to their small size, challenging replication, ease of preservation, and low immunogenicity, MSC exosomes are emerging as a promising therapeutic strategy for traumatic brain injury. This review explores the pathogenesis of traumatic brain injury, the underlying mechanisms of MSC exosome action, and the potential clinical applications of MSC exosomes in the treatment of traumatic brain injury.

**Keywords** Traumatic brain injury, Mesenchymal stem cell exosome, Research progress

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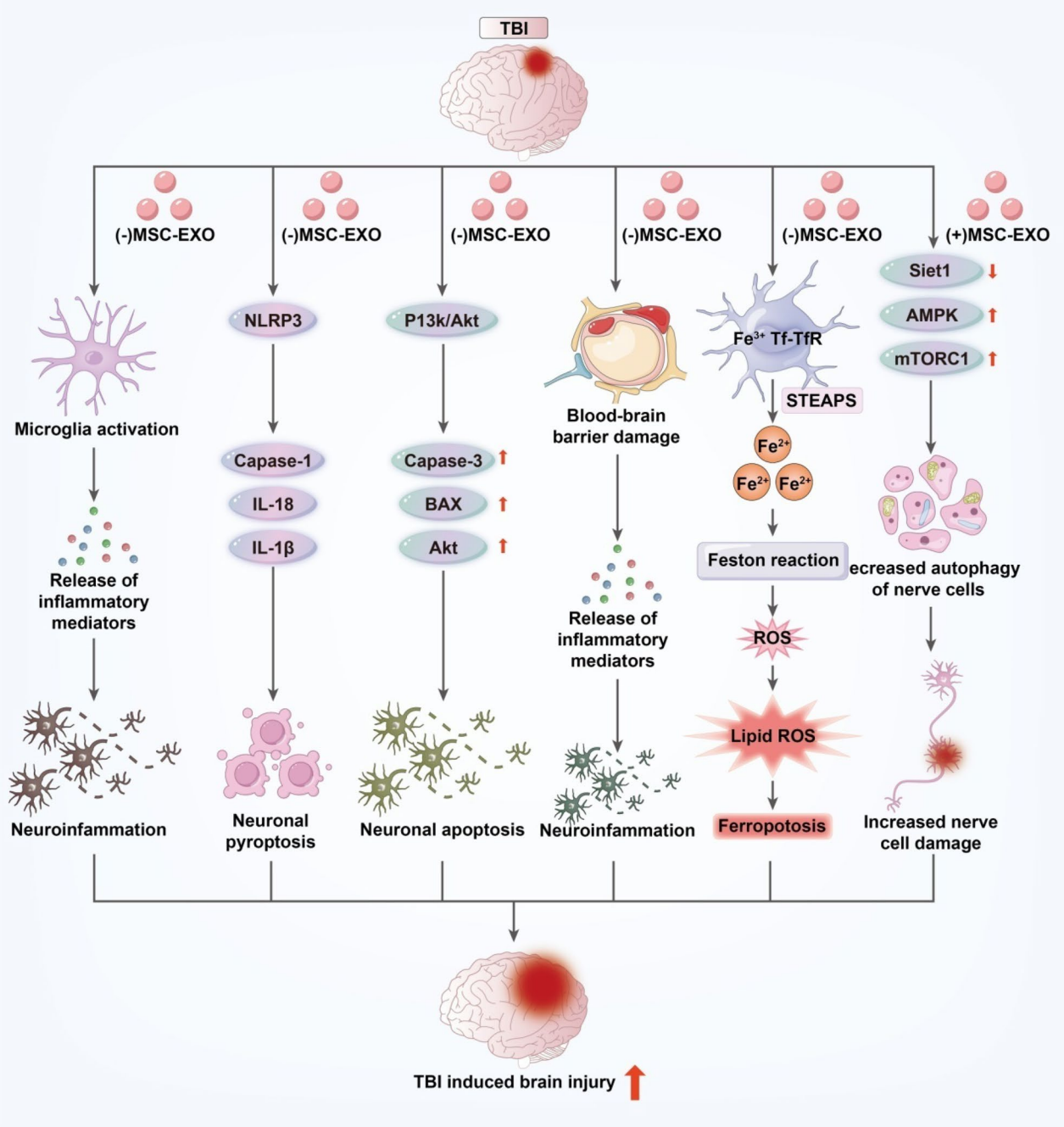
**Graphical abstract**




## Background

Traumatic brain injury (TBI) is a complex disorder that arises from neurological disturbances triggered by traumatic forces [1]. Brain trauma causes various pathological processes, including synaptic dysfunction, protein aggregation, mitochondrial impairment, oxidative stress, and central inflammation, contributing to its high morbidity and mortalityrates [2]. Annually, approximately one million cases of brain injury are reported worldwide, imposing a significant burden

on both families and society [2]. Current treatment options for TBI include surgical intervention, cell-based therapies, neuroprotective agents, hypothermic therapy, and electrical nerve stimulation [3]. However, owing to the diverse pathogenesis, clinical manifestations, and varying severity of the condition, existing therapeutic approaches often fail to yield satisfactory outcomes [4]. Therefore, enhancing the efficacy of TBI treatment remains a critical clinical challenge.

Mesenchymal stem cells (MSCs) are multipotent stem cells with self-renewal capabilities and the ability to differentiate into multiple cell types [5]. MSC-derived exosomes(MSC-EXOs) represent a novel multifunctional therapeutic modality capable of mediating intercellular signaling, promoting tissue regeneration, and exerting anti-inflammatory effects. These properties suggest the significant potential of MSC-EXOs in regenerative medicine [6]. MSC-EXOs have demonstrated efficacy in facilitating the repair of TBI, improving neuronal function, and improving patient prognosis, making them a promising therapeutic option [7]. This review examines the pathogenesis of TBI, the biological characteristics of MSC exosomes, their mechanisms of action, and their application in the treatment of TBI.

## Pathogenesis of TBI

TBI is a prevalent form of acquired brain injury and is classified into mild (14–15), moderate (9–13), and severe (3–8) categories based on the Glasgow Coma Scale (GCS) [8]. TBI originates as a primary injury but evolves into a secondary injury that exacerbates the initial damage. Primary injury may lead to cerebral hypoperfusion, hypoxia, hemorrhage, edema, and disruption of the blood–brain barrier, triggering inflammatory responses, altered metabolite release, and cerebral ischemic damage (Fig. 1) [9]. Astrocytes are central to the inflammatory response in TBI [10]. Research indicates that microglia can secrete anti-inflammatory factors through the M1/M2 phenotypic switching, interact with astrocytes, and contribute to neuronal repair after injury [11]. TBI induces a cascade of oxidative stress, excitotoxicity, mitochondrial dysfunction, and inflammation, which can impair brain function and cause long-term neuronal apoptosis [12]. These changes, which may persist for hours to years, often result in functional impairment and disability [13]. Following TBI, local inflammation plays a dual role: it helps clear necrotic tissue, promotes angiogenesis, and supports nerve repair post-injury [14]. However, chronic inflammation can exacerbate permeability, edema, and apoptosis of the cerebrospinal fluid barrier, thus worsening secondary brain damage and significantly contributing to the progression of TBI [15, 16]. Developing safer and more effective treatments for secondary brain injury remains an urgent clinical priority [17].

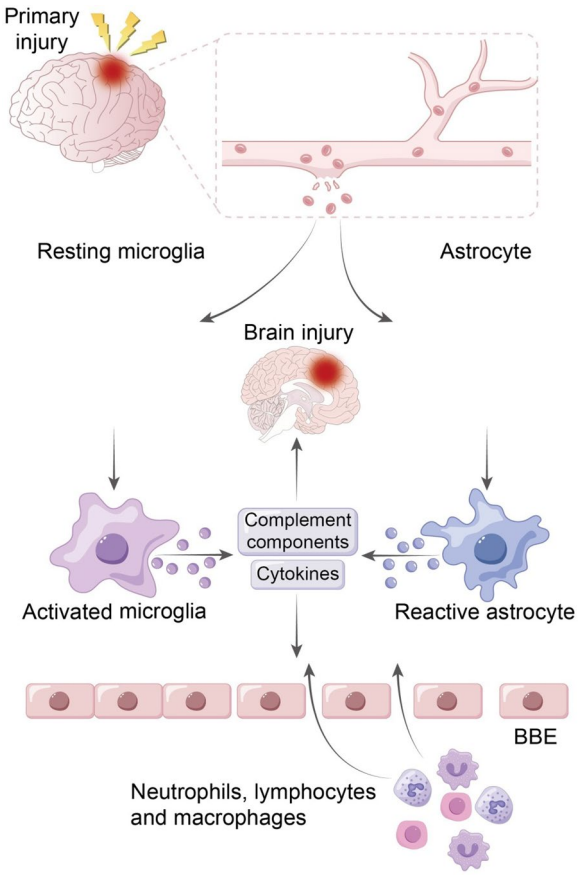



**Fig. 1** Mechanism of traumatic brain injury-induced brain damage

## Isolation and extraction of exosomes

Different substances exhibit unique sedimentation coefficients in solution, allowing centrifugal forces to be applied to induce precipitation. Differential centrifugation, which capitalizes on this principle, is currently the most widely used technique for isolating extracellular vesicles (EVs). Sequential centrifugation steps were employed: 300×*g* to remove cells and debris, 2000*g* to sediment apoptotic bodies, and 10,000 ×*g* to collect large vesicles. Finally, EVs were obtained by centrifugation at 100,000 ×*g*, followed by filtration through 0.22 μm or 0.45 μm pore-size membranes to enhance purity. If necessary, this process can be repeated with PBS resuspension to yield relatively pure EVs [18, 19]. The primary advantages of this method include effective separation of lipoproteins and proteins, high yield, and low cost. However, its disadvantages include prolonged processing time and the tendency of exosomes,

which are on the nanometer scale, to aggregate during high-speed centrifugation. Additionally, centrifugal shear forces may compromise the structural integrity of the exosomes. The purity of isolated EVs is highly sensitive to factors such as the sample viscosity, rotor type, and radius of rotation [20]. Technological advancements have led to the development of density-gradient centrifugation to address these limitations. Using common media such as iodixanol, a gradient with increasing density from top to bottom was established in a centrifuge tube. This approach can be further categorized into equal-density gradient centrifugation and rate zone centrifugation, which segregate particles into specific zones according to their densities and sedimentation rates [21].

Alternatively, polymers such as polyethylene glycol (PEG) can be employed to form a cross-linked network structure in solution, thereby enhancing the binding forces between hydrophobic proteins and lipid molecules and facilitating their separation from the solution. Simultaneously, the sugar chains of EV-membrane glycoproteins interact with lectins, altering their dispersibility and solubility. Consequently, EVs can be isolated using low-speed centrifugation [22, 23]. This method is simpler, less time-consuming, and results in less damage to EVs compared to ultracentrifugation. However, it suffers from low purity, especially when working with complex body fluids. Proteins, such as fibrinogen and lipoprotein particles, along with some vesicles, tend to aggregate and precipitate together, making it difficult to separate them effectively. This issue may have affected the accuracy of subsequent studies [24]. Consequently, efforts to improve EV purity continue, including methods such as using protease K to enhance the purity of samples [25]. Despite these advancements, this approach is not the preferred option for exosome extraction.

A rapid, time-efficient, and effective ultrafiltration method utilizes specialized pore size filter membranes to separate samples based on the size of EV molecules. This process allows for the retention of EVs while filtering out smaller molecules, such as proteins [26]. Ultrafiltration can be performed using either centrifugal or pressure-based methods. To address some of the limitations of traditional filtration techniques, an asymmetric flow-field separation method has been introduced. This technique applies force fields from multiple directions, creating an angled flow path between the filtered liquid and membrane, which enables the fluid to pass through the membrane at varying speeds. This configuration significantly reduced the likelihood of membrane blockage. Additionally, when combined with diverse detection methods, this approach can facilitate the sorting and identification of different vesicle subtypes [27–29]. However, challenges related to time consumption and yield require further refinement.

Exosomes contain unique membrane proteins that can be targeted by antigen antibody-specific recognition. By attaching specific antigens to filter membranes or magnetic beads, extracellular vesicle membrane proteins can be captured, thereby isolating EVs. This technique, known as immunoaffinity membrane separation, offers high specificity but is limited by its high cost and low yield, which have hindered its widespread application [30]. Other methods for isolating EVs, such as size-exclusion chromatography, molecular sieve chromatography, and emerging microfluidic techniques, offer distinct advantages and drawbacks. In clinical practice, a combination of multiple techniques is often employed to optimize the efficiency and purity of EV extraction.

Flow Field-Flow Fractionation (AF4) represents the most widely adopted flow field separation technique in extracellular vesicle research. Developed by Wahlund and Giddings in 1987, AF4 is a separation technique. Characterized by a broad separation range, AF4 eliminates the need for pre-treatment procedures (e.g., centrifugal filtration) on samples. This technique has gained popularity in separating and analyzing complex biological samples. AF4 has been applied to isolate extracellular vesicles from human mesenchymal and neural stem cell cultures, and to perform size-based separation and characterization of mouse melanoma cell-derived extracellular vesicles [31]. Despite these advantages, AF4 remains underutilized in extracellular vesicle isolation studies. By optimizing AF4 and integrating it with density ultracentrifugation, Hu et al. successfully isolated high-purity, intact extracellular vesicles from human plasma and serum, minimizing lipoprotein and non-extracellular vesicle contaminant interference. Subsequent proteomic analysis identified novel plasma exosome markers (e.g., MYCT1, MPIG6B, and TSPAN14), suggesting that certain traditional cell-derived exosome markers may not be suitable for plasma exosome detection [32]. AF4 employs a gentle separation environment, avoiding mechanical stress-induced damage to extracellular vesicle biological activity inherent in conventional separation methods. Integration of multi-angle light scattering (MALS) and fluorescence detection systems with AF4 enabled comprehensive characterization of separated extracellular vesicles. Detection results indicated that cross-flow velocity and channel dimensions exerted significant effects on extracellular vesicle fractionation quality. Conversely, focusing time demonstrated minimal influence on separation outcomes. Through optimizing AF4 parameters (cross-flow gradient, focusing time,

ultrafiltration parameters, sample volume, injection volume) and sample preparation protocols, Sitar et al. successfully separated and characterized human plasma-derived extracellular vesicles using AF4. This study highlighted the ability of AF4 to differentiate extracellular vesicles from HDL/LDL particles in human plasma, enhance product purity, and ensure reproducibility [33]. Recent advancements have demonstrated that miniaturized AF4 channels can achieve comparable separation efficiencies while reducing the processing time through dimensional optimization [34, 35].

Thermoelectrophoresis describes the directed migration of particles toward cooler regions in temperature-gradient environments, a well-documented physical phenomenon, and recent advancements have enabled its application in extracellular vesicle separation and enrichment protocols. Characterized by label-free operation, high sensitivity, and ease of use, thermoelectrophoresis offers distinct advantages for processing extracellular vesicles in complex biological matrices [36]. Sun et al. introduced a nanoplasmonic thermophoretic aptamer sensor incorporating fluorescently labeled aptamers for exosome targeting. Aptamer aggregation on exosome surfaces generates a fluorescence enhancement effect through plasmonic coupling. Fluorescence intensity correlates with target protein density on exosome surfaces. Laser irradiation induces thermophoretic aggregation of extracellular vesicles in solution. Aggregated vesicles produce stronger signals compared to single vesicles, enhancing detection sensitivity. This study employed seven cancer-specific exosome subtypes for aptamer labeling. In a 102-patient cohort study, the sensor achieved 95% sensitivity, 100% specificity, and 68% diagnostic accuracy for cancer classification. Requiring < 1 μL serum per test, this method enables minimally invasive early cancer screening, classification, and treatment monitoring [37]. Subsequent optimization enabled the in situ detection of exosomal miRNAs [38]. Leveraging thermophoretic aggregation to enhance miRNA fluorescence signals, this method achieves sub-femtomolar detection (0.36 fM) using only 0.5 μL serum samples. Demonstrating 88% sensitivity and 83% specificity, this approach shows significant potential for clinical translation. Thermal swimming-based analysis enabled rapid, sensitive, and cost-effective characterization of surface glycans on TNBC plasma exosomes. This method achieved 91% diagnostic accuracy and 96% accuracy in longitudinal treatment response monitoring [39]. Despite these advantages, thermophoretic separation faces challenges: (1) potential loss of small/low-charge vesicles; (2) incomplete removal of lipoprotein/protein aggregate contaminants; (3) lack of standardized protocols and quality control metrics for extracellular vesicle isolation. As with all emerging technologies, continued optimization of thermophoretic methods will likely establish them as key tools for extracellular vesicle research.

Extracellular vesicle separation methods have been developed based on their biophysical properties (size, density, charge, and composition), including ultracentrifugation, ultrafiltration, size exclusion chromatography, polymer precipitation, and immunoaffinity approaches, among which ultracentrifugation represents the gold standard for extracellular vesicle isolation and is well established and widely adopted by the research community; however, each method exhibits distinct advantages and limitations regarding separation purity, efficiency, throughput, and operational complexity [40–50] (Table 1). Thus, integrating complementary separation techniques represents a promising strategy for the vesicle isolation.

**Table 1** Comparison of different exosome separation methods

| Item | Purity | Productivity | Cost | Separation time | Refs |
|---|---|---|---|---|---|
| Ultracentrifugation | Low | Moderate | Moderate | 4–6 h | [40] |
| Ultrafiltration | Low | Very high | Very high | 1–2 h | [41] |
| Size-exclusion chromatography | Very high | Moderate | Moderate | 0.5–2 h | [42] |
| Precipitation polymerization | Low | High | Low | 2–12 h | [43] |
| Immunoaffinity chromatography | High | Low | High | 2–4 h | [44] |
| Microfluidics | High | High | High | 0.5–1 h | [45] |
| DNA aptamer-based exosome separation | High | Moderate | High | 2–4 h | [46] |
| Flow Field-flow fractionation | Moderate | Moderate | High | 1–2 h | [47] |
| Thermophoresis | High | Moderate | High | 0.5 - 1 h | [48] |
| Phospholipid recognition separation | High | Moderate | High | 2–3 h | [49] |
| Combined separation and purification method | High | Low | High | 1–2 h | [50] |

## Identification of exosomes

Following the isolation of EVs, their identification is typically based on their physicochemical properties, including size, morphology, concentration, and presence of specific protein markers. The most common methods for identification are fluorescence-activated cell sorting (FACS) and western blotting, both of which focus on the detection of specific protein markers carried by EVs. In particular, exosomes can be identified by their expression of characteristic proteins, such as the heat shock protein Hsp60 and the transmembrane protein superfamily members CD63, CD9, and CD81. This is achieved by binding EVs to beads, applying fluorescent antigen–antibody reactions, and analyzing the resulting interactions through flow cytometry [51]. Although this method is time-consuming, it offers the advantage of accurately identifying EVs by eliminating interference from impurities and allows for the precise quantification of EV concentration.

Proteomics enables characterization of both known and novel exosomal proteins across diverse abundance levels. Nano liquid chromatography-mass spectrometry (nanoLC-MS) integrates dual-pressure ion trap speed/sensitivity with Orbitrap's high resolution and mass accuracy, enabling deep proteomic profiling of complex biological matrices. This technology supports high-throughput qualitative and quantitative proteomic analysis [52]. MS-based proteomics uncovers protein functions, activities, and evolutionary relationships between extracellular vesicles and their parental cells. Extracellular vesicles carry diverse molecular cargoes, including proteins, RNAs, and miRNAs. Through surface protein-receptor interactions, extracellular vesicles deliver genetic payloads to target cells, regulating transcriptional and translational processes. Agarose gel electrophoresis separates nucleic acid fragments based on size via electrophoretic mobility. Analysis of extracellular vesicle-mediated gene regulation requires nucleic acid isolation/characterization techniques, including qPCR [53–56] and RT-qPCR. Complementary characterization methods include transmission electron microscopy (TEM) and dynamic light scattering (DLS), each with distinct principles and performance trade-offs for extracellular vesicle analysis (Table 2).

## Biological characteristics of exosomes of mesenchymal stem cells

Exosomes (MSCs) are characterized by their self-renewal capacity and multifunctional differentiation potential [62]. However, recent research has revealed more complex aspects of their reparative effects. Although only a small proportion of transplanted MSCs survive, their survival is typically short-lived, and they are often unable to migrate to the injured site or differentiate into permanent tissues [63]. Intriguingly, MSCs can exert therapeutic effects even when located far from the damaged area [63]. These observations suggest that MSCs do not directly replace damaged tissues but instead mediate biological effects through a variety of bioactive factors secreted by the cells. These factors include immune modulation, anti-inflammatory and anti-apoptotic activities, scar reduction, and the promotion of angiogenesis [62]. Exosomes, which are 30–100 nm in size, encapsulate the majority of these bioactive factors and are known for their significant biological functions [64, 65]. The formation of exosomes begins with the release of the cell membrane, which then forms early endosomes. These endosomes then accumulate granular substances that are secreted by cells. As early endosomes mature, they transform into late endosomes, which are characterized by the formation of cytoplasmic polycystic structures. These structures fuse with portions of the cell membrane leading to the formation of budding vesicles. These vesicles, now referred to as exosomes, are released into the extracellular space via the exocytic pathway [66, 67]. Exosomes can interact with recipient cells through ligand-receptor binding or by releasing their contents into target cells via endocytosis or by direct fusion with the cell membrane. These processes enable exosomes to modulate the biological behavior of target cells and facilitate intercellular communication [64]. The process of exosome formation and its interaction with the target cells are illustrated in Fig. 2.

In addition to proteins and lipids, exosomes are enriched in various nucleic acids [66]. MiRNAs, a class of small non-coding RNAs, regulate gene expression by binding to complementary sequences of target genes, leading to their degradation and inhibition of protein translation. This process influences key biological processes such as cell differentiation, proliferation, and apoptosis [68]. For example, miR-125a has been shown to promote angiogenesis [69], whereas miR-19a inhibits apoptosis [70]. Recent studies have demonstrated that the expression of miR- 133b and miR-22 in exosomes is upregulated under hypoxic and ischemic conditions, facilitating nerve repair through the modulation of the extracellular microenvironment[71], [62, 72]. These observations suggested the presence of a bidirectional regulatory pathway between MSCs and their surrounding microenvironment. However, precise identification of exosome content remains a challenge. Thus, future research should focus on further elucidating the composition and functional roles of exosomes, as well as their involvement in tissue repair mechanisms.

**Table 2** Comparison of different methods for identifying extracellular vesicles

| Technical name | Principle | Detection content | Advantage | Disadvantage | Refs |
|---|---|---|---|---|---|
| Transmission electron microscope (TEM) | A collimated electron beam is directed through ultrathin samples, where interactions with atomic structures induce angular scattering patterns that generate high-resolution transmission electron micrographs | Form, structure | Transmission electron microscopy (TEM) enables direct visualization of exosome ultrastructure and morphological features | Pre-analytical processing of samples involves labor-intensive protocols, limiting high-throughput applications.Key limitations include: 1) Inability to accurately quantify post-fixation exosome concentrations 2) Structural artifacts induced by aldehyde fixation 3) High capital costs associated with TEM systems | [57] |
| Dynamic light scattering (DLS) | During particle stochastic motion, constructive/destructive interference of scattered light generates time-correlated intensity fluctuations.These fluctuations are analyzed to derive particle diffusion coefficients, from which hydrodynamic diameters are calculated using the Stokes–Einstein equation | Particle size, concentration | Dynamic light scattering (DLS) has a detection limit of 10 nm and exhibits higher sensitivity to monodisperse particle populations | Light scattering signals from larger particles may overshadow those from smaller vesicles in polydisperse samples.This limitation restricts DLS applicability to polydisperse exosome preparations | [58] |
| Nanoparticle tracer analysis (NTA) | Laser illumination of exosome samples generates scattered light, which is visualized via microscopy with digital imaging to quantify particle number and concentration | Quantity, concentration and particle size distribution | Nanoparticle tracking analysis (NTA) enables direct real-time visualization of vesicles, providing accurate sizing for both monodisperse and polydisperse samples | Key limitations include: 1) Stringent sample purity requirements 2) Need for parameter optimization across instrument settings 3) Challenges in analyzing heterogeneous exosome populations | [59] |
| Western blot | Immunoassays detect target proteins in complex samples through antigen–antibody interactions | Surface marker detection | Enable precise identification of exosome subtypes | Biomarker profiles vary with extracellular vesicle cell origin | [60] |
| Nanoflow cytometry | Nanoflow cytometry leverages laser-based detection and fluorescence staining of surface markers (e.g., antigens, antibodies) to enable multi-parameter quantitative analysis of extracellular vesicles | Detecting particle size and surface markers | This technique enables rapid, high-throughput analysis of particle size/volume using low-concentration samples | Key limitations include: 1) Requires specialized instrumentation 2) Limited sensitivity for small exosomes | [61] |

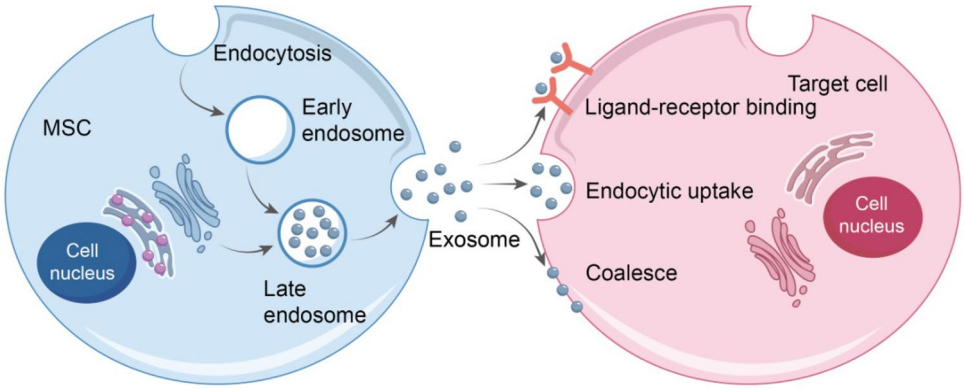



**Fig. 2** Processes of exosome formation and binding to target cells. *MSCs* mesenchymal stem cells

## Mechanism of action of exosomes in mesenchymal stem cells

Exosomes, secreted by nearly all brain cells, are categorized into neuronal, microglial, and astroglial exosomes based on their cellular origin [73]. Exosomes derived from these cells play a pivotal role in transforming microglia from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, thereby ameliorating the symptoms of TBI [74]. This review discusses the mechanisms by which exosomes from various cell types contribute to TBI treatment (Table 3) [75–90]. Compared with exosomes derived from central nervous system cells, those derived from peripheral MSCs have gained increased attention for their ability to enhance recovery from TBI. MSC-derived exosomes are particularly effective in inducing the M1-to-M2 shift in microglia, which is a key mechanism in TBI pathophysiology of TBI [91, 92]. Recent studies have suggested that MSC-derived exosomes can mitigate secondary neurodegeneration and neuroinflammation, promote neuronal regeneration, stimulate vascularization, and enhance motor function. The mechanisms underlying the action of exosomes on MSCs have been extensively analyzed.

### Vascular regeneration

Previous studies have demonstrated that angiogenesis and tissue regeneration are essential for restoring normal tissue function, and exosomes play a pivotal role in promoting endothelial cell migration, proliferation, and angiogenesis [93]. Exosomes have been shown to contain bioactive molecules such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and interleukin-8 (IL-8), with VEGF being particularly critical in endothelial cell proliferation and angiogenesis [94]. These exosome-derived signaling molecules can activate various signaling pathways [95]. For instance, hairy and enhancer of split 1 (HES1), a key downstream molecule of the Notch signaling pathway, regulates vascular remodeling and angiogenesis [96]. Additionally, Gonzalez-King et al. identifiedJagged-1 within exosomes as a regulator of angiogenesis via the Notch pathway [97]. Furthermore, Wnt proteins carried by exosomes have been shown to promote β-catenin translocation into the nucleus of endothelial cells, activating downstream effector molecules [98]. This process, facilitated by the Wntproteins in exosomes, accelerates angiogenesis and tissue repair. Shang et al. demonstrated that protein kinase B(AKT) overexpression in MSCs enhanced angiogenesis through activation of the platelet-derived growth factor (PDGF) signaling pathway [99]. Additionally, Moeinabadi-Bidgoli et al. reported that exosomes with elevated levels of extracellular matrix metalloproteinases promote endothelial cell migration and vascular network formation by activating the extracellular signal-regulated kinase (ERK)/AKT pathway [100].

Moreover, miRNAs present in exosomes have been implicated in angiogenesis promotion [101]. For example, exosomes carrying miRNA-125a and miRNA-30b have been found to bind to the 3'untranslated region of the delta-like 4 (Dll4) gene, inhibiting DLL4 expression and thereby facilitating vascular regeneration [102]. miRNA-21 downregulates PTEN, activates activating AKT, and regulates the expression of factors involved in angiogenesis and apoptosis [103]. The mechanism by which exosomes promote angiogenesis is illustrated in Fig. 3.

### Immune regulation

Following brain injury, the activation of glial cells, recruitment of white blood cells, and release of inflammatory mediators significantly influence not only

**Table 3** Mechanisms of exosomes from different cellular sources for the treatment of TBI

| Donorcells | Recipient cell | The cargo or molecules involved | Mechanism | Ref. |
|---|---|---|---|---|
| MSCs-exo | Microglial cell | miR-17-92 | Mitigate neuroinflammation while promoting angiogenesis and neuronal regeneration | [75] |
| | Microglial cell | NF-κB, p38/MAPK signaling pathways | Suppress microglial/macrophage activation, attenuate neuroinflammation and apoptosis, and enhance hippocampal neuronal proliferation | [76] |
| | Microglial cell | NF-κB | Attenuate neuroinflammation, suppress neuronal apoptosis, and facilitate neuronal morphogenesis | [77] |
| | Hippocampal neurons | microRNA-93 | This axis mediates glial activation, neuroinflammation, and BDNF-dependent hippocampal neuropathology | [78] |
| | Microglia and astrocytes | BDNF | This axis mediates glial activation, neuroinflammation, and BDNF-dependent hippocampal neuropathology | [79] |
| | Microglia | miR-26a-5p | Exosomal miR-26a-5p suppresses microglial apoptosis by targeting CDK6 | [80] |
| Neuro | Microglial cell | miR-21-5p | Induce microglial polarization while suppressing neuroinflammation | [81] |
| | Neuro | miR-21-5p | Suppress neuronal autophagy to preserve neuronal integrity | [82] |
| MDEs | Microglial cell | miR-124-3p/Rela protein/Apolipoprotein E | Mitigate traumatic brain injury (TBI)-induced neurodegeneration and enhance cognitive recovery | [83] |
| | Neuro | miR-124-3p/Rela protein/Apolipoprotein E | TBI inhibition reduces neurodegeneration | [84] |
| | Neuro | miR-124 | Attenuation of neuronal apoptosis via miR-124/USP14 axis regulation | [85] |
| | Neuro | miR-5121 | RGMa modulation downregulates GAP43/PSD-95 expression and facilitates neuronal growth | [86] |
| ADEs | Microglial cell | miR-873-5p/NF-κB | Induce microglial M2 polarization to suppress neuroinflammation | [87] |
| | Neuro | Slit connexin α1 truncated monomer – 20k | Inhibition of neuronal apoptosis and attenuation of post-injury dendritic damage | [88] |
| | Cerebral vascular endothelial cells | Protein phosphatase/protein kinase B | Suppress apoptosis to preserve blood–brain barrier integrity | [89] |
| | Microglial cell | – | Facilitate microglial polarization to reduce neuroinflammation | [90] |

*miR* microRNA, *NF-κB* nuclear factor-kappaB, *p38 MAPK* mitogen-activated protein kinase

the surrounding glial cells and neurons but also adjacent immune cells, such as neutrophils, macrophages, and lymphocytes (Fig. 4) [104]. Research has demonstrated that MSC-derived exosomes modulate inflammatory responses by downregulating and upregulating key inflammatory mediators while also facilitating the differentiation of $CD^{4+}$ T cells into regulatory T cells [105]. Lin et al. further indicated that MSC-derived exosomes promote the differentiation of Th1/Th2 cells, which is accompanied by an increase in regulatory T cells, thereby enhancing the proliferation of peripheral mononuclear cells and CD3+T lymphocytes and attenuating inflammatory responses [106]. Microglia and astrocytes are critically involved in brain injury and are capable of producing proinflammatory cytokines [107]. Studies have shown that MSC-derived exosomes can mitigate brain injury induced by inflammatory responses and contribute to the repair of white matter microstructures through the modulation of microglial and astrocyte activation [108].

Exosome components have also been shown to modulate immune function to varying extents [109]. For instance, VEGF exerts anti-inflammatory effects by inhibiting the expression of proinflammatory cytokines [110]. Prostaglandin E2 and transforming growth factor beta (TGF-β) can significantly suppress NK cellfunction [111, 112]. IL-10 inhibits the Th1 immune response while upregulating the expression of anti-inflammatory factors by suppressing the activation of macrophages and neutrophils [113]. Additionally, these factors may interact with antigen-presenting cells, such as dendritic cells, influencing antigen presentation. IL-10 inhibits dendritic cell activation via the Janus kinase 1 (JAK1)/signal transducer and activator of transcription (STAT)3

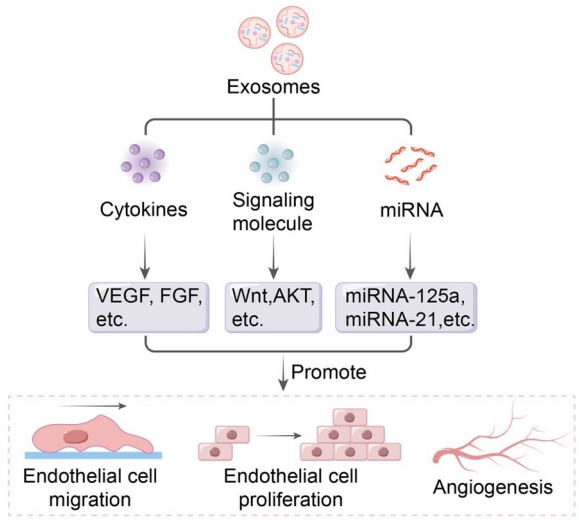



**Fig. 3** Mechanism of exosomes promoting vascular regeneration. *Akt* protein kinase B, *VEGF* vascular endothelial growth factor, *FGF* fibroblast growth factor, *miR* microRNA

signaling pathway, working synergistically with other antigenic factors to maintain the immature immune tolerance of dendritic cells [114]. Exosomes not only harbor a wide array of immunomodulatory proteins and factors but also carry miRNAs that play significant roles in immune regulation [109]. Phinney et al. demonstrated that MSC-derived exosomes contain miRNAs capable of inhibiting macrophage activation and exerting anti-inflammatory effects by blocking the toll-like receptor pathway. Figure 5 illustrates these processes [115].

Immune cell populations, including monocytes/macrophages, T cells, and natural killer cells, play critical roles in angiogenesis. These cells regulate neovascularization formation and stability through growth factor/cytokine secretion and inflammatory signaling mediation. Furthermore, immunosuppressive agents modulate immune system activity, thereby indirectly influencing angiogenic processes. In CD4+ T cell-deficient breast tumor models, reduced pericyte coverage and increased tumor hypoxia were observed, suggesting CD4+ T cell deficiency induces vascular dysfunction [116]. Dual anti-CTLA4/PD1 therapy, traditionally considered to primarily target T cells [117–119], induces tumor vascular normalization [119]. Immune cells exhibit both antiangiogenic and proangiogenic properties (Fig. 6) [119–121]. Thus, mesenchymal stem cell-derived exosomes are likely to modulate vascular remodeling through immune cell-mediated inflammatory signaling.

### Promoting myelination and axon growth

Oligodendrocytes, essential components of the central nervous system [122], contribute significantly to myelin production, which is critical for maintaining the structural integrity and functional capacity of myelin [123]. Myelin sheath and axonal injury resulting from nerve damage impair the conduction of nerve impulses [124]. Otero-Ortega et al. demonstrated that exosomes enhance axonal regeneration; stimulate oligodendrocyte proliferation, differentiation, and migration; and promote myelination [125]. Zhang et al. demonstrated that distal neurons and axons are adsorbed [126], and that exosomes containing miRNAs with axon-promoting properties can exert effects on axonal growth. Specifically, miR-17-92, which is highly expressed in exosomes, promotes axonal growth when delivered via MSC transfection

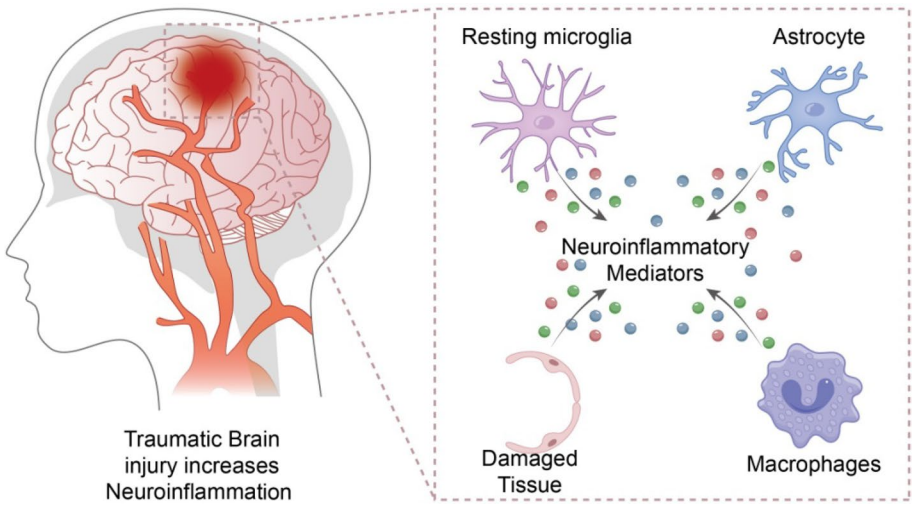



**Fig. 4** Mechanism of traumatic brain injury-induced neuroinflammation

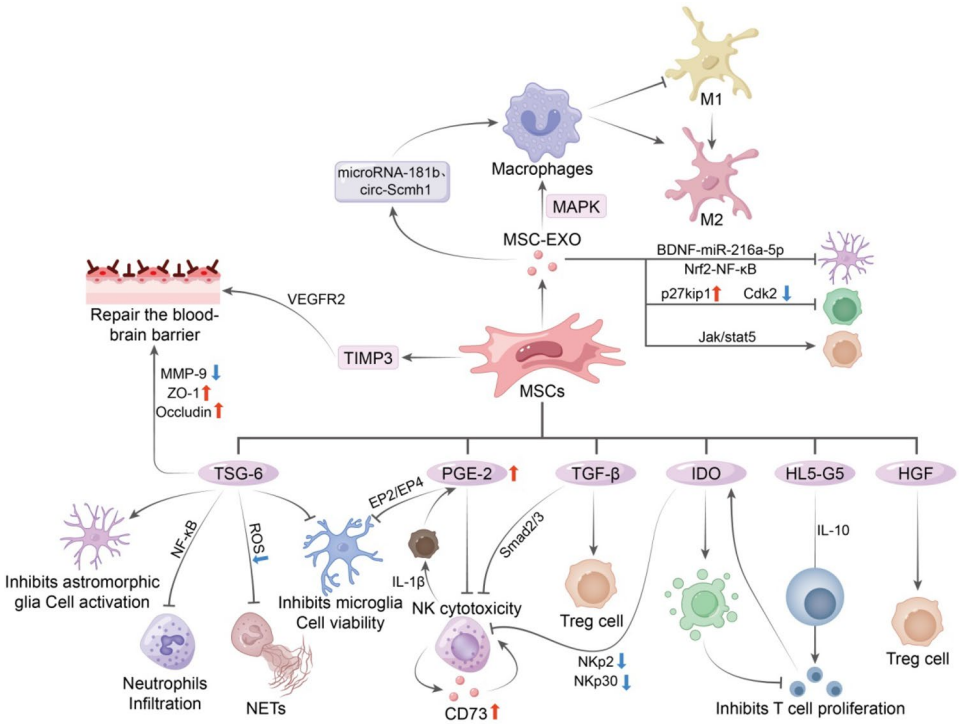



**Fig. 5** Immunomodulation by exosomes from plasmablast stem cells in traumatic brain injury. *MSCs* mesenchymal stem cells, *MSC-EXO* mesenchymal stem cell exosomes, *NETs* neutrophil extracellular traps, *TSG-6* TNF-α stimulates gene/protein 6, *PGE-2* prostaglandin E2, *TGF-β* transforming growth factor-β, *IDO* Indoleamine 2,3-dioxygenase, *HL5-G5* leukocyte antigen-G5, *HGF* hepatocyte growth factor, *MMP-9* matrix metalloproteinase-9, *ZO-1* blocking small band protein-1, *Occludin* tight junction closure protein, *VEGFR2* endothelial growth factor receptor 2, *MAPK* mitogen-activated protein kinase, *BDNF* brain-derived neurotrophic factor, *TIMP3* tissue metalloproteinase inhibitor 3, *Jak/Stat5* protein tyrosine kinase/signal transduction and transcription activator 5, *IL* interleukin, *Nrf2* nuclear factor E2 related factor 2, *ROS* reactive oxygen species, *NF-κ B* nuclear factor kappa B, *Treg cells* regulatory T cells

[127]. This study revealed that miR-17-92regulates the AKT/mammalian target of rapamycin (mTOR)/Glycogen Synthase Kinase-3β (GSK-3β) signaling pathway, promoting their phosphorylation, activating downstream signaling cascades, and facilitating axonal repair [128]. Moreover, miR-133b, been shown to enhance axonal growth, synaptic plasticity, and brain injury repair by modulating the expression of tyrosine hydroxylase and dopamine transporters in damaged neurons [129].

### Inhibiting cell apoptosis

Tissue injury often leads to cellular damage and heightened apoptosis [130]. Previous studies have indicated that MSC transplantation significantly improves ischemic neuron survival and reduces neuronal apoptosis [131]. MSCs exert anti-apoptotic effects through exosomes (Fig. 7). Studies have suggested that exosomes regulate Leukemia-2 (BCL-2)and pro-apoptotic BCL2-Associated X (BAX) gene expression, upregulating the BCL-2/BAX ratio, thereby inhibiting apoptosis [132]. Song et al. found that under oxidative stress, exosomal cytokines, including IL-8 and TNF-α, enhance mitochondrial membrane potential and suppress apoptosis via the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway [133]. Exosomes have also been shown to release anti-apoptotic miRNAs, particularly miR-19a andmiR-21-5p, which mitigate apoptosis through the activation of AKT, ERK, and other signaling pathways [134–136]. Furthermore, Wang et al. demonstrated thatexosomalmiR-21 and miR-210 downregulate caspase-3, contributing to apoptosis inhibition [137, 138], whereas after cerebral ischemia, miR-133a-3p levels in exosomes increase, interacting with DNA methylated cytosine-guanine (CpG) sites to further suppress apoptosis [139].

### Inhibitingferroptosis of nerve cells

Ferroptosis, a form of programmed cell death driven by lipid peroxidation and iron-mediated processes, is characterized by excessive accumulation of reactive oxygen species (ROS) within cells, leading to mitochondrial structural damage. Unlike apoptosis, pyroptosis, necrosis, or autophagy, which are typically inhibited by specific blockers, ferroptosis cannot be

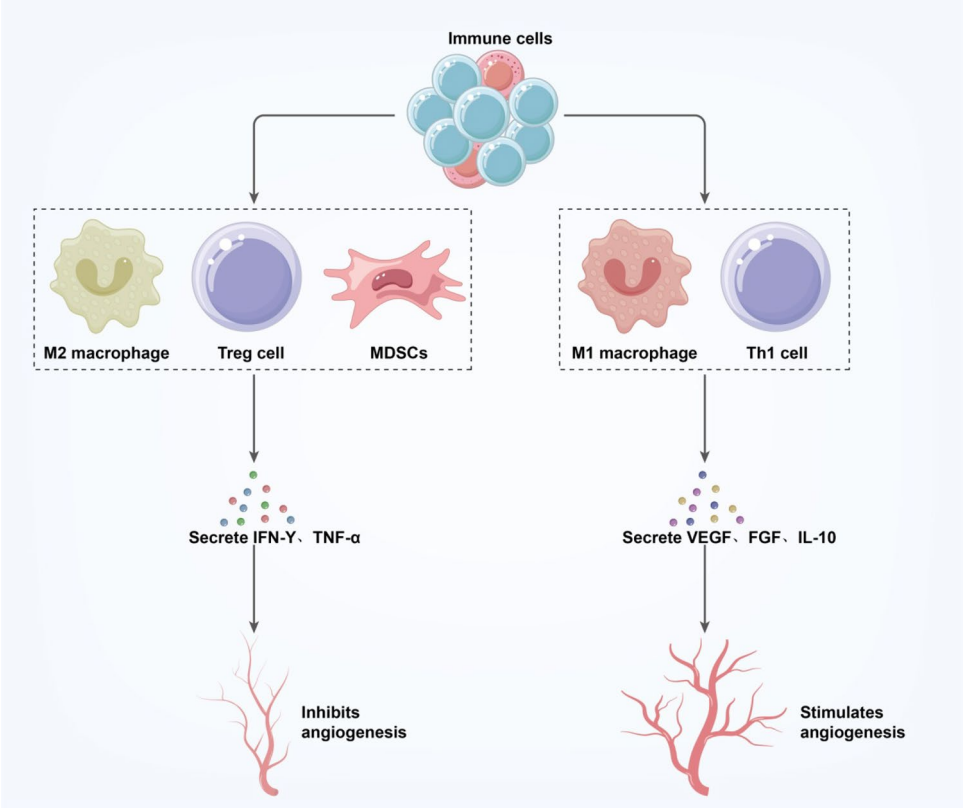



**Fig. 6** Immune cell-mediated regulation of vascular regeneration. *VEGF* vascular endothelial growth factor, *FGF* fibroblast growth factor, *MDSCs* myeloid-derived suppressor cells

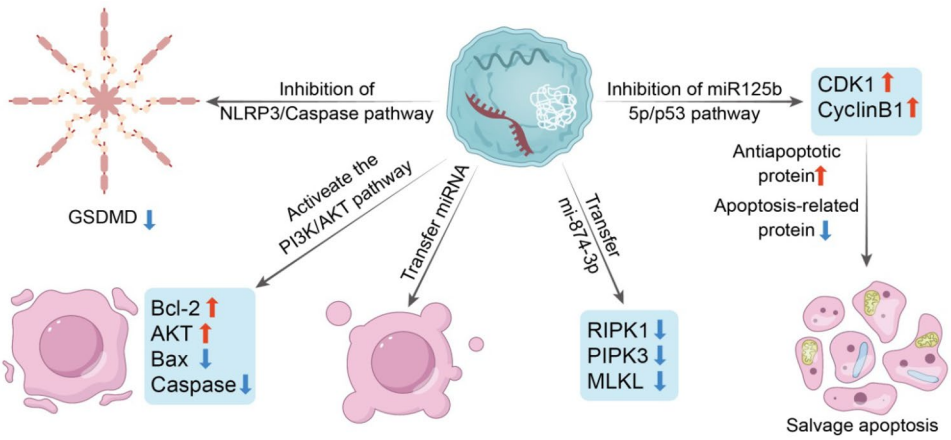



**Fig. 7** Mechanism by which extracellular vesicles reduce traumatic brain injury-induced neuronal apoptosis. *BCL2* B-cell lymphoma 2, *Akt* protein kinase B, *BAX* BCL2 associated X, *CDK1* cyclin-dependent kinase 1, *RIPKs* receptor interacting protein kinases, *MLKL* mixed lineage kinase domain-like protein

suppressed by these agents and can only be mitigated by antioxidants and iron chelators [140]. The key pathways regulating ferroptosis include iron metabolism disorders, lipid peroxidation, and glutathione (GSH) depletion [140]. The regulatory mechanisms of ferroptosis are illustrated in Fig. 8. Ferroptosis plays a significant role in the physiological and pathological regulation of various acute and chronic neurological disorders [141].

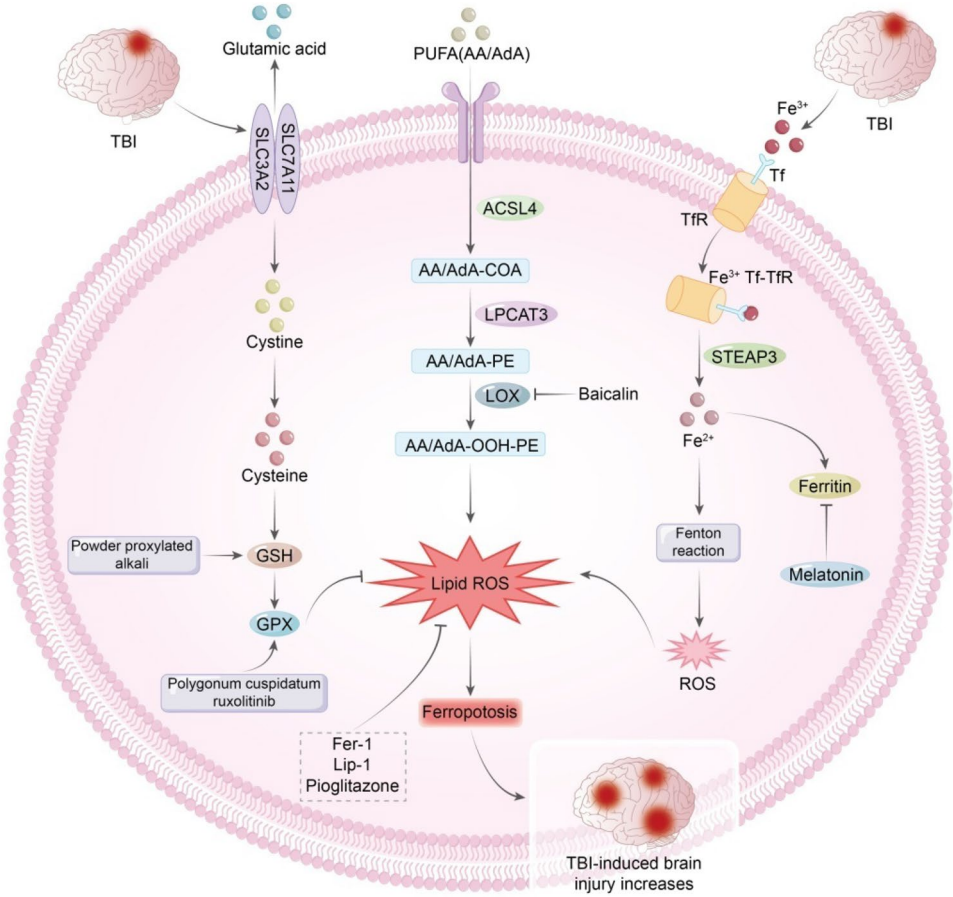



**Fig. 8** Mechanism of traumatic brain injury-induced ferroptosis. *GSDMD* Gasdermin-D, *TBI* traumatic brain injury, *FLC3 A2* solute carrier family 3 member 2, *SLC7 A11* solute carrier family 7a member 11, *PUFA* polyunsaturated fatty acids, *DHA* docosahexaenoic acid 22:6n-3, *AA* arachidonic acid 20:4n-6, *ACSL4* Acyl-CoA synthetase long-chain family member 4, *LPCAT3* lyso-phosphatidylcholine acyltransferase-3, *LOX* lysyl oxidase, *AdA-PE* AdA-containing phosphatidylethanolamines, *STEAP3* six-transmembrane epithelial antigen of prostate 3, *ROS* reactive oxygen species, *Fer-1* Ferrostatin-1, *GSH* glutathione, *Lip-1* liproxstatin-1, *TfR* transferrin receptor, *GPX* glutathione peroxidase family

In recent decades, iron deposition has been observed in experimental models of TBI [140]. Animal studies have further demonstrated that inhibiting ferroptosis can effectively prevent neurodegeneration and neurological dysfunction following TBI (Table 4) [142–149]. Administration of ferrostatin-1 (Fer-1) via lateral ventricular injection reduced iron deposition, mitigated neurodegenerative changes, and decreased injury volume in damaged tissues, resulting in improved long-term outcomes in motor and cognitive functions after TBI [150]. However, as lateral ventricular injection is not a viable clinical approach, future studies should explore alternative administration routes, such as intraperitoneal and tail vein injections of Fer-1, and assess the therapeutic time windows. Recent research has indicated that overexpression of miR- 125b-5p inhibits BRAC 1 Associated C Terminal Helicase 1 (Bach1), promotes activation of the nuclear factor-E2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway, and alleviates neuronal ferroptosis [151]. Moreover, MSC-derived exosomes have been shown to deliver miRNA-194, which inhibits Bach1 and activates the Nrf2/HO-1 signaling pathway, thereby alleviating ferroptosis in neurons [151]. Based on these findings, it is hypothesized that MSC exosomes may also protect neurons from ferroptosis by modulating the Nrf2/HO-1 pathway, offering potential therapeutic benefits in TBI-induced brain injury.

### Regulating neuronal pyroptosis

Following TBI, the pyrin domain (PYD)-containing protein(NLRP) 3 inflammasome and apoptosis-associated speck-like protein (ASC) assemble to activate caspase-1, which subsequently cleaves the precursors IL-1β and IL-18 to form their mature forms. These cytokines are released through the GSDMD-N-mediated membrane pores, triggering the onset of inflammatory

**Table 4** Effects and possible mechanisms of drugs regulating ferroptosis on traumatic brain injury

| Drug | Animal model | Dosage | Neuroprotection | Potential mechanisms | References |
|---|---|---|---|---|---|
| Lipstatin-1 | Mice | 10 mg/kg | Mitigate traumatic brain injury (TBI)-induced lesion volume, attenuate neurodegeneration, and ameliorate cognitive deficits | Iron deposition reduction-mediated ferroptosis inhibition | [142] |
| Iron somatostatin 1 | Mice | 3μg/L | Attenuation of injury lesions and enhancement of long-term motor/cognitive function | Iron deposition reduction-mediated ferroptosis inhibition | [143] |
| Polydatin | Mice | 50 mg/kg | Alleviate acute neurological deficits and enhance subacute motor recovery in TBI mouse models | Inhibiting ferroptosis | [144] |
| Baicalin | Male C57BL/6 mice | 50 mg/kg | Enhance spatial memory acquisition capacity | Attenuated ferroptosis-induced cell death and phospholipid oxidation | [145] |
| Rusoletinib | Mice | 0.44 mg/kg | Attenuate neurodegenerative alterations, reduce cerebral edema, mitigate lesion volume, and restore motor/memory functions | Ferroptosis inhibition confers neuroprotective effects | [146] |
| Pioglitazone | Mice | 5 mg/kg | Reduce brain injury area and neuronal loss | Traumatic brain injury (TBI)-induced neuronal ferroptosis is ameliorated via cyclooxygenase-2 inhibition-mediated PPAR-γ upregulation in mice | [147] |
| Tetrandrine | Mice | 30–60 mg/kg | Enhance neurological function while reducing cerebral edema and brain damage | Autophagy regulation mitigates ferroptosis | [148] |
| Melatonin | Mice | 10 mg/kg | Mitigate lesion volume, suppress neurodegeneration, ameliorate cognitive deficits, and reduce anxiety-like behavior | The circPtpn14/miR-351-5p/5-LOX axis alleviates brain injury by attenuating lipid peroxidation, thereby exerting anti-ferroptotic and anti-endoplasmic reticulum stress effects | [149] |

*PPAR-γ* peroxisome proliferator-activated receptor γ, *MT2* membrane-type-2 matrix metalloproteinase, *LC3II* light chain 3, *BECN1* Beclin 1, *p62* protein sequestosome p62, *circRNAs* circular RNAs, *5-LOX* 5-lipoxygenase, *PE* phosphatidylethanolamine

cell pyroptosis (Fig. 9) [152, 153]. In addition to NLRP3, NLRP1 and caspase-11 expression was upregulated after TBI. Caspase-11, primarily expressed in mice, participates in an atypical pyroptosis pathway and is mainly activated by lipopolysaccharides (LPS) derived from the cell wall of gram-negative bacteria during infection [154]. Besides NLRP3, the expression of NLRP1 and Absent in Melanoma 2 (AIM2) is also elevated in both patients with TBI and animal models. Inhibition of inflammasome activation through drug treatments, neutralizing antibodies, or genetic interventions has been shown to suppress pyroptosis, thereby protecting animal models of TBI (Table 5) [154–167]. Xiong et al. demonstrated that Treg cells target NF-κB activating protein (NKAP) through extracellular vesicle miR-709 to reduce microglial pyroptosis and promote the recovery of motor function after spinal cord injury (SCI) [168]. Another study found that BMSC-derived EXOs protect pericytes by inhibiting cell pyroptosis and enhancing blood–brain barrier integrity, which in turn promotes neuronal survival and axonal regeneration, ultimately improving motor function in rats with SCI [169]. Based on these findings, we hypothesized that BMSC-derived EXOs might provide neuroprotection against TBI-induced brain damage by inhibiting neuronal pyroptosis.

### Regulating neuronal autophagy

Research has demonstrated that autophagy exerts neuroprotective effects in TBI [170, 171]. In a rat model of hydraulic shock brain injury, autophagy was activated through the accumulation of microtubule-associated protein 1 light chain 3 (LC3-II), autophagosomes, and autolysosomes following brain injury, thereby contributing to neuroprotection [170]. Conversely, Luo et al. utilized a free-fall method to establish a TBI mouse model and administered the autophagy inhibitors 3-Methyladenine (3-MA) and Bafomycin A1 prior to injury. They observed an increase in LC3-II and Beclin1 levels, along with a decrease in p62 levels, which correlated with improvements in water maze learning ability and reduced cell apoptosis, thus confirming the protective role of autophagy against brain damage [171]. Recent research has further revealed that extracellular vesicles derived from neural stem cells possess the capacity to regulate autophagy. Therefore, it is hypothesized that neural stem cell-derived extracellular

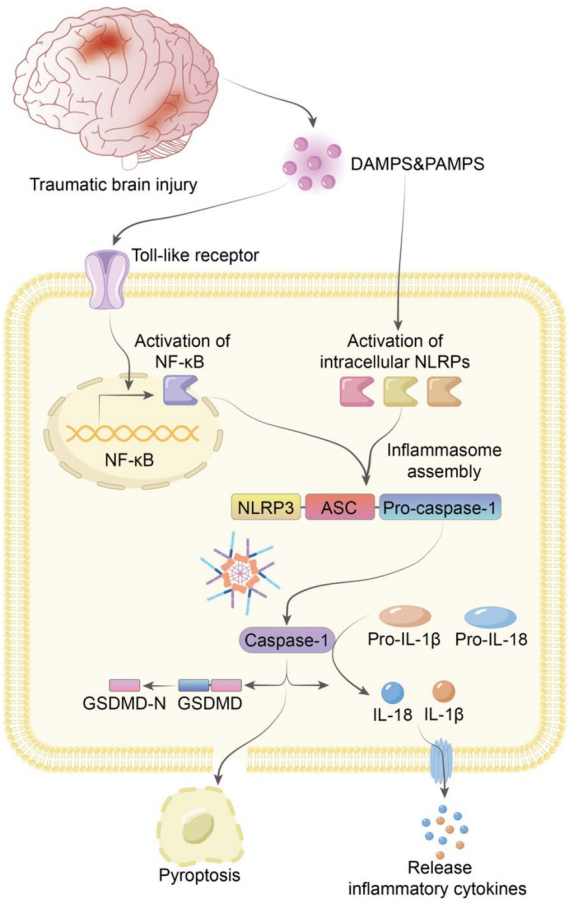



**Fig. 9** Mechanism of TBI-induced neuronal pyroptosis. *DAMPs* dangerous molecular patterns, *PAMPs* pathogen-related molecular patterns, *NF-κB* nuclear factor kappa B, *NLRPs* NOD-like receptor protein family, *ASC* apoptosis-related spot like proteins containing cysteine protease recruitment domains, *Caspase-1* cysteine containing aspartic acid protease 1, *IL* interleukin

vesicles may confer neuroprotection in TBI by modulating neuronal autophagy, as depicted in Fig. 10.

## Application of mesenchymal stem cell exosomes in the diagnosis and treatment of traumatic brain injury

### Diagnosis

Currently, the diagnosis of craniocerebral injury primarily relies on medical history, neurological examination, and imaging techniques [172]. Computed tomography(CT) scans have significant clinical value in TBI because of their accuracy in localizing lesions, sensitivity, and ability to assess prognosis [173]. However, CT has limitations such as its inability to effectively detect diffuse brain injuries, for which magnetic resonance imaging (MRI) is essential [174]. MRI is highly sensitive, non-invasive, and advantageous for evaluating the structural integrity of the blood–brain barrier [174], although it is hindered by higher costs and potential surgical contraindications.

Recent advancements in research methods have led to continuous improvements in the detection of craniocerebral injuries [175]. Studies have suggested that exosomes hold promise for the diagnosis and treatment of TBI [176]. Exosomes offer several advantages, including high sensitivity, strong specificity, extended circulatory half-life, easy release into body fluids within 24 h, noninvasive sampling, abundant content, and dynamic monitoring of disease progression [177].

Furthermore, exosomes have smaller particle sizes, lower immunogenicity, and the ability to cross the blood–brain barrier, making them suitable carriers for targeted drug delivery to the nervous system [178]. Exosomes have been proposed as diagnostic biomarkers for TBI as they mediate neuronal cell death and inhibit axon growth and synaptic repair [81]. Yin et al. demonstrated that miR-21-5p expression was upregulated in microglia and neurons after brain injury [81]. Additionally, when PC12 cells were co-cultured with BV2 cells, microglia took up miR-21-5p-containing exosomes secreted by PC12 cells, promoting microglial polarization [81]. This M1-type polarization leads to the secretion of neuroinflammatory factors, inhibits neuronal proliferation, and results in aggregation of the microtubule-binding protein P-Tau [81]. These findings suggest that exosome-derived miR-21-5p plays a pivotal role in the pathogenesis and progression of severe TBI by modulating inflammatory factors, positioning it as a key biomarker for evaluating TBI. Elevated blood-based central nervous system-derived exosomal protein biomarkers of traumatic brain injury–cognitive impairment (TBI–CogI) remain detectable decades post-injury. These composite biomarkers discriminate between TBI and CogI states and include neurodegenerative proteins and inflammatory cytokines [179]. Elucidating the etiology of TBI–CogI is critical for developing targeted therapeutics, and tau pathology severity correlates with neurodegeneration and cognitive decline during TBI progression, identifying pathological tau as a key diagnostic biomarker and therapeutic target [180].

Mesenchymal stem cell (MSC)-derived exosomes exert therapeutic effects primarily via microRNA (miRNA) transfer.MSC-EVs enter target cells via endocytosis/fusion, delivering miRNAs and bioactive cargoes to modulate M2 polarization, oxidative stress, inflammation, and ferroptosis-related signaling pathways [181–187] (Table 6). Select miRNAs exhibit neuroprotective effects in brain injury models and may also serve as biomarkers for assessing treatment efficacy and prognosis in TBI patients.

**Table 5** Effects of different intervention inflammasome methods on TBI-induced brain injury

| Animal model or patients | Intervention measures | Inflammasome | Final result | References |
|---|---|---|---|---|
| Mice | MSC-derived extracellular vesicles | NLRP3 | Ease neuroinflammation and brain dysfunction | [155] |
| Mice | Parthenolide | NLRP1 and NLRP3 | Relieved neural function deficits, brain edema and neuron apoptosis and improved the memory and learning function of TBI mice | [156] |
| Rats | Anti-ASC antibody | NLRP1 | Reduced caspase-1 activation, X-linked inhibitor of apoptosis protein cleavage, and processing of interleukin-1beta, resulting in a significant decrease in contusion volume | [157] |
| Mice | NLRP1 knockout | NLRP1 | No difference in motor recovery, cell death, or contusion volume | [158] |
| Mice | ACT001 | NLRP3 | Down-regulated microglial neuroinflammatory response | [159] |
| Rats | Dexmedetomidine | NLRP3 | Improve cognitive function, and inhibited the neuroinflammation in brain tissue as well as the expressions of NLRP3 and caspase-1 | [160] |
| Mice | Artesunate | NLRP3 | Reduced the TBI-induced lesion through the modulation of neurotrophic factors (BDNF, GDNF, NT-3) that play a key role in neuronal survival and anti-inflammatory action | [161] |
| TBI patients | Degradation of NETs | NLRP1 | Ameliorate NETs-induced neuronal pyroptotic death after TBI | [154] |
| Rats | Resveratrol | NLRP3 | Attenuate the inflammatory response and relieve TBI by reducing ROS production and inhibiting NLRP3 activation | [162] |
| Mice | Hyperbaric oxygen | NLRP-3 | Alleviates inflammatory response | [163] |
| NLRP3-/- mice | NLRP3 knockout | NLRP3 | Revealed a more conserved brain structure with reduced damage by inhibit NLRP3 activities | [164] |
| NOX-/- mice | NOX2 knockout | NLRP3 | Reduce the area of trauma by inhibiting NLRP3 activation | [165] |
| Mice | Pioglitazone | NLRP3 | $educed cerebral edema and immune response after TBI by downregulating the effects of NLRP3 | [166] |
| Mice | Deletion of WTAP | NLRP3 | Not affect neurological function but promoted functional recovery after TBI by. suppressing NLRP3 induced neuroinflammation | [167] |

*NLRP- 1* NLR family pyrin domain containing 1, *NLRP3* NOD-like receptor family pyrin domain containing 3, *ASC* apoptosis-related spot like proteins containing cysteine protease recruitment domains, *Nox2* NADPH oxidases 2, *TBI* traumatic Brain Injury, *NOX* NADPH oxidases, *ROS* reactive oxygen species, *BDNF* brain-derived neurotrophic factor, *GDNF* Glial cell line-derived neurotrophic factor, *NT-3* neurotrophin-3

### Treatment

MSC transplantation technology has advanced significantly [188]; however, challenges persist, including issues related to aging, functional loss, low transplantation efficiency, reduced survival rates post-transplantation, and ethical concerns surrounding cell-based therapies [189]. Mesenchymal stem cell (MSC)-derived exosomes display functional similarities to parental cells while maintaining more stable membrane structures (Table 7) [190–192], which is attributed to their unique lipid/protein composition, environmental adaptability, and antioxidant capacity (Table 4) [190]. These properties allow extracellular vesicle membranes to retain structural and functional integrity within complex bodily fluids, facilitating intercellular communication and cargo transport. MSC exosomes are small, highly active, widely distributed, and capable of efficiently crossing the blood-spinal fluid barrier, positioning them as promising vectors for drug delivery to the brain [6].

MSC-derived exosomes have been shown to promote nerve repair, exert anti-inflammatory effects, and protect cells [193]. Zou et al. demonstrated that bone marrow-derived MSCs significantly reduced neurovascular remodeling and inflammatory response following TBI [194]. Three-dimensional (3D) cultures produce higher exosome yields than conventional 2D systems [195]. Exosomes derived from 3D cultures demonstrate superior therapeutic efficacy compared to their 2D-derived counterparts, which is attributed to differential cargo transfer [196]. Furthermore, hypoxic MSC-derived exosomes contain environment-responsive molecular cargoes secreted by MSCs, which have therapeutic potential in ischemia–reperfusion

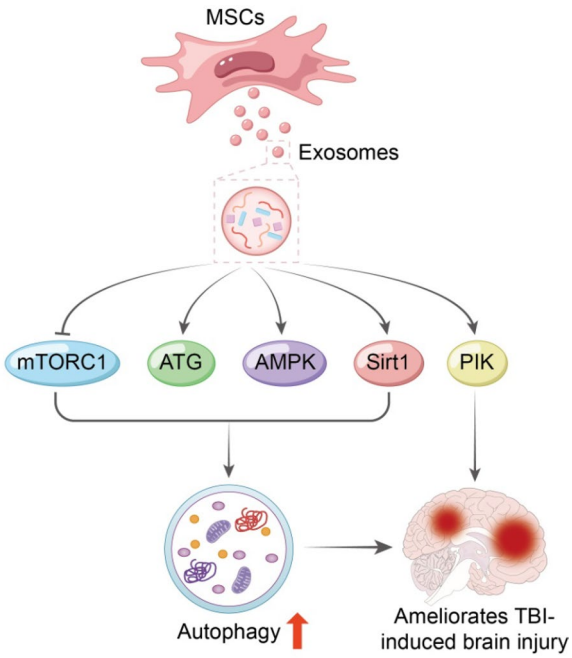



**Fig. 10** Regulatory mechanism of extracellular vesicles from mesenchymal stem cells on TBI-induced neuronal autophagy. *mTORC1* mechanistic target of rapamycin complex 1, *ATG* anti-thymocyte globulins, *AMPK* AMP-activated protein kinase, *SIRT1* sirtuin 1, *PIK* phosphatidylinositol (PI) kinase

injury (IRI) [197]. However, the underlying mechanisms remain unclear [198]. William et al. were among the first to utilize Yorkshire pig models of TBI and hemorrhagic shock and discovered that transplantation of human-derived MSCs facilitated neural function recovery in pigs [199]. However, only a small fraction of MSCs differentiate into neuronal cells, and the repair process largely depends on a range of cytokines, whereas exosomes exert therapeutic effects primarily through paracrine signaling [200]. Studies have confirmed that MSC-derived exosomes provide therapeutic benefits comparable to MSC transplantation for craniocerebral injury repair [201]. In contrast to traditional cell therapies, exosomes offer advantages, such as the absence of cell proliferation, reduced immunogenicity, and ease of storage. Exosomes exhibit higher uptake efficiency by recipient cells compared to synthetic nanocarriers because of their inherent stability and ability to evade phagocytic clearance and immune recognition. As a natural, endogenous transport system, exosomes effectively deliver various therapeutic agents, including easily degradable RNAs, making them an ideal mode of drug delivery [202].

Previous studies have demonstrated that MSCs can promote dendritic cell immune tolerance by modulating macrophageM2-type polarization, reducing the secretion of TNF-α and IL-10, inhibiting polymorphonuclear leukocyte (PMN)infiltration, and inducing dendritic cell tolerance [202]. Yang et al. administered MSC-derived exosomal miR- 124 via tail vein injection in rats, resulting in enhanced M2 polarization of microglia and facilitation of neuronal regeneration in the hippocampus [181]. Additionally, Sun et al. observed that oxidative stress in bone marrow-derived MSCs significantly improved motor and cognitive functions in rats, increased hippocampal neuron density, and promoted angiogenesis and nerve repair [203]. Notably, 100 μg MSCs demonstrated superior efficacy compared to both 50 μg and 200 μg doses, and adipose-derived

**Table 6** Mechanism of action of extracellular vesicle microRNAs secreted by mesenchymal stem cells

| microRNA | Regulate mechanism | Effector cell | Effect | Refs |
|---|---|---|---|---|
| miR-124 | TLR4/NF-κB pathway inhibition and PI3 K/AKT signaling activation | Microglia | Induce M2 macrophage polarization to suppress inflammatory responses | [181] |
| miR-212-5p | PTGS2 expression inhibition | Neuronal cells | Preserve neuronal integrity and enhance neurological function | [182] |
| miR-17-92 | Concurrent PTEN downregulation and PI3 K/AKT/mTOR signaling activation | Neuronal cells | Enhance neurogenesis, oligodendrogenesis, and synaptic plasticity | [183] |
| MiR-126 | PI3 K/AKT/eNOS pathway activation | Endothelial cells | Enhance vascular angiogenesis and preserve blood–brain barrier integrity | [184] |
| MiR-132-3p | PI3 K/AKT/eNOS pathway activation | Endothelial cells | Enhance vascular angiogenesis and preserve blood–brain barrier integrity | [185] |
| MiR-532-5p | Ang-1/Tie-2 signaling pathway inhibition | Pericyte | Enhance vascular angiogenesis and preserve blood–brain barrier integrity | [186] |
| MiR-21 | Simultaneous STAT3 and NF-κB expression inhibition | Neuronal cells | Suppress inflammatory responses and facilitate neural remodeling | [187] |

*PTGS2* prostaglandin endoperoxide synthase 2, *PTEN* phosphatase and tensin homolog, *mTOR* rapamycin, *eNOS* endothelial NO synthase, *Ang- 1* angiotensin 1, *TLR4* toll-like receptor 4, *NF-kappaB* nuclear factor-kappaB, *PI3 K* phosphatidylinositol 3-kinase, *Akt* protein kinase B, *Tie- 2* epidermal growth factor homology domain 2, *STAT3* signal transducer and activator of transcription 3

**Table 7** Comparison between extracellular vesicles derived from MSCs and stem cell membranes

| Item | Exosome membrane | MSC membrane |
|---|---|---|
| Lipid composition | Enriched with cholesterol and sphingomyelin, these components contribute to membrane rigidity and stability [190] | MSC membranes exhibit structurally complex lipid compositions enriched with unsaturated fatty acids, rendering them vulnerable to oxidative stress [191] |
| Protein composition | Abundant in transmembrane and membrane-associated proteins (e.g., tetraspanins CD9/CD63/CD81), exosome membranes form stable protein networks [190] | MSC membranes contain a diverse repertoire of proteins, though not all contribute to stable network formation [191] |
| Environmental adaptability | Exosome membranes must maintain stability within complex bodily fluids post-release, thereby ensuring compact and robust structures [190] | Unlike exosome membranes, MSC membranes must adapt to intracellular environmental changes without requiring long-term extracellular stability [192] |
| Antioxidant capacity | Exosome membranes are enriched with antioxidant enzymes (e.g., superoxide dismutase), which mitigate oxidative stress [192] | While MSC membranes contain antioxidant enzymes, their concentrations and activities are typically lower than those of exosome membranes [192] |

*MSC* mesenchymal stem cell

MSC exosomes exhibited comparable efficacy to bone marrow-derived MSC exosomes, although the former are more readily accessible [204]. Further studies have indicated that MSCs can drive M2-type macrophage polarization and alleviate central inflammation through the p38MAPK signaling pathway [205]. Recent research also suggests that MSC exosomes significantly mitigate brain edema, reduce lesion size, decrease intracranial pressure, and attenuate central inflammatory responses in pig brain tissue subjected to hemorrhagic shock [206]. Additionally, early MSC exosome treatment has shown anti-inflammatory and anti-apoptotic properties, significantly accelerating recovery in inpatients [207]. In a study on oxidative therapy using bone marrow MSC exosomes in macaques with cortical injury, MSC exosomes alleviated motor deficits [208]. These exosomes are capable of delivering a wide range of functional molecules, including proteins, lipids, DNA, RNA, and metabolites, to target cells, thereby modulating multiple genes and biological processes and playing a pivotal role in neuroprotection [209]. Studies have revealed that miR-21, miR- 30, miR- 124, miR- 133, and miR- 138 are key players in the pathophysiology of craniocerebral trauma [210]. Emerging evidence indicates that extracellular vesicle (EV)-derived circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) regulate neuronal growth/repair, and modulate nervous system development and signaling within the mouse brain extracellular milieu [211, 212]. Therefore, MSC-derived exosomes represent a promising approach for repairing TBI-induced neuronal injury.

Engineered exosomes enriched with specific microRNAs (miRNAs) are being actively investigated for stroke and traumatic brain injury (TBI) therapies because of their potential to enhance therapeutic efficacy through miRNA-mediated mechanisms [213]. Engineered C3-EPm-|TKNPs| extracellular vesicles (EVs) efficiently delivered pioglitazone (PGZ) to mitigate mitochondrial damage via mitoNEET, thereby reversing behavioral deficits in TBI mouse models [214]. These results establish C3-EPm-|TKNPs|-derived nanodrugs as promising translational candidates for neuroinflammatory intracranial disorders [214]. The incorporation of bone marrow mesenchymal stem cell (BM-MSC)-derived exosomes into hyaluronan-collagen hydrogels induces angiogenesis and neurogenesis through sequential processes: (1) endogenous neural stem cell (NSC) recruitment, (2) neuronal differentiation/vascularization, and (3) synergistic promotion of axonal regeneration, remyelination, synaptogenesis, and brain structural remodeling, ultimately restoring neurological function in TBI [215]. Collectively, these investigations demonstrated that engineered exosomes enriched with specific miRNAs/lncRNAs augment MSC-exosome therapeutic potency in TBI, improving both experimental and clinical outcomes.

Intercellular communication mediated by exosomes represents a frontier research area. Exosomal cargos exhibit cell-type specificity and typically contain nucleic acids, proteins, and lipids [216]. Exosomes traffic through the circulatory system to deliver cargo to local or distant cells, thereby modulating recipient cell functions, and represent promising drug delivery platforms because of their capacity to cross biological barriers (e.g., the blood–brain barrier), low toxicity, and immunogenicity [217]. Recent studies have increasingly recognized exosomes as regulators of blood–brain barrier (BBB) homeostasis, influencing processes such as tumor progression [218], angiogenesis [219], and immune surveillance [220]. Thus, exosomal intercellular communication represents a central mechanism underlying exosome-based therapeutic strategies. For example, traumatic brain injury (TBI)-derived exosomes exhibit osteoinductive properties that accelerate bone repair.

## Prospects and challenges of exosomes from mesenchymal stem cells

Neonatal craniocerebral injuries present a significant challenge in the clinical practice. While hypothermic treatment has demonstrated some promise, its clinical application remains limited, particularly in children with moderate to severe TBI, owing to a narrow therapeutic window and the risk that some patients may not benefit. Consequently, there is an urgent need to explore novel therapeutic options. MSC transplantation has shown potential in promoting nerve injury repair; however, challenges such as tumorigenesis, embolization, and low graft survival remain. Recent studies indicate that exosomes, as cell-free therapeutic alternatives, may offer more effective therapeutic benefits than MSCs. For instance, intravenous administration of EVs has been shown to mitigate nerve and tissue damage in fetal sheep models, bypassing the risks associated with live-cell therapy [221].

Exosomes offer several advantages over MSCs: (1) They contain fewer membrane proteins, thereby reducing immunogenicity and enhancing their recognition and phagocytosis by immune cells. (2) Exosomes can be combined with MSCs as a cell-free therapeutic modality to mitigate the risk of tumor formation and thrombosis. (3) Exosomes can be stored at ultra-low temperatures (e.g., − 20 °C) for up to six months without losing biological activity [222]. (4) The bilayer phospholipid membrane in exosomes protects their cargo from rapid degradation by inflammatory factors and RNA, facilitating the delivery of siRNA and drugs

to target organs. (5) Exosomes are not metabolized and are unaffected by the internal environment, ensuring a sustained therapeutic function. (6) These lipid-bound nanoscale vesicles can readily penetrate the blood vessel wall and cross the blood–brain barrier [223, 224].

Despite these advantages, exosomes, as novel agents for nerve repair, still face several unresolved challenges. The mechanisms underlying their therapeutic effects remain unclear, necessitating further investigation of their pharmacodynamic material basis and biological functions. Additionally, certain substances within exosomes, such as TNF-α and IL-6, exhibit potent toxicity, and the potential harmful effects of these components on the body must be thoroughly understood to minimize their side effects [225]. Emerging studies also suggest that exosomes can elicit adverse reactions, underscoring the need to refine current isolation and purification methods to ensure their safety and efficacy. Moreover, future research should focus on exploring the impact of different cell sources and delivery modes for exosomes, as well as the optimal dosing frequency, considering factors such as in vivo half-life and therapeutic outcomes [225]. Recent findings have indicated that MSC-derived exosomes, particularly under hypoxic conditions, may enhance their therapeutic efficacy, targeting ability, and safety.

Accumulating evidence indicates that stem cells from diverse species, sources, passages, and culture conditions display phenotypic heterogeneity, differential adipogenic/osteogenic differentiation capacities, and variable regenerative potentials [226, 227]. Furthermore, existing safety/efficacy evidence primarily stems from preclinical studies, which inherently differ from human organ systems. While initial clinical trials have been performed, critical considerations include standardized patient selection criteria, cell sourcing protocols, dosing regimens, and administration methods. Additionally, large-scale, multicenter, randomized controlled trials with extended follow-ups are required to establish translational feasibility.

Recent clinical trials have expanded research on exosomes across diverse therapeutic areas [192, 228]. Landscape analysis of ongoing clinical trials (GlobalData, 2025) identified oncology (54%), central nervous system disorders (13%), infectious diseases (13%), and immunology (8%) as primary therapeutic areas for exosome-based therapeutics. Among 420 exosome-based therapeutics in clinical development, over 65% are in early stage (preclinical to Phase I) development and have not been approved by the Food and Drug Administration (FDA). Exosome-based therapies for neurological and cardiovascular diseases have emerged as rapidly evolving research frontiers. Although no curative therapies exist for Alzheimer's disease, traumatic brain injury (TBI), or Parkinson's disease, exosomal bioactive molecules hold promise for promoting neuronal growth/repair as novel therapeutic modalities.

The future development of extracellular vesicle (EV) separation technology will necessarily advance toward increased efficiency, precision, and automation, confronting four key challenges [229–231]: (1) Current EV isolation methods struggle to achieve both high purity and yield simultaneously; for instance, immune affinity-based isolation yields high-purity EVs but with low recovery and potential vesicle damage, whereas polymer precipitation methods offer high yields but introduce contaminating impurities, and future innovations should focus on hybrid separation strategies that integrate complementary techniques. (2) Subpopulation-Specific Isolation EVs display marked heterogeneity in surface markers, size, and cargo, resulting in distinct biological functions; however, efficient methods for isolating functional EV subpopulations remain underdeveloped, constraining translational applications. Future approaches should prioritize biomarker-driven isolation techniques, including antibody-conjugated microspheres or DNA aptamers, to enable targeted EV subpopulation capture. (3) The standardization and Automation Diverse EV isolation methods and complex workflows hinder reproducibility. Standardized separation protocols and robust quality control metrics are essential, as automated separation platforms enhance efficiency, minimize human error, and accelerate the translation of EV research to clinical settings. (4) Emerging Separation TechnologiesBeyond traditional approaches, emerging separation technologies demonstrate significant potential; for instance, label-free and non-destructive EV isolation can be achieved via acoustophoresis, dielectrophoresis, and magnetophoresis. Nanomaterial-based separation technologies enhance isolation efficiency and specificity, and future efforts should focus on elucidating mechanisms and optimizing applications of these technologies to advance more efficient and user-friendly EV isolation protocols.

Current extracellular vesicle (EV) identification methods lack standardized detection criteria [232, 233]. Accumulating evidence indicates that neither surface morphology characterization nor transmembrane protein detection alone can definitively identify EVs, particularly for concentration and purity assessments [233]. However, the current consensus indicates that the combined characterization of particle size distribution, morphological features, and marker proteins represents the gold standard for EV identification, enabling both qualitative and basic quantification.

The current clinical management of traumatic brain injury (TBI) comprises symptomatic support, pharmacological intervention, and surgical resection [234–236]. The complexity of the nervous system contributes to an incomplete understanding of post-TBI pathological mechanisms [234], consequently hindering the establishment of universally applicable treatment protocols, despite extensive clinical evidence [236]. Thus, the development of novel, safe, and efficacious therapeutic approaches to TBI is imperative.

In summary, while the composition and biological functions of exosomes remain incompletely understood, further investigation of their molecular mechanisms, targets, signaling pathways, and potential adverse reactions is essential. Advanced experimental methods should be employed to elucidate the full therapeutic potential of exosomes in TBI. In the future, efforts should also focus on optimizing exosome separation and purification techniques, modifying exosomes for tissue specificity, and conducting large-scale multicenter clinical trials to establish their clinical viability in TBI treatment.

**Abbreviations**

| | |
|---|---|
| MSCs | Mesenchymal stem cells |
| TBI | Traumatic brain injury |
| ROS | Reactive oxygen species |
| EVs | Extracellular vesicles |
| TNFa | Necrosis Factor alpha |
| IL- 6 | Interleukin 6 |
| NLRP | Pyrin domain (PYD)-containing protein |
| EVs | Extracellular vesicles |
| DAMPs | Danger-associated molecular patterns |
| PAMPs | Pathogen-related molecular patterns |
| NF-κB | Nuclear factor kappa B |
| NLRPs | NOD-like receptor protein family |
| ASC | Apoptosis-related spot like proteins containing cysteine protease recruitment domains |
| Caspase- 1 | Cysteine containing aspartic acid protease 1 |
| IL | Interleukin |
| mTORC1 | Mechanistic target of rapamycin complex 1 |
| ATG | Anti-thymocyte globulins |
| AMPK | AMP-activated protein kinase |
| SIRT1 | Sirtuin 1 |
| PIK | Phosphatidylinositol (PI) kinase |
| miR | MicroRNA |
| NF-κB | Nuclear factor-kappaB |
| p38 MAPK | Mitogen-activated protein kinase |
| LC3-II | Microtubule-associated protein 1 light chain 3 |
| Akt | Protein kinase B |
| VEGF | Vascular endothelial growth factor |
| FGF | Fibroblast growth factor |

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**Author contributions**
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## Declarations

**Ethics approval and consent to participate**
Not applicable.

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**Competing interests**
The authors declare that they have no competing interests.

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