


# Novel Therapeutic Mechanisms and Strategies for Intracerebral Hemorrhage: Focusing on Exosomes

Shandong Jiang<sup>1,2</sup>, Libin Hu<sup>1,2</sup>, Hang Zhou<sup>1,2</sup>, Jianan Wu<sup>1,2</sup>, Jiayin Zhou<sup>1,2</sup>, Xian Yu<sup>1,2</sup>, Gao Chen<sup>1,2</sup> 

<sup>1</sup>Department of Neurosurgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310016, People's Republic of China; <sup>2</sup>Key Laboratory of Precise Treatment and Clinical Translational Research of Neurological Diseases, Hangzhou, 310016, People's Republic of China

Correspondence: Gao Chen, Department of Neurosurgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Clinical Research Center for Neurological Diseases of Zhejiang Province, Hangzhou, 310009, People's Republic of China, Email [d-chengao@zju.edu.cn](mailto:d-chengao@zju.edu.cn)

**Abstract:** Intracerebral hemorrhage (ICH) is a primary, non-traumatic cerebral event associated with substantial mortality and disability. Despite advancements in understanding its etiology and refining diagnostic techniques, a validated treatment to significantly improve ICH prognosis remains elusive. Exosomes, a subtype of extracellular vesicles, encapsulate bioactive components, predominantly microRNAs (miRNAs), facilitating and regulating intercellular communication. Currently, exosomes have garnered considerable interests in clinical transformation for their nanostructure, minimal immunogenicity, low toxicity, inherent stability, and the ability to traverse the blood-brain barrier. A wealth of studies has demonstrated that exosomes can improve the prognosis of ICH through anti-apoptosis, neurogenesis, angiogenesis, anti-inflammation, immunomodulation, and autophagy, primarily via the transportation or overexpression of selected miRNAs. More importantly, exosomes can be easily customized with specific miRNAs or bioactive compounds to establish delivery systems, broadening their potential applications. This review focuses on the therapeutic potential of exosomes in ICH, reviewing the mechanisms of molecular biology mediated by certain miRNAs, discussing the benefits, challenges, and future prospects in ICH treatment. We hope comprehensive understanding of exosomes based on miRNAs will provide new insights into the treatment of ICH and guide the translation of exosome's research from laboratory to clinical practice.

**Keywords:** intracerebral hemorrhage, exosomes, extracellular vesicles, microRNAs, stem cells

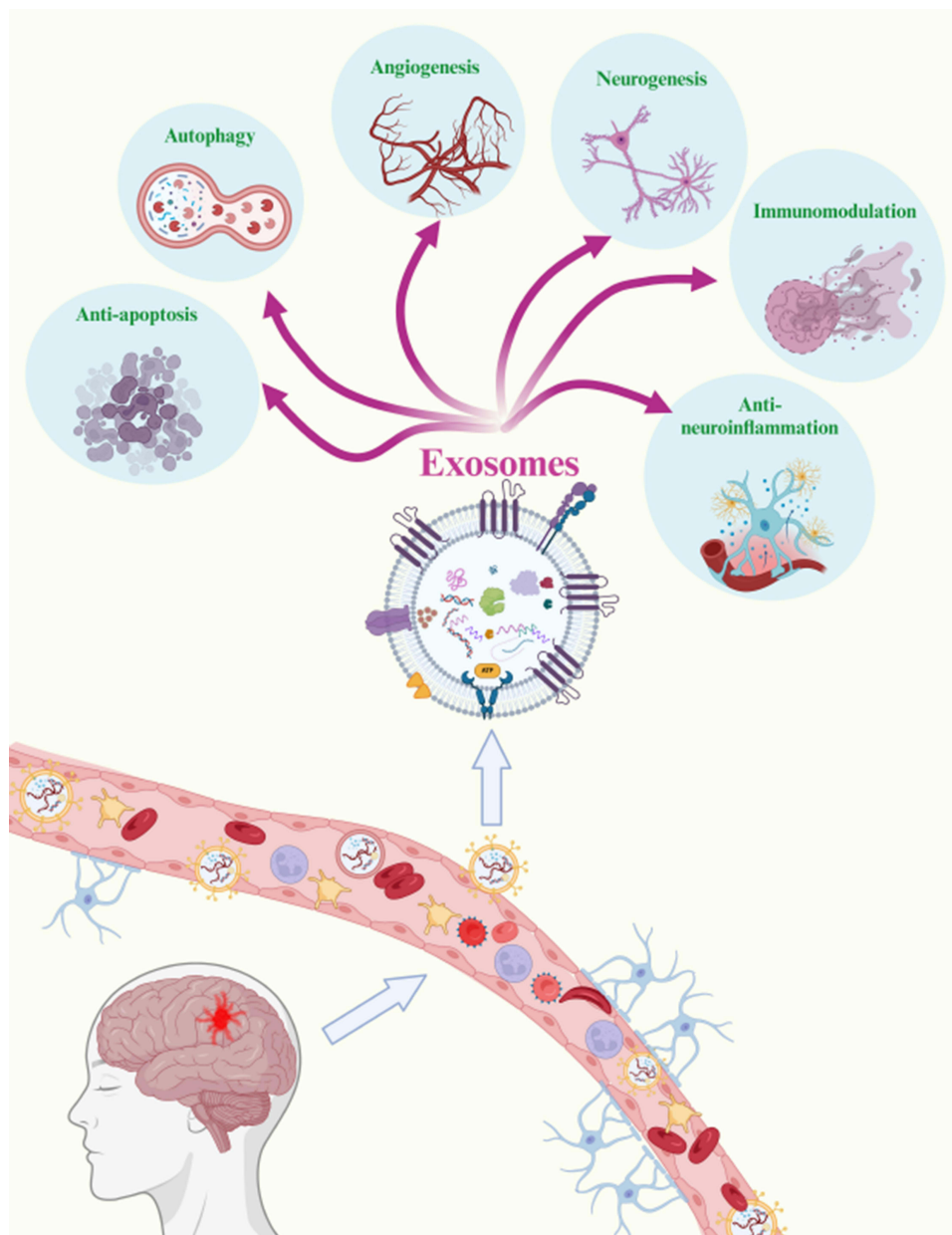
## Introduction

Spontaneous intracerebral hemorrhage (ICH) represents a catastrophic form of stroke, characterized by blood extravasation within the brain parenchyma. It accounts for 15% of all stroke-related cases.<sup>1-3</sup> Despite considerable advancements in the management of ICH and its associated complications, an effective clinical intervention that can significantly ameliorate ICH prognosis remains wanting. In the ICH pathogenesis landscape, hematoma size is a recognized determinant of ICH severity and a robust prognostic indicator, underscoring the potential benefits of early hematoma clearance for prognosis improvement.<sup>4</sup> However, recent clinical trials suggest that neither early open nor endoscopic surgical interventions for hematoma evacuation confer significant advantages in functional recovery compared to medical management of ICH.<sup>5</sup> This highlights the limited success of mechanical hematoma removal in enhancing ICH outcomes. Consequently, researchers are fervently exploring viable strategies to expedite hematoma resolution and hasten neurological functional recovery via endogenous pathways.

A plethora of potential therapeutic drugs or agents that could promote hematoma degradation have been identified. However, the blood-brain barrier (BBB) restricts their access to the target parenchyma, thus limiting their utility in neurological disease management. This challenge may be circumvented by exosomes, which not only reply on their own biological effects but also given their inherent BBB crossing potential and broad surface-engineering capability, as confirmed by numerous studies and techniques.<sup>6-8</sup> The interaction between exosome surface ligands and brain endothelial cell receptors is thought to be the main mechanism.<sup>9</sup>

Exosomes are lipid bilayer-delimited particles naturally secreted from cells, either through vesicle budding into endosomes or direct vesicle budding from the plasma membrane (PM).<sup>10</sup> They are secreted by almost all cell types, including stem cells,

## Graphical Abstract



macrophages, microglia, astrocytes, and so forth,<sup>11</sup> and are present in various body fluids like blood, cerebral spinal fluid (CSF), semen, saliva, plasma, serum, and bronchial fluid.<sup>12</sup> Based on their natural targeting capabilities different types of natural exosomes from different donor cells can adhere to various targeting cells. Besides, exosomes can be amended through their parental cells to express a targeting moiety on their surface, or supplemented with desired biological activity.<sup>13</sup> Owing to their ability to cross biological barriers, innate stability, and high delivery efficiency,<sup>14</sup> exosomes facilitate crucial intercellular communications, including proliferation, differentiation, intercellular signaling, and immune regulation.<sup>15</sup>

Exosomes contain a myriad of biological molecules, including DNA, RNA, microRNAs, circular RNAs (circRNAs), amino acids, metabolites, and cytosolic proteins, which play pivotal roles in intercellular communication.<sup>16</sup> Circular RNAs are a type of noncoding RNA that are particularly abundant in brain tissues, which are produced by the back

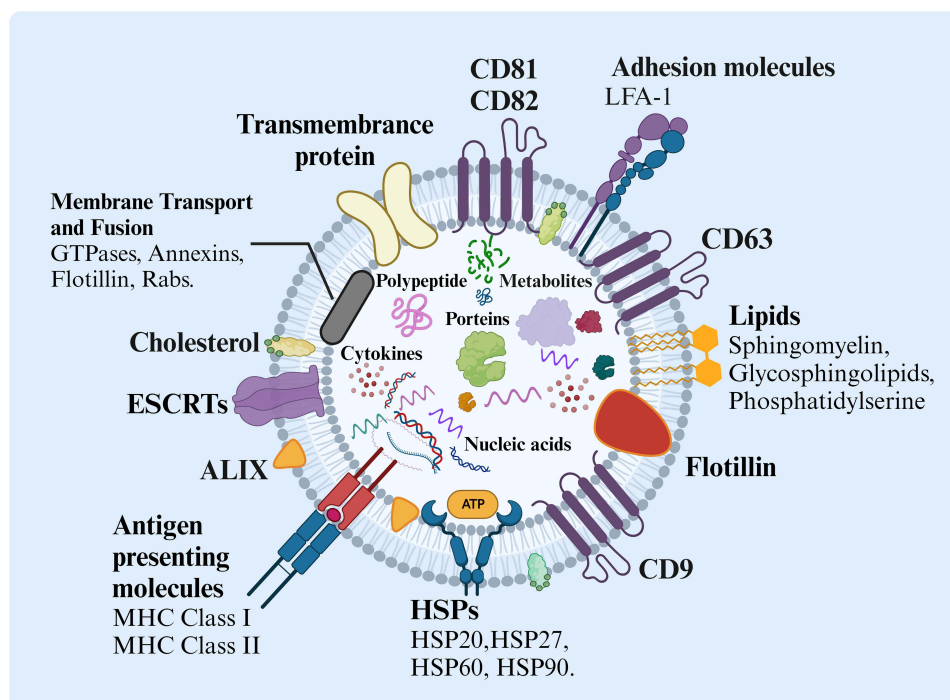
splicing of long RNA transcripts (including mRNAs and long noncoding RNAs).<sup>17</sup> It has demonstrated that exosomal circRNAs are associated with intracranial vascular disease. Jiang X<sup>18</sup> first revealed that five plasma brain-derived exosomal circRNAs serve as ideal biomarkers for the diagnosis of acute ischemic stroke and may also be potential targets for therapeutic interventions. Besides, circRNAs have critical influence on critical molecular and cellular processes underpinning pathogenesis of intracranial aneurysms, make circRNAs an enticing avenue for innovative therapeutic approaches, offering as potential diagnostic biomarkers.<sup>19</sup> However, among the various components of exosomes, microRNAs (miRNAs) are pivotal to their biological function. They play a significant role in cell proliferation, inflammation, apoptosis, and other biological processes.<sup>20–22</sup> As single-stranded non-coding RNAs of 20 to 24 nucleotides, miRNAs are not translated into proteins themselves but regulate protein translation by targeting mRNA, thereby controlling gene expression at the epigenetic level.<sup>20</sup> One or both strands of mature miRNA bind to argonaute 2 (Ago2), forming part of the RNA-induced silencing complex (RISC), which targets mRNA for cleavage or translational repression.<sup>21,22</sup> While new miRNAs frequently emerge, they are seldom lost.<sup>23</sup> However, miRNAs are inherently unstable and prone to degradation, especially in the extracellular environment. This leads to their uneven distribution in various extracellular fluids, impacting the stability and efficiency of signal transmission.<sup>24</sup> Without a carrier, it is challenging for miRNAs to be secreted into extracellular fluids or to be specifically recognized and absorbed by recipient cells.<sup>25</sup> However, when transported in the form of exosomes, miRNAs can be absorbed by proximate or distant cells, influencing their gene expression and biological function. This is considered the third cellular communication pathway, following cell contact-dependent signaling and soluble molecule-mediated signaling.<sup>26</sup> The bilayer lipid membrane structure of exosomes protects the encapsulated miRNA from degradation by ribonuclease, thereby maintaining miRNA stability. Furthermore, miRNAs can be efficiently packaged into exosomes, facilitating their precise delivery to recipient cells and enabling their participation in intercellular communication.<sup>27</sup> As research progresses, evidence has emerged showing that exosomes promote recovery after ICH by suppressing cell apoptosis,<sup>28</sup> promoting angiogenesis,<sup>29</sup> reducing inflammation,<sup>30</sup> modulating cell phenotype,<sup>31</sup> and autophagy.<sup>32</sup> These effects are mediated by the various miRNAs contained within exosomes. Thus, due to their nanoscale size, low expression of membrane-bound proteins, and remarkable therapeutic effects, exosomes are emerging as an excellent candidate for ICH treatment.

Despite the promise of exosomes in the treatment of central nervous system diseases, research has primarily focused on ischemic stroke and Alzheimer's disease, with ICH receiving less attention. This review, therefore, concentrates on the therapeutic roles and mechanisms of exosomes in ICH, summarizing the therapeutic mechanisms of miRNAs and other exosomal contents in improving ICH prognosis and complications. With the advancement of material technology, exosome-based targeted drug transport systems have also been widely studied, which broaden the clinical application of exosomes in the treatment of ICH. We also discussed the advantages, challenges, and prospects of exosomes in the treatment of ICH. This review aims to support and guide the translation of exosome research from laboratory to clinical practice.

## Composition and Biogenesis of Exosomes

Exosomes, membrane-bound carriers with a spheroid shape of diameters ranging from 40 to 160 nm, reflect the nature and physiological state of their donor cell (Figure 1).<sup>33</sup> During membrane fusion, exosomes incorporate various surface proteins and markers from parental cell. The main membrane-bound proteins included in exosomes belong to the tetraspanin family (CD9, CD63, CD81, and CD82),<sup>34</sup> endosomal sorting complexes required for transport (ESCRTs) proteins, heat shock proteins (HSPs), actin, and adhesion molecules like integrin lymphocyte function-associated antigen 1 (LFA-1).<sup>35,36</sup> In addition, proteins such as MHC Class I and II are specific to the donor cell type.<sup>37</sup> Exosomes also possess several endosome membrane proteins like flotillin and ALIX, also known as PDC61-interacting proteins.

Exosomes originate either through the invagination of the plasma membrane (PM) or direct budding from the PM (Figure 2).<sup>38</sup> Specifically, exosomes are generated through a process involving the invagination of the plasma membrane via endocytosis, which forms early endosomes. This leads to the creation of intracellular multivesicular bodies (MVBs) or late endosomes containing intraluminal vesicles (ILVs), a process mediated by Rab27a.<sup>39</sup> ILVs sequester specific proteins, lipids, and cytosolic components, a process first discovered through the study of vesicular secretion of the transferrin receptor (TFR) by mature reticulocytes.<sup>40</sup> Following the transportation of MVBs to the PM via the cytoskeletal and microtubule network, and subsequent exocytosis post fusion to the PM, ILVs are ultimately secreted into the extracellular environment as



**Figure 1** The structure and biological composition of exosomes. Exosomes are composed of various proteins: tetraspanins, antigen presenting molecules, adhesion molecules, heat shock proteins (HSPs), ESCRT components, membrane transport, and fusion molecules; proteins in endosome membranes such as flotillin and ALIX. Components in the exosome lumen include proteins, polypeptides, nucleic acids (DNA, RNA, mRNA, noncoding RNA), cytokines, and metabolites. Exosomes also constitute multiple lipids such as cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine. Nucleic acids among DNA, mRNAs, non-coding RNAs, etc. The figure was created with <https://app.biorender.com/>.

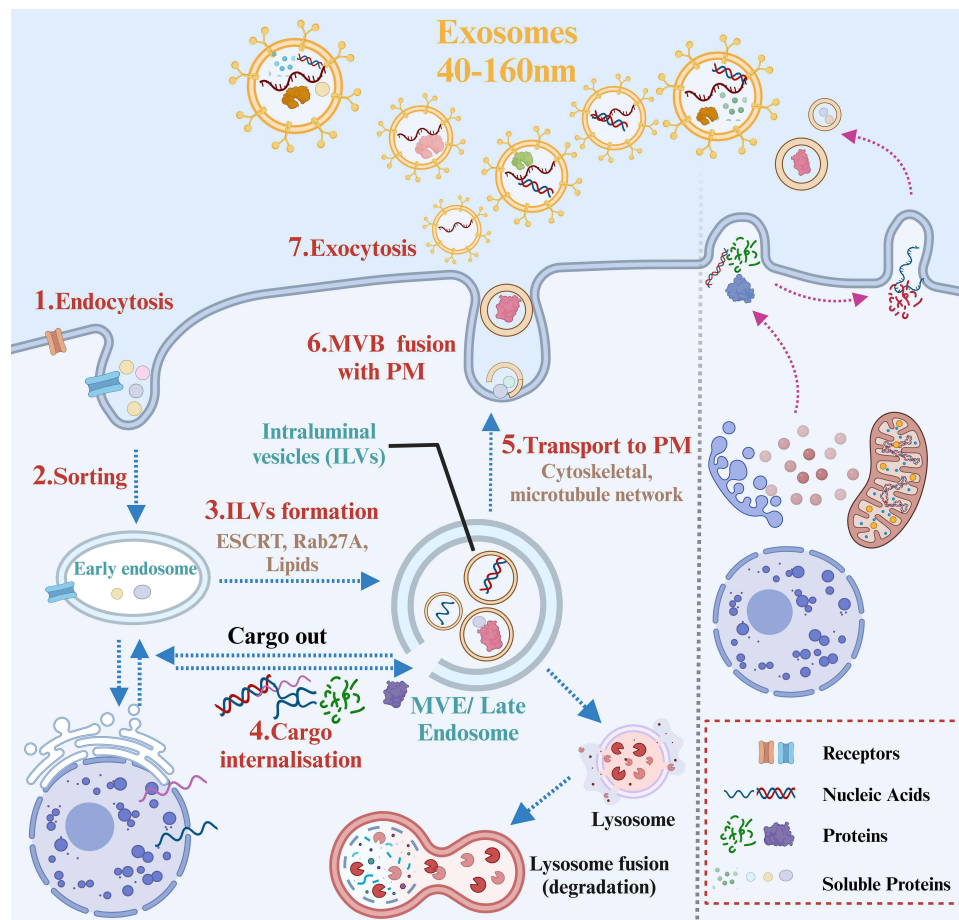
**Abbreviations:** HSPs, Heat shock proteins; MHC, Major histocompatibility complex; I=LFA-1, Lymphocyte function-associated antigen; ESCRTs, Endosomal sorting complexes required for transports.

exosomes.<sup>34</sup> In addition to fusing with the PM, MVBs can also merge with lysosomes and autophagosomes for degradation<sup>35</sup>. Furthermore, MVBs intersect with other intracellular vesicles and organelles, contributing to the diversity in the constituents of exosomes.<sup>34</sup> Notably, exosomes can also be generated by direct vesicle budding from the plasma membrane through exocytosis. This complex process demonstrates the intricate nature of exosome formation and release, highlighting their potential as precise delivery systems for intercellular communication.

Through complex biogenesis process, exosomes contain a diverse array of biological molecules, including proteins, nucleic acids (DNA, RNA, noncoding RNA), cytokines, and metabolites, reflecting their varied cellular origins.<sup>16</sup> Among these, miRNAs are one of the most abundant RNA species in exosomes. They play significant roles in multiple biological processes, including exocytosis, neurogenesis, and angiogenesis, and participate in exosome-mediated cellular communications.<sup>41</sup> All cells, both prokaryotes and eukaryotes, release extracellular vesicles (EVs), particularly exosomes, as part of intercellular communication and disease development.<sup>27</sup> Exosomes can transfer a range of these biological macromolecules into target cells, inducing specific signaling cascades that favor cell bioactivity.<sup>42</sup> These biological macromolecules are encased within a lipid bilayer, often enriched in cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine.<sup>43</sup> The lipid content not only protects the macromolecules from degradation but also enhances bioavailability and minimizes adverse reactions. Furthermore, the lipid composition influences cargo sorting, exosome secretion, structure, and signaling, making exosomes an efficient and precise delivery system for intercellular communication.<sup>43</sup> As research progresses, the potential of exosomes in the treatment of conditions such as intracerebral hemorrhage is becoming increasingly apparent.

## Pathophysiologic Connection Between ICH and Exosomes

Understanding the pathophysiology of ICH is vital for devising effective treatment and management strategies. The relationship between the mechanism of ICH and exosomes is depicted in Figure 3.<sup>44</sup> ICH-related pathological brain



**Figure 2** Biogenesis of exosomes. In the process of biogenesis, Within the endosomal system, soluble proteins were internalized by endocytosis<sup>1</sup> to sort into early endosomes,<sup>2</sup> which then mature into late endosomes or MVBs.<sup>3</sup> Late endosomes/MVBs are specialized endosomal compartments rich in ILVs, which sequester proteins, lipids, cytosolic compartments, and potential exosome cargoes.<sup>4</sup> MVBs containing exosome cargoes get transported to the PM via cytoskeletal and microtubule network,<sup>5</sup> fuse with the cell surface<sup>5</sup> and the ILVs then get secreted as exosomes.<sup>6</sup> The figure was created with <https://app.biorender.com/>.

**Abbreviations:** ILVs, intraluminal vesicles; MVBs, Multivesicular Bodies; PM, Plasma membrane.

injury involves both primary and secondary injuries. The primary brain injury occurs when blood vessels rupture to form a hematoma, resulting in direct mechanical damage to the brain tissue due to compression. Post-ICH, the surrounding brain tissue will be faced with ischemic and hypoxic, leading to a shift in cellular metabolism from aerobic phosphorylation to anaerobic glycolysis.<sup>45</sup> Exosomes can transmit miR-126,<sup>46</sup> miR-125a, miRNA-181b-5p/TRPM7 axis, and others to promote angiogenesis, which is beneficial to improve prognosis by compensating for brain ischemia. Continuous bleeding or hematoma expansion exacerbates mechanical compression injury and ischemic changes in the peripheral brain tissue, accelerating neurological deficits due to increased neuron and glial cell death. Exosomes can transport miR-124,<sup>47</sup> miR-17-92,<sup>48</sup> and other miRNAs to promote functional recovery via facilitating neural plasticity, myelination, and neurite remodeling. When cell mitochondria or cytomembranes are damaged, excessive release of excitatory neurotransmitters and dysfunction of sodium, potassium, and calcium ions occur, leading to nerve cell death and apoptosis, creating a vicious cycle of brain damage. Reducing apoptosis levels is beneficial for reducing neuron death and functional deficits. Exosomes, loaded with miR-134, could reduce oligodendrocyte apoptosis by upregulating caspase-8, which suppresses the extrinsic apoptosis signaling cascade.<sup>49</sup>

Secondary brain injury is caused by the physiological response to the hematoma and the toxic components originating from the hematoma.<sup>50</sup> After ICH, the bleeding site and surrounding brain tissue induce inflammation primarily through peripheral leukocyte diapedesis and activation of astrocytes and microglia, excessively releasing proinflammatory factors, including Interleukin (IL), tumor necrosis factor (TNF), nuclear factor (NF), matrix metalloproteinases (MMPs), nitric oxide



factor–thrombomodulin, which is mediated by C3 and C5 of complement system.<sup>59,60</sup> Exosomes derived from thrombin-activated platelet, containing miRNA-223, may also play an important role in regulating neuroinflammation.<sup>61</sup>

The main approach to hematoma absorption is dependent on hemoglobin breakdown; however, degradation products, including red cell lysate, iron ions, and biliverdin, can lead to secondary damage. High concentrations of iron ions can increase ROS levels and direct brain injury mediated by oxidative stress.<sup>62</sup> Free radical generation could contribute to the iron imbalance-induced brain injury,<sup>63</sup> which consists of a vicious circle. Exosomes can break this vicious circle by reducing the levels of TNF- $\alpha$  and ROS.<sup>64</sup> The process of autophagy after ICH is a complex pathological process, which initiates an increase in ROS, Ca<sup>2+</sup> ions, and excitatory neurotransmitters. Accumulation of red blood cell breakdown products and leukocyte infiltration also facilitate the process.<sup>36</sup> Modest autophagy is beneficial to protect cells against stressful circumstances and facilitate cell survival. Exosomes can reduce the level of apoptosis via transporting miR-21 or miR-25-3p into target cells.<sup>65</sup> Furthermore, the disruption of the BBB caused by physical compression of the hematoma or hemoglobin breakdown products leads to increased BBB permeability in the hemorrhagic area, resulting in edema and peripheral leukocyte infiltration.<sup>66</sup>

Exosome are also related with stroke through regulating intercellular communication in brain-derived cells. A recent study show that microglia are the main source in brain under physiological conditions for extracellular vesicles (sEVs), including exosomes. This situation changed at 24h after induced stroke-reperfusion, where a significant increase of astrocytic sEVs was observed.<sup>67</sup> Furthermore, the result further show that EVs isolated from murine brain and brain-derived cell lines are enriched in prion protein (PrP) and PrP fragment(PrP-C1), with consequences in the regulation of vesicle uptake by recipient cells. Accordingly, when sEVs lack PrP, their uptake by neurons is increased, and conspicuously, they are also more readily taken up by microglia and astrocytes. Moreover, PrP-KO-sEVs were found to colocalize with lysosomes much faster than WT-sEVs. Therefore, increased release of sEVs and exosomes by astrocytes together with elevated levels of PrP in sEVs may play a role in intercellular communication at early stages after stroke, which may be a novel therapeutic method for stroke and ICH.<sup>67</sup>

In summary, following blood vessel rupture, hemoglobin enters the brain, forming a hematoma which induces pathological changes such as toxicity, edema, inflammation, oxidative stress, and apoptosis, leading to neurological deficits. The mechanism of brain injury after ICH is complex, but we can still improve the therapeutic effect of ICH by affecting one or more pathological pathways. Exosomes alleviate brain injury via angiogenesis, neurogenesis, anti-apoptosis, anti-inflammatory, immunomodulation, autophagy, and other ways, promoting the recovery of nerve function and reducing the mortality and disability rate of ICH. This potential has attracted increasing attention for its application prospects and potential conversion value.

## The Effects and Mechanism of Exosomes in the Treatment of ICH

ICH treatment initially focuses on hematoma removal via endogenous pathways and protecting surrounding brain parenchyma. Subsequent stages concentrate on repairing damaged neural cells, primarily through reducing inflammation, promoting angiogenesis, and neurogenesis.<sup>68</sup> Exosomes can improve ICH prognosis by promoting angiogenesis, neurogenesis, anti-apoptosis, anti-inflammatory, and immunomodulation, aligning with ICH's pathological mechanisms. Understanding the association between exosomes and these mechanisms could elucidate their therapeutic effect. In the following sections, we will discuss these mechanisms in detail.

## Association Between Exosomes and Angiogenesis After ICH

Following an ICH event, vascular integrity is compromised, leading to ischemic stress in adjacent tissues and blood vessels. This stress further exacerbates brain damage. Angiogenesis, the formation of new vascular buds from pre-existing blood vessels, is a complex process that can enhance blood perfusion in the ischemic area.<sup>69</sup> This increased blood supply helps maintain neuronal energy metabolism, thereby preventing neurons from entering a harmful cycle of apoptosis and death. As such, promoting cerebral angiogenesis under ischemic or hemorrhagic conditions may be a viable strategy to mitigate brain functional damage after ICH.

Interestingly, research has shown that exosomes from various cell sources, including mesenchymal stem cells (MSCs), cardiac progenitor cells, endometriotic stromal cells, and human induced pluripotent stem cells (iPSCs), can

**Table 1** The Source, Component, and Mechanism of Exosomes in the Treatment of ICH Through Angiogenesis

| Source of Exosomes            | Component               | Mechanism                                       | Experiment Type | Model  | Reference |
|-------------------------------|-------------------------|-------------------------------------------------|-----------------|--------|-----------|
| BMMSCs                        | miR-126                 | VEGF or fibroblast growth factor                | In vivo         | ICH    | [75,76]   |
| miR-126 modified BMMSCs       | Over-expressing miR-126 | PI3K/Akt signaling pathway                      | In vitro        | HUVECs | [75]      |
| ADMSCs                        | /                       | transferring miR-125a, miRNA-181b-5p/TRPM7 axis | In vivo         | /      | [77]      |
| Mouse brain endothelial cells | /                       | promote endothelial capillary formation         | In vivo         | ICH    | [46]      |

**Abbreviations:** BMMSCs, Bone marrow mesenchymal stem cells; ADMSCs, adipose-derived mesenchymal stem cells; ICH, intracerebral hemorrhage; HUVECs, Human Umbilical Vein vessel Endothelial Cells; ICH, intracerebral hemorrhage.

stimulate angiogenesis.<sup>70,71</sup> Angiogenesis is a multistep process involving cell proliferation, migration, invasion, vessel formation, and the maturation and stabilization of new sprouts. This process is mediated by activated endogenous progenitor cells, exogenous stem cells, and therapeutic agents like pro-angiogenic miRNAs.<sup>72,73</sup> Certain miRNAs, such as miR210, miR126, miR132, and miR21, found in exosomes, can promote angiogenesis by inhibiting gene expression post-transcriptionally.<sup>29</sup> For example, exosomes secreted by bone marrow mesenchymal stem cells (BMMSCs) transplanted into mice with ischemic stroke have been shown to enhance neurological and motor function recovery. This improvement is potentially mediated by miR-126, an endothelial cell-specific miRNA known to play a critical role in vascular stabilization.<sup>46,74</sup> Exosomes derived from miR-126 overexpressing BMMSCs have been shown to promote the proliferation, migration, and angiogenesis of human umbilical vein vessel endothelial cells (HUVECs) by regulating the PI3K/Akt signaling pathway.<sup>75</sup> Other studies have found that the effect of miR-126 is responsive to angiogenic growth factors, including VEGF or fibroblast growth factor.<sup>75,76</sup> Exosomes from human adipose-derived mesenchymal stem cells (ADMSCs) have been shown to increase the length, number, and branches of microvessels by transferring miR-125a and the miRNA-181b-5p/TRPM7 axis. Overexpression of TRPM7 reverses the effects of miRNA-181b-5p-exosomes on migration and cerebral capillary microvessel formation. Furthermore, it has been reported that exosomes originating from mouse brain endothelial cells can significantly enhance endothelial capillary formation, leading to higher blood vessel density, larger arterial diameter, and improved neurological cognitive function after ICH.<sup>46</sup> A summary of the current knowledge of exosomes in the treatment of ICH via related angiogenesis mechanisms is provided in Table 1.

## Association Between Exosomes and Neurogenesis After ICH

Neurogenesis, a process that includes the proliferation of neural stem cells (NSCs), migration of immature neurons and neuroblasts, differentiation into adult neurons, and extension of neurites, culminates in the formation and stabilization of synapses.<sup>78</sup> Notably, neurogenesis after a stroke is closely associated with angiogenesis.<sup>79,80</sup> Angiogenesis serves as the foundation for neurogenesis, and in turn, neurogenesis is a prerequisite for functional recovery.<sup>81</sup> The inhibition of cerebral angiogenesis can negatively impact neurogenesis in both hemorrhagic and ischemic brains. Therefore, encouraging neurogenesis under ischemic or hemorrhagic conditions could be an effective strategy to reduce brain functional damage following ICH.

As previously discussed, neurogenesis and angiogenesis often occur concurrently. Exosomes derived from mouse brain endothelial cells have been linked to improved synaptic remodeling and enhanced neurological cognitive function, attributed to the formation of endothelial capillaries and increased blood vessel density.<sup>46</sup> Exosomes carrying miR-126 also foster neuronal formation and axonal growth, thereby aiding the recovery of neurological and motor functions<sup>74</sup>. Preclinical studies indicate that the systemic administration of exosomes can support the endogenous rewiring of neuronal circuitry, white matter remodeling, angiogenesis, and neurogenesis in the injured brain.<sup>82</sup> Exosomes derived from MSCs have been shown to effectively enhance neurological recovery, particularly in spatial learning and motor functions after ICH. This could be attributed to their role in promoting endogenous neurogenesis, including the formation of neuroblasts and mature neurons in the subventricular zone, and myelination in the striatum.<sup>83</sup> In line with these findings, exosomes derived from miRNA 133b-overexpressing MSCs have demonstrated the ability to enhance neural plasticity and increase myelination, thereby facilitating functional recovery and neurite remodeling plasticity.<sup>84</sup> A summary of the current understanding of exosomes in the treatment of ICH via the related mechanism of neurogenesis is provided in Table 2.



**Table 2** The Source, Component, and Mechanism of Exosomes in the Treatment of ICH Through Neurogenesis

| Source of Exosomes            | Component  | Mechanism                                       | Experiment Type | Model             | Reference |
|-------------------------------|------------|-------------------------------------------------|-----------------|-------------------|-----------|
| BMSCs                         | miR-126    | VEGF or fibroblast growth factor                | In vivo         | ICH               | [75,76]   |
| Mouse brain endothelial cells | miR-126    | Promote endothelial capillary formation         | In vivo         | ICH with diabetes | [46]      |
| MSCs                          | unmeasured | Promote myelin formation and neurons mature     | In vivo         | ICH               | [83]      |
| miR-133b-overexpressing MSCs  | miR-133b   | Synergistic effect among astrocytes and neurite | In vitro        | ICH               | [84]      |

**Abbreviations:** BMSCs, Bone marrow mesenchymal stem cells; MSCs, mesenchymal stem cells; ICH, intracerebral hemorrhage.

## Association Between Exosomes and Anti-Apoptosis After ICH

Apoptosis, or programmed cell death, is a gene-controlled process that maintains internal environmental stability. In the context of ICH, apoptosis and cell death, which contribute to neuronal cell loss, serve as secondary injuries and are significant factors in high mortality and morbidity rates. As such, anti-apoptotic strategies could play a crucial role in mitigating and reversing the detrimental effects of ICH.

A study conducted *in vitro* has demonstrated that exosomes secreted by MSCs, loaded with miR-134, can reduce oligodendrocyte apoptosis following oxygen-glucose deprivation (OGD). This is achieved through the upregulation of Caspase 8, which suppresses the extrinsic apoptosis signaling cascade.<sup>49</sup> Interestingly, the introduction of miR-134 inhibitors to exosomes reduces the expression of Caspase 8 but increases procaspase-8 and caspase-8 cleaved product proteins, which promotes OGD-induced apoptosis.<sup>49</sup> Another study revealed that exosomes released by astrocytes can be absorbed by neurons, resulting in a decrease in apoptosis levels and an increase in mitochondrial performance, thereby aiding the functional recovery of damaged neurons.<sup>85</sup> Similarly, microglia-derived exosomes can also reduce neuronal apoptosis when taken up by neurons.<sup>11</sup> *In vivo* experiments further substantiate the anti-apoptotic properties of exosomes. A study by Heyu found that injecting MSCs transfected with miR-21 into ICH rats could mitigate neuronal apoptosis and improve neural function.<sup>28</sup> This treatment also significantly reduced hematoma and edema sizes. Further research indicated that exosomes derived from miR-21 overexpressing MSCs, when absorbed by neurons, can lessen post-ICH damage by reducing the activation of the NF- $\kappa$ B pathway. This suggests that exosomes secreted by miR-21-modified MSCs can alleviate neuronal apoptosis through miR-21, thereby protecting neurological function after ICH.<sup>28</sup> Mice treated with exosomes derived from miR-133b modified MSCs exhibited improved cognitive and motor function compared to the control group. This improvement is attributed to the resistance to neuronal apoptosis by inhibiting RhoA expression and activating the ERK1/2/CREB pathway in hemorrhagic brain tissue.<sup>86</sup> Moreover, exosomes overexpressing miR-146a-5p from MSCs can enhance neural function after ICH by reducing neuronal apoptosis and inhibiting the proinflammatory phenotype polarization of microglia.<sup>87</sup> A summary of the current understanding of exosomes in the treatment of ICH via the related mechanism of anti-apoptosis is provided in Table 3.

**Table 3** The Source, Component, and Mechanism of Exosomes in Treatment of ICH Through Anti- Apoptosis

| Source of Exosomes        | Component                   | Mechanism                                             | Experiment Type   | Model | Reference |
|---------------------------|-----------------------------|-------------------------------------------------------|-------------------|-------|-----------|
| MSCs                      | Loading with miR-134        | Upregulation of Caspase 8                             | In vitro          | OGD   | [49]      |
| Astrocytes                | /                           | Taken up by neurons                                   | In vitro          | OGD   | [85]      |
| Microglia                 | /                           | Assimilated by neurons                                | In vitro          | OGD   | [11]      |
| miR-133b modified MSCs    | Over-expressing miR-133b    | Inhibiting RhoA expression and activating ERK1/2/CREB | In vivo and vitro | ICH   | [86]      |
| miR-21 modified MSCs      | Loading with miR-21         | Reducing NF- $\kappa$ B                               | In vivo           | ICH   | [28]      |
| miR-146a-5p modified MSCs | Over-expressing miR-146a-5p | /                                                     | In vivo           | ICH   | [87]      |

**Abbreviations:** MSCs, mesenchymal stem cells; OGD, oxygen glucose deprivation; ICH, intracerebral hemorrhage; BMSCs, bone marrow mesenchymal stem cells.

## Association Between Exosomes and Anti-Inflammation After ICH

Neuroinflammation plays a significant role in the pathogenesis of ICH. In the early stages of ICH, microglia activation, cell death, and the breakdown of products initiate an inflammatory cascade that results in increased neuronal death. Microglia are the initial inflammatory cells that respond to the injury caused by ICH. The subsequent infiltration of blood-derived cells, including monocytes that transform into macrophages within the lesion, further intensifies the neuroinflammatory cascade.<sup>88</sup> Within 12 hours post-ICH, inflammatory macrophages and dendritic cells largely outnumber microglia in the hemorrhagic area.<sup>54</sup> The extensive neuroinflammatory response induced by ICH appears to exacerbate brain damage.<sup>81</sup> As such, reducing neuroinflammation is considered a potential therapeutic target.

Several studies have shown that exosomes can carry miRNAs, proteins, or other contents, which can confer anti-inflammatory effects.<sup>31</sup> For instance, exosomes derived from MSCs can mitigate the inflammatory response and improve prognosis following ICH by suppressing IL-1 $\beta$  levels.<sup>30</sup> In a study involving diabetic rats with ICH, exosomes derived from BMSCs alleviated neuroinflammation induced by hemorrhage via the transfer of miR-183-5p.<sup>52</sup> Moreover, the administration of exosomes secreted from angiotensin-converting enzyme 2-overexpressed endothelial progenitor cells could provide a therapeutic effect on acute ICH. This is achieved by alleviating functional neurological deficits and post-stroke inflammation through the transfer of ACE2 into the hemorrhage area, leading to down-regulated TNF- $\alpha$  and NF-kB, and upregulated IrB $\alpha$  levels.<sup>53</sup> The result was further verified by using DX600—an ACE2 inhibitor.<sup>89</sup> Exosomes derived from thrombin-activated platelets also play a crucial role in neuroinflammation. Upon the occurrence of ICH, thrombin is released, activating platelets that secrete exosomes containing miRNA-223. This triggers the activation of the NF-KB and MAPK signaling pathways. Through a series of signaling cascade pathways, the expression of miR-223 leads to the down-regulation of adhesion molecules, including ICAM-1.<sup>61</sup> As several studies have shown that adhesion molecules exacerbate inflammatory conditions during clot formation inside blood vessels, inhibiting the generation of these adhesion molecules via exosomes could potentially improve inflammatory damage following a stroke.<sup>90,91</sup>

Furthermore, exosomes can influence neuroinflammation through systemic effects within and between organ systems. Evidence suggests that exosomes from peripheral cells affect the interaction between peripheral and brain inflammation.<sup>92</sup> Peripheral inflammation triggers the choroid plexus epithelium to release exosomes into the CSF, which then cross the blood–cerebrospinal fluid barrier to transfer their pro-inflammatory cargo into astrocytes and microglia, leading to brain inflammation.<sup>82</sup> Inhibiting exosome secretion to block this exosome-mediated communication can attenuate brain inflammation.<sup>92</sup> Additionally, the brain-gut axis can regulate brain parenchyma inflammation under physiological conditions through extracellular vesicles secreted by bacteria. For instance, the K12-derived *E. coli strain W3110* can reduce inflammation levels by inhibiting NLRP3 inflammasome activation, which prevents mitochondrial damage and iron death.<sup>93</sup> This demonstrates how the exosome system can affect organisms at a systemic level. A summary of the current understanding of exosomes in the treatment of ICH via the related mechanism of anti-neuroinflammation is provided in Table 4.

**Table 4** The Source, Component, and Mechanism of Exosomes in Treating ICH Through Anti-Neuroinflammation

| Source of Exosomes          | Component  | Mechanism                                                                  | Experiment Type | Model             | Reference |
|-----------------------------|------------|----------------------------------------------------------------------------|-----------------|-------------------|-----------|
| MSCs                        | /          | Suppressing IL-1 $\beta$ levels                                            | In vivo         | ICH               | [30]      |
| BMSCs                       | miR-183-5p | /                                                                          | In vivo         | ICH with diabetic | [52]      |
| EPCs                        | /          | Down-regulated TNF- $\alpha$ and NF-kB and up-regulated IrB $\alpha$ level | In vivo         | ICH               | [89]      |
| EPCs                        | ACE2       | Reduce hematoma size and cerebral edema                                    | In vivo         | ICH               | [53]      |
| Thrombin activated platelet | miRNA-223  | Down-regulated adhesion molecules                                          | In vivo         | ICH               | [82,92]   |
| Bacteria                    | /          | Inhibition of NLRP3 inflammasome activation                                | In vivo         | Ischemic stroke   | [93]      |

**Abbreviations:** MSCs, mesenchymal stem cells; EPCs, endothelial progenitor cells; BMSCs, bone marrow mesenchymal stem cells; ICH, intracerebral hemorrhage.

## Association Between Exosomes and Immunomodulation After ICH

Microglia display two distinct functional phenotypes: the proinflammatory and anti-inflammatory phenotypes. During the initial stages of ICH, there is a marked increase in the expression of TNF- $\alpha$  in the brain parenchyma surrounding the hematoma, a characteristic marker gene of the proinflammatory phenotype.<sup>94</sup> This prompts microglia to secrete a multitude of pro-inflammatory cytokines such as nitric oxide synthase (NOS), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and monocyte chemoattractant protein 1 (MCP1), thereby stimulating a nervous system inflammatory response.<sup>95</sup> Conversely, microglia with an anti-inflammatory phenotype secrete interleukin-10 (IL-10), interleukin-1R (IL-1R), and transforming growth factor- $\beta$  (TGF- $\beta$ ), which contribute to systemic protection and repair.<sup>96,97</sup> Research has demonstrated that immunomodulation, including the inhibition of proinflammatory phenotypic polarization and acceleration of anti-inflammatory phenotype polarization in microglia, plays a neuroprotective role and could be a potentially important strategy in the treatment of ICH.<sup>98</sup> Additionally, some studies have shown that the activation and polarization of microglia can be controlled, further substantiating the potential for recovery through immunomodulation.<sup>99</sup>

Exosomes secreted by microglia contain a large number of miRNAs, such as miR-126-5p, miR-21-3p, miR-212, among others, which are implicated in the polarization of microglia from a proinflammatory phenotype to an anti-inflammatory phenotype.<sup>57</sup> Exosomes derived from MSCs have been shown to inhibit microgliosis and astrogliosis, and promote immunomodulation.<sup>100</sup> Further research has indicated that these exosomes facilitate the shift in microglia phenotype from proinflammatory to anti-inflammatory by regulating the CysLT2R-ERK1/2 pathway.<sup>101</sup>

Similarly, exosomes secreted by BMSCs possess immunomodulatory and anti-inflammatory capabilities, which can be therapeutically beneficial in ICH. This is achieved through the regulation of miR-146a-5P, which may further target IRAK1 and NFAT5 genes, inhibiting the activation of inflammatory signaling pathways.<sup>58</sup> Another study confirmed that exosomes from BMSCs exert immunomodulation to protect brain parenchyma through the modulation of exosomal H19.<sup>102</sup> Moreover, exosomes from BMSCs also regulate the polarization of microglia towards the anti-inflammatory phenotype by downregulating the expression of IL-1 $\beta$ , CD16, CD11b, and NOS, while upregulating CD206.<sup>103</sup> A summary of the current understanding of exosomes in the treatment of ICH via the immunomodulation of microglia is provided in Table 5.

## Association Between Exosomes and Autophagy After ICH

Autophagy is a process where proteins or organelles are engulfed, endocytosed into vesicles, merged with lysosomes to form autophagic lysosomes, and then the contents within them are degraded. This process fulfills the metabolic requirements of the cell and facilitates the renewal of certain organelles. Numerous studies have indicated that both insufficient and excessive levels of autophagy can exacerbate cell death, whereas moderate autophagy can protect cells against stressful conditions and promote cell survival.<sup>104</sup> Autophagy is considered an early-stage mechanism in the pathophysiology of stroke and inflammatory diseases, regulating immune cell homeostasis.<sup>105</sup> Conversely, the production of certain inflammatory biomolecules, such as TNF- $\alpha$ , IL-1 $\alpha$ , and IFN, can influence the progression of autophagy. Following the onset of ICH, inflammation, oxidative stress, and the accumulation of free iron can trigger apoptosis and autophagy.<sup>36</sup> Autophagy may initially exacerbate brain injury, but later stages may be neuroprotective due to the removal of cellular debris.<sup>106</sup> In vitro experiments have shown that inhibiting autophagy significantly reduces the inflammatory

**Table 5** The Source, Component, and Mechanism of Exosomes in Treating ICH Through Immunomodulation

| Source of Exosomes | Component                              | Mechanism                                                       | Experiment Type | Model | Reference |
|--------------------|----------------------------------------|-----------------------------------------------------------------|-----------------|-------|-----------|
| Microglial         | miRNA-126-5p, miRNA-21-3p, and miR-212 | M1-to-M2 polarization                                           | In vivo         | /     | [57]      |
| MSCs               | /                                      | CysLT2R-ERK1/2 pathway                                          | In vivo         | /     | [100,101] |
| BMSCs              | Regulation of miR-146a-5P              | Targeting IRAK1 and NFAT5 genes                                 | In vivo         | ICH   | [58]      |
| BMSCs              | Exosomal H19 modulates                 | /                                                               | In vivo         | ICH   | [102]     |
| BMSCs              | /                                      | Down-regulating IL-1 $\beta$ , CD16, CD11b; up-regulating CD206 | In vivo         | ICH   | [103]     |

**Abbreviations:** BMSCs, Bone marrow mesenchymal stem cells; MSCs, mesenchymal stem cells; ICH, intracerebral hemorrhage.

**Table 6** The Source, Component, and Mechanism of Exosomes in the Treatment of ICH Through Reducing Autophagy

| Source of Exosomes | Component | Mechanism                               | Experiment Type   | Model  | Reference |
|--------------------|-----------|-----------------------------------------|-------------------|--------|-----------|
| Astrocyte          | /         | Inhibiting OGD-induced neuron autophagy | In vivo and vitro | ICH    | [108]     |
| MSCs               | miR-21    | Alleviate neuronal apoptosis            | In vivo           | ICH    | [32]      |
| ADMSCs             | miR-25-3p | Alleviate neuronal apoptosis            | In vivo           | Stroke | [65]      |

**Abbreviations:** MSCs, mesenchymal stem cells; OGD, oxygen glucose deprivation; ADMSCs, adipose-derived mesenchymal stem cells; ICH, intracerebral hemorrhage.

response induced by OGD. Similarly, *in vivo* studies have reported a significant reduction in the cerebral infarction area through the acceleration of the autophagic response at target sites.<sup>107</sup> Both *in vivo* and *in vitro* experiments have confirmed that exosomes derived from astrocytes can mitigate ischemia-induced neuronal damage by inhibiting OGD-induced neuronal autophagy.<sup>108</sup> Another study suggested that exosomes secreted by ADMSCs could reduce autophagy in a stroke mouse model by delivering miR-25-3p into target cells.<sup>65</sup> Therefore, maintaining a balanced state of autophagy and inflammation may be a potential treatment strategy for ICH. A summary of the current understanding of exosomes in the treatment of ICH via autophagy-related mechanisms is provided in Table 6.

## The Therapeutic Effect of Exosomes in Complications After ICH

ICH, a severe type of stroke, occurs when blood leaks into the brain parenchyma, contributing to 15% of total stroke-related cases.<sup>1-3</sup> While most survivors of ICH experience some degree of spontaneous functional recovery, the improvement is often modest and insufficient for independent daily living, with only 25% of survivors managing to live independently within a year following ICH.<sup>109</sup> Complications following ICH include visual impairment, ischemic cerebral infarction, hematoma expansion, cerebral hernia, seizures, hydrocephalus, subarachnoid hemorrhage, and venous thrombotic events.<sup>110</sup>

Research has demonstrated that exosomes can enhance the recovery from ischemic infarction. For instance, in mice with focal cerebral ischemia, intravenous administration of RVG exosomes loaded with miR-124 reached the peri-ischemic area and improved blood perfusion by promoting angiogenesis and neurogenesis.<sup>111</sup> Another study revealed that MSC-derived exosomes could directly influence axonal growth in the ischemic region by inhibiting Argonaut 2 via miR-17-92, leading to axonal growth through the activation of the PTEN/mTOR signaling pathway.<sup>48</sup> Additionally, components of exosomes derived from HUMSCs have been shown to possess potential therapeutic roles in treating epilepsy,<sup>112</sup> reducing tissue damage, and enhancing tissue repair.<sup>113</sup>

When blood infiltrates the brain tissue and enters the subarachnoid space, secondary subarachnoid hemorrhage (SAH) occurs. Studies have shown that exosomes also have significant therapeutic effects on SAH through anti-apoptotic pathways. One study found that exosomes secreted by human umbilical cord MSCs with miR-206 knockdown mediated the TrkB/CREB signaling pathway by targeting BDNF, significantly improving neurological deficits and inhibiting apoptosis to prevent early brain damage caused by SAH.<sup>114</sup> Another study observed that miR-26b-5p-modified exosomes inhibited apoptosis and the expression of inflammatory mediators during SAH through the MAT2A-mediated p38 MAPK/STAT3 signaling pathway in early brain injury.<sup>115</sup> Recently, a study reported that exosomes from BMSCs reduced neuroinflammation and exerted a neuroprotective effect in brain tissues post-SAH by inhibiting the NF- $\kappa$ B and activating AMPK pathways.<sup>103</sup> A summary of the current understanding of exosomes in the treatment of complications after ICH is provided in Table 7.

## Application of Exosomes-Based Delivery System in Treating ICH

In the above article, we summarize the inherited properties of exosomes are plausible and beneficial in ameliorating ICH and its complications through kinds of miRNAs by molecular biological mechanisms. Due to the transmembrane capacity of exosomes, they can also be effective carriers to directional transport of the active substances or drugs cross BBB into parenchymal hemorrhage area. BBB is a physiological barrier primarily composed of non-fenestrated endothelial cells that prevent potentially harmful substances from entering the brain environment. Due to tight junctions

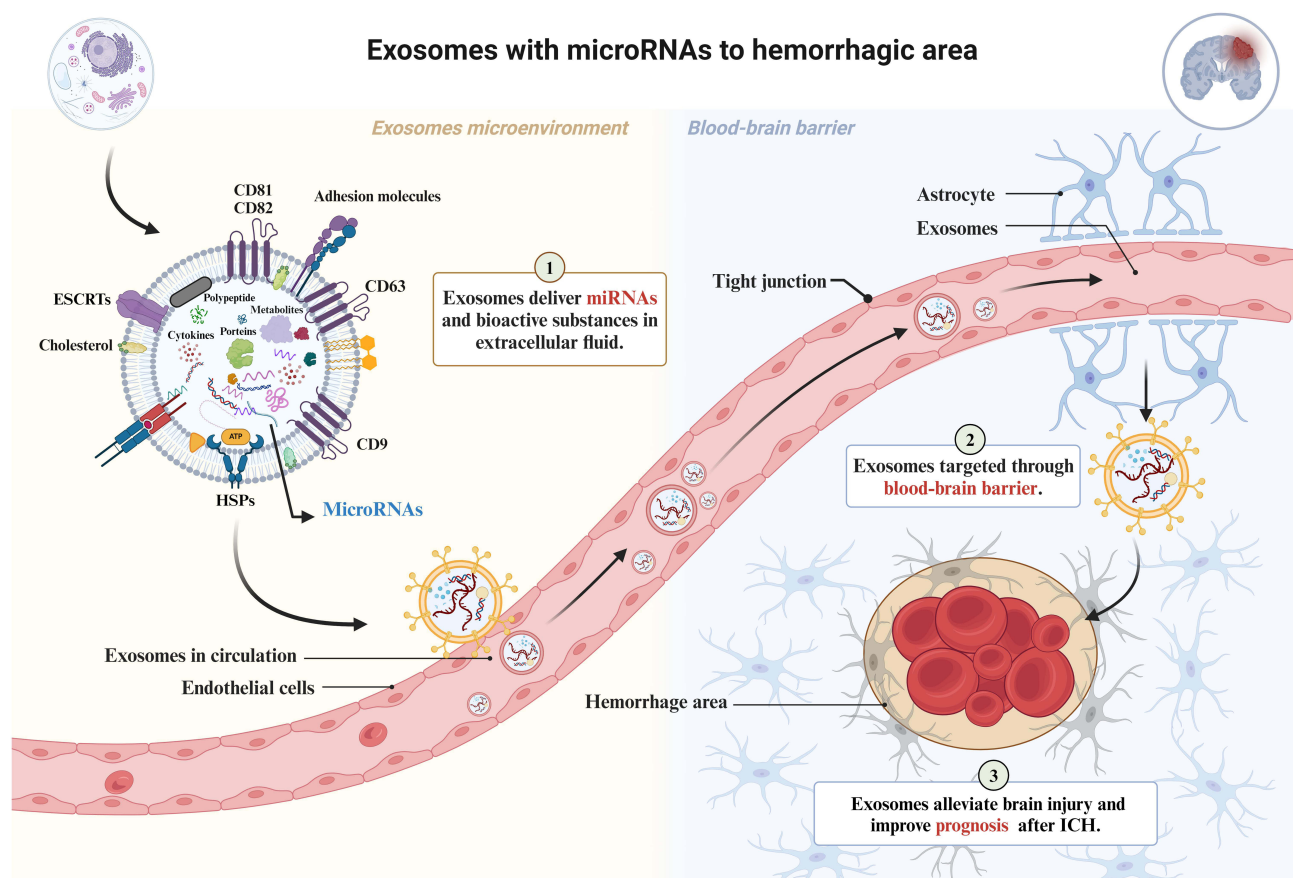
**Table 7** The Source, Component, and Mechanism of Exosomes in the Treatment of Complications After ICH

| Source of Exosomes                  | Component                                      | Mechanism                                                            | Experiment Type    | Model                       | Reference      |
|-------------------------------------|------------------------------------------------|----------------------------------------------------------------------|--------------------|-----------------------------|----------------|
| Exogenous MSCs                      | miR-124<br>miR-17-92                           | Enriched in neurons<br>Activation of the PTEN/mTOR signaling pathway | In vivo<br>In vivo | Cerebral ischemia<br>Stroke | [111]<br>[48]  |
| Exogenous Human umbilical cord MSCs | Overexpressing MiR-26b-5p<br>miR-206 knockdown | MAPK/STAT3 signaling pathway<br>TrkB/CREB signaling pathway          | In vivo<br>In vivo | SAH<br>SAH                  | [115]<br>[114] |
| BMSCs                               | /                                              | Inhibit NF- $\kappa$ B but activate AMPK pathways                    | In vivo            | SAH                         | [103]          |

**Abbreviations:** BMSCs, bone marrow mesenchymal stem cells; MSCs, mesenchymal stem cells; SAH, subarachnoid hemorrhage.

and selective behavior of BBB, it prevents the penetration of most chemotherapeutic agents or biotech drugs. Therefore, despite the availability of a wide variety of disease ameliorating agents, only 2% of drugs can traverse the BBB.<sup>116</sup> Exosome-based delivery systems, a novel drug delivery system, may provide a solution to the transmembrane capacity issue of exosomes. There are several routes for exosomes interact with BBB, including trafficking through macropinocytosis, lipid raft or nonspecific exosome-endothelium interaction, and receptor-mediated transcytosis (Figure 4).<sup>117</sup>

How to load bioactive cargo and establish an effective targeted transport system? The origin, payload type, and loading technique are crucial for ensuring the efficient therapeutic application of exosomes. Despite the low uploading efficacy, it is feasible to co-incubate exosomes with a hydrophobic drug in a suitable medium, which allows the drug to



**Figure 4** Exosomes-based delivery system with miRNAs or bioactive substances to hemorrhage area. Exosomes deliver miRNAs from extracellular fluid into capillaries, such as miR-126, miR-125, miR-124, miR-17-92 and etc.<sup>1</sup> targeted cross the BBB,<sup>2</sup> then enter the cerebral hemorrhage area to promote nerve function recovery and improve prognosis by various biological effects.<sup>3</sup> The figure was created with <https://app.biorender.com/>.

diffuse through the exosomal membrane. Typical successful examples of the pattern are curcumin, which was successfully loaded after a 5-min incubation at room temperature,<sup>118</sup> or the catalase enzyme, which was successfully loaded after 18 h of incubation in PBS at room temperature.<sup>119</sup> Transfecting or infecting parental cells with DNA encoding therapeutically active compounds is also an effective approach. When parental cells released exosomes, therapeutically active compounds will be warped by exosomes.<sup>13</sup> Mechanistic studies showed that exosomes secreted from genetically-modified parental cells contained the encoded therapeutic protein, as well as its gene-synthetic material including DNA, mRNA and transcription factor involved in the encoded gene expression.<sup>120</sup>

Recently, the therapeutic effects of cargo-loaded exosomes have been reported by numbers of studies. A recent study showed that iron oxide and other magnetic nanoparticles packaged in exosomes significantly accumulated at stroke-affected brain tissue,<sup>121</sup> showing a 5.1-fold higher accumulation at the injury site. Exosomes loaded with an anti-inflammatory small molecule compound, curcumin, were shown to protect mice from brain inflammation.<sup>122</sup> Additionally, exosomes isolated from BMSCs were also loaded with curcumin, which strongly suppressed inflammatory lesions and apoptosis in the ischemic brain after ICH.<sup>123</sup> Another therapeutic avenue involves the use of exosomes to transport exogenous siRNA or miRNA. For example, exosomes were used to transport siRNA targeted to miR-150, due to its promotional effect on VEGF.<sup>124</sup> It was demonstrated that exosomes loading siRNA attenuated angiogenesis in mice, mediating by the neutralization of miR-150 down-regulated VEGF levels in mice.<sup>124</sup> Mice treated with exosomes derived from miR-133b modified MSCs exhibited improved cognitive and motor function compared to the control group.<sup>86</sup> Moreover, exosomes overexpressing miR-146a-5p from MSCs can enhance neural function after ICH by reducing neuronal apoptosis and inhibiting the proinflammatory phenotype polarization of microglia.<sup>87</sup> The therapeutic effects and mechanisms of exosomes-based therapy on ICH will be elaborated in the following article. A summary of the current understanding of exosomes-based delivery system in the treatment of ICH is provided in Table 8.

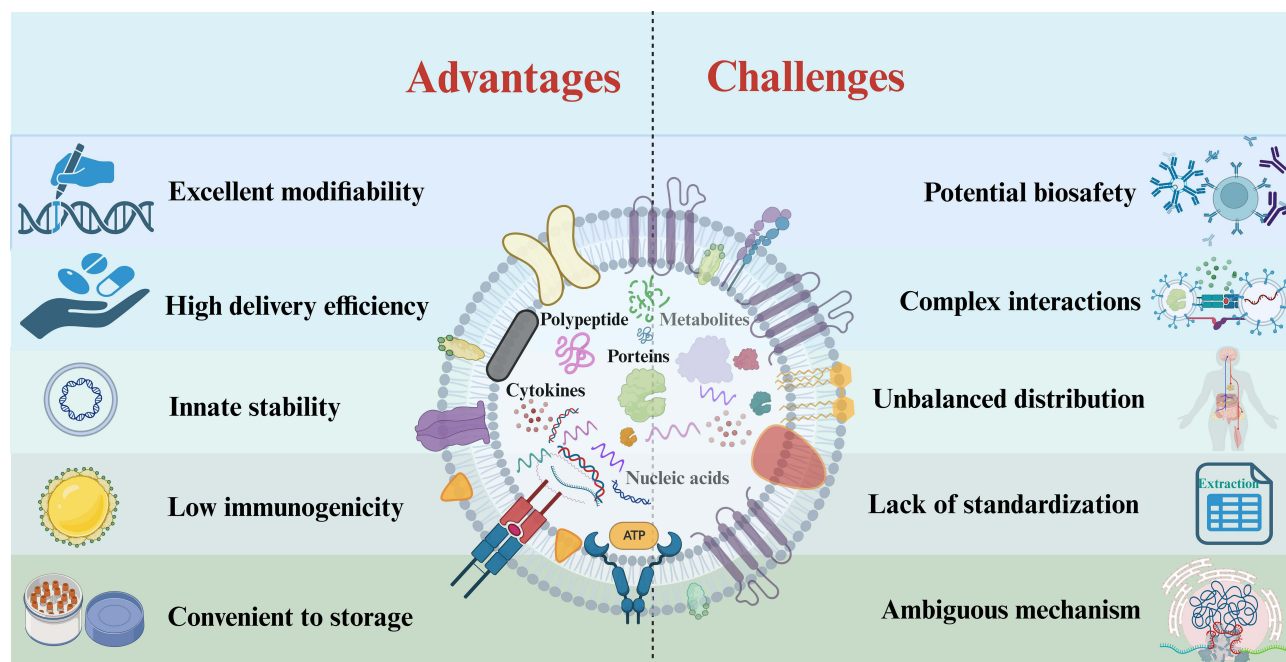
## Advantages and Challenges of Exosomes in Treating ICH

Compared to other treatments like stem cell therapy, exogenous systemic injection of exosomes offers several advantages: I: They have a stronger biological effect as they can cross biological barriers and directly fuse with target cells.<sup>125</sup> II: They are more convenient to store and can be kept at  $-80^{\circ}\text{C}$  for extended periods due to the protective lipid bilayer.<sup>125</sup> III: They have a higher transport capacity and can be modified to carry specific cargoes like drugs, cytokines, or miRNAs.<sup>126</sup> IV: They offer higher biosafety due to their low immunogenicity as cell-free therapy.<sup>127</sup> V: They can be administered in various ways, including intravenously, intramuscularly, transnasally, and subcutaneously, making it easy to control the concentration, dosage, transport route, and administration time. VI: They have excellent editability/modifiability. Exosomes can be used as carrier particles for certain miRNAs, drugs, and existing or newly created compositions to target cross BBB, maximizing clinical benefit and reducing drug concentration. VII: They have a stronger ability to pass the BBB. Exosomes have natural BBB crossing potential through the interaction between exosome surface ligands and brain endothelial cell receptors.<sup>128</sup> They can serve as a good delivery vehicle for both hydrophobic and hydrophilic drugs through specific proteins in the exosome membranes and natural homing ability.

**Table 8** The Mechanism and Effects of Exosomes Based Delivery System in the Treatment of ICH

| Exosomes Based System                      | Mechanism                                                                                          | Effects                                     | Reference |
|--------------------------------------------|----------------------------------------------------------------------------------------------------|---------------------------------------------|-----------|
| Oxide-Exo-BMSCs/magnetic nanoparticles-Exo | /                                                                                                  | Accumulated at stroke-affected brain tissue | [121]     |
| Curcumin-Exo-BMSCs                         | Suppressed inflammatory lesions and apoptosis                                                      | Protect from brain inflammation             | [122,123] |
| SiRNA-Exo                                  | Down-regulated VEGF levels                                                                         | Attenuated angiogenesis                     | [124]     |
| miR-133b-Exo-MSCs                          | /                                                                                                  | Improved cognitive and motor function       | [86]      |
| miR-146a-5p-Exo-MSCs                       | Reducing neuronal apoptosis and inhibiting the proinflammatory phenotype polarization of microglia | Enhance neural function after ICH           | [87]      |

**Abbreviations:** Exo, exosomes; BMSCs, bone marrow mesenchymal stem cells; MSCs, mesenchymal stem cells; VEGF, vascular endothelial growth factor; ICH, intracerebral hemorrhage.



**Figure 5** Major advantages and challenges of exosomes in the treatment of ICH. Exosomes have the advantages of high biological effect, targeting crossing BBB and convenient transformation, so they have broad application prospects in ICH. However, there are still certain challenges in clinical translation, including ambiguous mechanisms, potential biosafety, unbalanced distribution, and lack of standardization. The figure was created with <https://app.biorender.com/>.

Despite these unique features and advantages in treating CNS diseases, there are fewer implications and research in the treatment of ICH compared to ischemic stroke. This may be due to several challenges in translating lab findings to clinical practice: I: Complex interactions. Current studies are mostly limited to a certain substance in exosomes from a single source. However, exosomes contain various bioactive substances, and whether there are synergistic effects of multiple substances in exosomes for the treatment of ICH needs further exploration because the interactions between nerve cells are intricate.<sup>129</sup> II: Potential biosafety. Although exosomes have low immunogenicity in terms of structure and chemical components, more experimental studies and repeated verifications are necessary to demonstrate their biosafety and efficacy. III: Lack of standardization. There is no uniform standard technology for the production of exosomes. Different techniques for production and isolation can result in significant heterogeneity and purity differences, hindering the reproducibility and reliability of experimental results and posing a major challenge for their entry into clinical applications.<sup>130</sup> IV: Ambiguous mechanism. Questions remain about how exosomes achieve cell-to-cell communication, how they cross the BBB and enter the target neurocyte, and how they transport cargo to the central nervous system. V: Unbalanced distribution. In an exosomal biodistribution study, after the intravenous administration of natural exosomes into the body, they were rapidly removed from the target site and accumulated in organs of the reticuloendothelial system such as the lung, liver, and spleen.<sup>131,132</sup> The uneven distribution in vivo limits the biological effects of exosomes. The major advantages and challenges of exosomes in clinical translation are summarized in Figure 5.

## Discussion and Prospect

As reviewed above, exosomes derived from various sources, loaded with specific miRNAs and acting through different mechanisms, have shown potential therapeutic effects in improving prognosis after ICH (Tables 1–8). The evidence suggests that exosomes derived from different cells, capable of transporting specific components across the BBB under ischemic or hemorrhagic conditions, offer a promising strategy for ICH treatment. Despite exosomes-based therapy owns unique advantages in nervous system diseases, researchers primary focus on brain tumors, Alzheimer, and other neurological disorders like Parkinson. The research and clinical transformation of ICH is relatively insufficient, which may attribute to sever symptoms, high disability, and mortality of ICH, which limit the development and clinical

translation of drugs. With the recent advances in nanomedicines and drug delivery, promoting hemostasis, neuroprotection, and nerve regeneration through exosome may provide insight into hemorrhagic stroke treatment.

While exosome treatment for ICH has shown excellent results in animal experiments, indicating substantial therapeutic potential and application prospects, several questions and challenges remain. In this review, we mainly focused on the therapeutic effects of exosomes through exogenous administration. However, the concept of promoting endogenous exosome secretion as a treatment strategy remains largely unexplored. In certain experimental models, such as an asthma mouse model, heat stimulation promoted exosome secretion from mastocytes, leading to significant reduction in asthma symptoms, which was mediated by promoting the expression of Rab3a and FcεRI, the key factors related to exosome secretion.<sup>133</sup> Similarly, in tumor cells, exosomes produced by cells growing on a hard extracellular matrix effectively promoted tumor growth.<sup>134</sup> At present, it has not been reported that promoting exosome secretion by a certain kind of cells can improve the recovery of neural defects and improve the healing after ICH. However, there are shreds of evidence that exosomes from peripheral cells can affect the interaction between peripheral and brain inflammation through promoting the activation of astrocytes and microglia, leading to brain inflammation.<sup>82,92</sup> Whether a similar strategy could be employed to promote exosome secretion to improve recovery from neural defects and healing post-ICH is an area ripe for exploration.

Furthermore, while some mechanisms of exosome targeting into brain tissue have been discussed, exosomes tend to accumulate in the lung, liver, and spleen. Enhancing the targeted abilities of exosomes to the brain and avoiding these organs is a challenge. Engineered exosomes designed to enhance targeting across the BBB through unique receptors may provide a solution. Surface engineering of various membrane proteins and components, such as CD9,<sup>135</sup> CD63,<sup>136</sup> PTGFRN,<sup>137</sup> LAMP2B,<sup>138</sup> and phosphatidylserine,<sup>43</sup> could facilitate receptor-mediated transcytosis. Additionally, neurotropic virus-derived peptides like RVG or peptides that bind to specific membrane proteins could be used for exosome modification. Currently, various technologies to efficiently incorporate drugs and active pharmaceutical ingredients into exosomes are being actively developed.<sup>139,140</sup> In preclinical studies, engineered exosomes possessing targetability to the brain have shown promising results for CNS delivery. However, for the successful development of clinically approved exosome therapeutics for CNS diseases, there need to be more evidence and studies to establish imaging methods for quantitative/qualitative monitoring of exosomal delivery to the brain parenchyma *in vivo* as well as uncover the mechanisms underlying the BBB crossing of surface-modified exosomes.<sup>25</sup>

Many preliminary works have proven that exosomes were sufficient to promote neurovascular remodelling and functional recovery in ischemic and hemorrhagic stroke. However, the clinical investigations of exosome-based therapy in stroke have just begun to explore, and well-designed clinical trials are warranted to promote exosome translation to clinic.<sup>141</sup> Clinical trial NCT03384433 was designed to explore the therapeutic effect of exosomes on stroke. Researchers are aiming to evaluate the administration of exosomes derived from allogenic MSC enriched with miR-124 on improvement of disability in patients with acute ischemic stroke which is still under recruiting. Similarly, NCT05035134 aims to explore application of circulating exosomes in early diagnosis and prognosis evaluation after ICH. The purpose of this trial is to observe the relationship between the changes of circulating exosomes and the development and outcome of the disease in patients with ICH, then to search for early serum markers and potential intervention targets for disease monitoring in patients with ICH.

## Conclusion

In conclusion, exosomes not only offer potential treatment for multiple pathogenic mechanisms post-ICH and improve prognosis, but also show promise in addressing long-term complications of ICH including SAH, epilepsy, and etc. Although there are many challenges to be overcome before clinical application of exosomes, their potential in ICH treatment is promising and novel. Understanding the mechanisms of exosomes and miRNAs in the process of ICH is critical for guiding their clinical applications. We hope that exosomes-based cell-free therapy will provide a new avenue for ICH treatment in the future.



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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

All authors have declared no conflicts of interest in this work.

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