

Research Progresses in the Inhibitory Effect of Bone Mesenchymal Stem Cell-Derived Exosome on Blood-Brain Barrier Disruption following Intracerebral Hemorrhage in Rats

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Abstract

Mesenchymal Stem Cells (MSCs) are a type of non-hematopoietic progenitor cells which have self-replication capacity and multilineage differentiation. They have widely applied in studies of various diseases due to their effects in damaged tissue repair, neuroprotection and immunoregulation. MSCs can secrete exosomes through multiple ways in the physiological or pathological state. Many researches' results on MSC-Exo show that it possesses many functions similar to MSCs, such as immunoregulation and regeneration promotion of damaged tissues. Hence, MSC-Exo is believed to have considerable research potentials in regenerative medicines. This study reviewed the research progresses on biological characteristics and functions of MSC-Exo.

Keywords

Bone Mesenchymal Stem Cell-Derived Exosome, Blood-Brain Barrier, Intracerebral Hemorrhage

1. Introduction

Mesenchymal Stem Cells (MSCs) are a type of non-hematopoietic progenitor cells which have self-replication capacity and multilineage differentiation. They have extensive distributions in bone marrow [1] [2], fat, liver, spleen, thymus, umbilical cord blood, placenta, Wharton's jelly, brain, lungs and dental pulps

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[3]-[7]. They have widely been applied in studies of various diseases due to their effects in damaged tissue repair, neuroprotection and immunoregulation. Previous research opinions agree that stem cells repair damaged tissues mainly through homing differentiation. In 2010, Lai *et al.* [8] separated Mesenchymal Stem Cells derived Exosomes (MSC-Exo) for the first time. Subsequent studies have proved the important biological functions of MSC-Exo [9]. MSC-Exo is a membranous vesicle which is secreted by MSCs and can carry and deliver bioactive substances (e.g. endogenous proteins, lipids, mRNA, siRNA, rRNA and micro RNA (miRNA)) which are wrapped in the lipid bilayers through internalization of target cells, receptor-ligand interaction or plasma membrane fusion. MSC-Exo is not only an important media for communicating among cells [10] [11], but also participates in maintaining the homeostasis of tissues. MSCs can secrete exosomes through multiple ways in the physiological or pathological state. Some study has pointed that MSCs are the cells which secrete exosomes the mostly [9]. May research results on MSC-Exo show that it possesses many functions similar with MSCs, such as immunoregulation and regeneration promotion of damaged tissue. Hence, MSC-Exo is believed to have considerable research potentials in regenerative medicines.

Exosomes which are secreted by Neural Stem Cells (NSCs) can strengthen neuroplasticity and promote nerve regeneration and functional recovery after injury. They provide a therapeutic effect by regulating the functions of neurons and gliocytes in the local microenvironment and remote target cells [12]. Therefore, NSCs-Exo has remarkable clinical application potentials and they are a new potential treatment for nervous system disease. This study reviewed the research progresses on biological characteristics and functions of MSC-Exo.

2. Biological Characteristics of MSC-Exo

2.1. Basic Features of MSC-Exo

Exosomes refer to vesicles (diameter: 40 - 100 nm) which are secreted by cells under different stress states and they are derived from the endosome system of cells. Firstly, cells sink through endocytosis and form the early endosome. Subsequently, the endosome membranes sink again to form intra-cavity vesicles which selectively receive proteins and lipids from the cytoplasm to form the late endosome. Next, the late endosome membrane and cytomembrane integrate to release a lot of vesicles out of cells through exocytosis, which are exosomes. The process for MSCs secretion of exosomes is the same with the process of other cells, which is also a process of "invagination-fusion-exsecretion" [13] [14]. MSC-Exo is wrapped by lipid bilayer and presents as cup-like minutes under an electron microscope. They can enrich in the 1.10 - 1.19 g/ml sucrose density gradient solution through the 100,000 g ultracentrifugation [15]. The MSC-Exo surface contain membrane-related proteins (e.g. CD9, CD63, CD81 and CD83, MHC-I and MHC-II), heat shock proteins (Hspa8, Hsp70 and Hsp90), Guanosine Triphosphate (GTP), and multivesicular biogenic marker proteins (Alix and

TSG101) [15] [16] [17], as well as metabolic enzymes (e.g. GAP-DH, LDHA, PGK1, aldolase and PKM), cytoskeletal proteins (actin, moesin and syntenin) as well as carrier proteins (e.g. albumin). Besides, MSC-Exo also expresses relevant molecules of MSCs, such as CD29, CD44, CD90 and CD73 [18]. In addition to proteins, MSC-Exo also is enriched of multiple biomacromolecules related with its biological source, including liposomes (e.g. phosphoglyceride, lysophosphatidic acid and cholesterol), ceramide, fatty acid chains, mRNA, non-coding RNA (miRNAs, tRNAs, rRNAs), and so on [19] [20] [21]. Exosomes are derived from the combination of vesicles and cytomembrane. Hence, secretion of exosomes is sensitive to different physicochemical substances and environmental conditions. For example, gamma knife-irradiation, calcium ion proteins, heparinase, statins, hypoxia and acidosis all can increase secretion of exosomes [19]. The hypoxia condition in placenta MSC media, depolarization of potassium ion dependent nerve cells and T-cell activation caused by TCR/CD3 crosslinking all can induce abundant secretion of exosomes [22].

2.2. Unique microRNA in MSC-Exo

Nucleic acids in MSC-Exo mainly include mRNA, miRNA, DNA, and so on [23]. MSCs can generate biological effect with target cells through miRNA in MSC-Exo [24]. Ti *et al.* [25] analyzed the miRNA expression spectra of MSC-Exo based on the gene chip technology and identified 42 miRNAs with abnormal expressions, including 15 upregulated. Specifically, expressions of miR-21, miR-146a and miR-181 are upregulated the most. According to the big data analysis, miR-21, miR-146a and miR-181 not only participate in cell development, congenital immunoreaction, inflammatory reaction toll-like receptor (TLR) pathway and IL-6 mediated cell signaling pathway [26], but also participate in the immune system activation and inflammation occurrence in the process of damaged tissue repair. miRNAs in MSC-Exo exchange biological information among adjacent cells as a molecular switch and its content is changed significantly [27]. Micro-environmental factors, such as different cell sources as well as different calcium ions and growth factor concentration, all can influence miRNAs composition in exosomes, thus influencing the information transmission and regulation performance [28]. Montecalvo *et al.* [29] pointed out that miRNA in exosomes has high stability because of their unique membrane structure. They can resist strong environmental interferences, such as low-acid pH value, low-temperature storage and RNase-mediated degradation reaction. So far, abnormal expressions of exosomes-derived miRNA have been detected in many diseases. Therefore, miRNA in exosomes is viewed as the biomarker in many pathogenetic processes [30].

2.3. Separation, Identification and Storage of MSC-Exo

It usually uses supercentrifugation combined with sucrose density gradient to separate and purify exosomes from supernate of MSC media and from different

biological fluids. It can eliminate solid precipitates of cells and big particles and separate exosomes under the ultra-high centrifugal force of 100,000 g [31]. In addition, High Performance Liquid Chromatography (HPLC), ultrafiltration method, antibody capture method and exosomes separation kit based on lectin (e.g. Exo Quick) are also good choices [32]. Some study believes that low-pH is conducive to separate exosomes and increase output [33]. There are many methods to identify exosomes, including Atomic Force Microscope (AFM), Scanning Electron Microscope (SEM), transmission electron microscope (TEM), Dynamic Light Scattering (DLS), Flow Cytometry (FCM), Nanoparticle Tracking Analysis (NTA), western blotting or Elisa method [32]. It usually suggested that the separated MSC-Exo shall be detected or frozen under a low temperature as soon as possible. Konala *et al.* [34] studied stability of exosomes under different storage conditions of -20°C , 4°C and 37°C , and found that exosomes under 4°C and 37°C shrank in volume, with some structural changes. Sokolova *et al.* [35] discovered that exosomes which were gained through ultracentrifugation can be stored under -20°C for 6 months without any cryoprotectant. The size and structure have no significant changes even after many freezing (-20°C)-thawing cycles.

3. Characteristics and Applications of NSCs

NSCs are primary progenitor cells of nervous system in different development stages. They can renew by themselves and differentiated to neurons, astrocytes and oligodendrocytes. Therefore, NSCs play a vital role in the formation of central nervous system. NSCs which are separated from brain tissues or NSCs after pluripotent stem cell induced amplification are used to treat cerebral nerve injury [36] [37] [38] [39]. This mainly develops the role by mediating nerve regeneration and plasticity and weakening neuroinflammation [40] [41] [42]. Although NSCs transplantation therapy has extremely promising application prospects, their clinical applications are restricted by cell dedifferentiation, immunological rejection, stem cell tumorigenesis and low survival rate of stem cells. As a result, the substitutive therapy of NSCs-Exo attracts considerable attentions, which can be used to nerve injury and neurodegenerative disorders.

NSCs-Exo and other cells are mainly gained through 5 pathways: 1) supercentrifugation, including differential centrifugation, density gradient centrifugation combining with saccharose or iodixanol; 2) ultrafiltration and size exclusion chromatography; 3) polymer precipitation method using hydrophilic polymer, such as polyethylene glycol; 4) immunoaffinity capture chromatography; 5) microcontroller chip separation method [43] [44]. Currently, researchers usually adopt supercentrifugation.

According to clinipathologic analysis of many neurodegenerative disorders, exosomes can serve as media of disease development. It is reported that exosomes secreted by nerve cells mediate the development of diseases by delivering specific pathogenic particles, such as β -amyloid peptide and tau proteins in Alz-

heimer disease [45] [46] and α -synuclein in Parkinson's disease [47], from the primitive cells to other cells. On the other hand, exosomes also have some neuroprotective effect, promote peripheral nerve regeneration and repair nerve damages. Besides, NSCs-Exo carries and delivers bioactive RNA, proteins and lipids. They can not only participate in synaptic transmission and neuron depolarization [48] as well as information exchange between neurons and neuroglia [49], but also regulate the synapsis function and maintain neurovascular integrity and myelinogenesis [50]. Under this background, exosomes which are released by NSCs have attracted wide attentions.

NSCs-Exo has the following functions: 1) mediating biological effect. They regulate cell growth and apoptosis through the specific miRNA, having some neuroprotection. Based on sequencing, Stevanato *et al.* [51] found 113 miRNAs in exosomes which are secreted by human NSCs. Results show that hsa-miR-1246, hsa-miR-4488, hsa-miR-4508, hsa-miR-4492 and hsa-miR-4516 have relatively high expression levels and hsa-miR-1246 is a target miRNA [52] that plays an important role in regulating cell growth and apoptosis [53]. In addition, other studies have pointed out that the high expressions of miR-21a and miR-125b in NSCs-Exo can promote differentiation and regeneration of nerve cells [54] [55]; 2) Mediating communication between NSCs and microenvironment. Cossetti *et al.* [56] found in a study that exosomes mediate communication between NSCs in mice and microenvironment and proinflammatory factor activate gamma interferon signal transduction pathway in NSCs, so that specific components of the gamma interferon pathway act on the target cells through exosomes [56]; 3) Mediating virus invasion into cells through non-receptor modes. For example, exosomes which are excreted by NSCs are added in the coxsackie virus and Adenovirus Receptor (CAR) defect cells and Adenovirus type 5 (AD5) for co-culture, which can promote invasion of Adenovirus type 5 into the coxsackie virus and Adenovirus Receptor defect cells [57]; 4) Playing a role as independent metabolic units. Exosomes can change concentration of key nutrients in the microenvironment, thus influencing physiological functions of peripheral cells [18]; 5) Influencing the aging process. It is found in a study that hypothalamus NSCs in mice influence the aging process after birth by releasing exosomes which carry specific miRNA into Cerebrospinal Fluid [58]; 6) Influencing morphological formation of microglial cells. According to the latest study, NSCs-Exo in the subventricular zone of mice is rich in miR-9, miR-26 and miR-181. These miRNAs specific target microglial cells influence the morphology, functions and proliferation in nervous system after birth [59] [60]. Therefore, NSCs-Exo has considerable promising application prospects to treat nervous system disorders.

The NSCs-Exo is superior to NSCs in promoting tissue regeneration and repair [61]. Therefore, NSCs-Exo has been widely used to study treatment for multiple nerve diseases. Compared with NSCs transplantation therapy, treatment based on NSCs-Exo has following advantages: 1) exosomes can be delivered to different regions of brain through nasal inhalation [62]; 2) Exosomes

disassemble quickly after releasing drugs since they cannot duplicate *in vivo*. Hence, it is hardly to develop tumor cancerization after treatment with exosomes [63]; 3) Exosomes are vesicles which have extremely low possibility of small vascular obstruction after intravenous injection and penetrate the blood-brain barrier (BBB) [64] [65]; 4) Relatively low immunogenicity [65]. Obviously, it is feasible to use exosomes which are produced by large cell factories to treat diseases [66]. NSCs-Exo provides a cell-free therapy for nervous system disorders, without evident side-effects.

4. Applications of Exosomes in Cerebral Stroke Treatment

Stroke is one of major causes of deaths and disability. However, there's no drug that can promote neural functional recovery on the market yet. For most survivors from stroke, it takes a long rehabilitation process and long-term drug therapy. Hence, it is necessary to develop a substitutive therapy that can repair cerebral damages of patients with stroke. WEBB *et al.* [67] found that intravenous injection of NSCs-Exo into mice after thromboembolic stroke could promote repair of damaged cells, tissues and functions. NSCs-Exo promotes polarization of microglial cell into M2 phenotype, thus promoting clearance of debris and decrease chronic inflammation [68] [69]. Hence, NSCs-Exo can provide neuroprotection, relieve movement and memory disorder. Subsequently, WEBB *et al.* [70] continued to explore the therapeutic effect of NSCs-Exo in the pig stroke model. After treatment with intravenous injection of exosomes, they found that damaged tissues and functions of the pig stroke model are improved significantly, and the Alba integrity is maintained.

Xin *et al.* [71] found that intravenous infusion of NSCs-exosomes to patients with cerebral stroke can promote neurogenesis, neurite regeneration and angiogenesis. MSC-Exo can provide neuroprotective effect through multiple ways since it can penetrate through BBB, while most drugs cannot [72]. Zhang *et al.* [73] provided two-week treatment of MSC-Exo to rats in the middle cerebral artery occlusion model and found that neurological functions of the model animals were improved significantly and the modified neurological severity scores (mNss) was increased to some extent. After four-week treatment of MSC-Exo, the quantity of axons surrounding the Ischemic Boundary zone (IBz) was increased significantly and nerve regeneration signals could be detection/This proved that MSC-Exo could induce axon development [72]. Besides, some study found that MSC-Exo contains enkephalin with high activity and can decrease the newly synthesized β -amyloid beta protein content in cells after it invades into nerve cells [73]. Hence, it shows potentials in treatment of Alzheimer disease. These functions of MSC-Exo provide a new possibility to treat the neurodegenerative diseases.

BBB which has a high differential permeability strictly restricts the delivery of macromolecules and almost micromolecules from other organs to the brain [74]. With small volume and biocompatibility, exosomes can penetrate through BBB and has very high flexibility in treatment of central nervous system disorders. It can delivery proteins and RNA to brains through nasal inhalation, intravenous

injection, abdominal injection and intracranial injection [74] [75]. HANEY *et al.* [75] treated the Parkinson's disease in mice by nasal administration of catalase-loaded exosomes and results demonstrated that catalase-loaded exosomes weakened the neuritis of mice with Parkinson's disease as a "drug" and it had significant neuroprotective effect. Some study proved that intravenous injection of exosomes which carry with specific miRNA and siRNA can target at nerve cells in brain and deliver miRNA and siRNA to target cells to treat mice with Alzheimer's disease [75] [76]. Joshi [77] pointed out that NSCs-Exo can penetrate through BBB effectively as a biological nano-carrier. In a word, there are two methods applied to study the therapeutic effect of miRNA of exosomes in central nervous system disorders. One is direct nasal inhalation or injection of exosomes in which there are miRNA good for the treatment. The other can load miRNA which can regulate disease-related gene expressions or specific miRNA that can promote nerve regeneration into exosomes selectively, and then make an *in vivo* study.

To sum up, NSCs-Exo treatment for stroke has five characteristics. It has neuroprotection to improve behavior and activity, eliminate intracranial hemorrhage conversion of ischemic diseases, weaken cerebral injury volume and brain swelling, and promote functional recovery after injury. Although there are few studies on NSCs-Exo treatment for stroke, It still great potential.

5. Conclusion

Although there are few studies on MSC-Exo, it has shown strong potential in medical treatment and exciting therapeutic effects in some animal models. Exosomes are the ideal carrier of drugs or gene transmission. They can release mRNA and miRNA into cytoplasm directly through membrane fusion with target cells, or uptake bioactive substances through endocytosis of target cells. Alternatively, they can recognize specific receptors on the cell surface and regulate the microenvironment of the disease, thus changing the initiation and development of the disease. MSC-Exo is a relatively unique type of exosomes and has some characteristics of stem cells. These studies prove that MSC-Exo can repair damaged tissues, inhibit inflammatory responses and regulate the immune system. However, the influences of MSC-Exo on tumors are still controversial and have to be further studied. Compared with MSC, MSC-Exo has a smaller volume, higher structural stability, lower immunogenicity and zero infection risk [57] [58]. Hence, MSC-Exo is highly appreciated as a cell-free therapy in studies and clinical studies; it is expected to enter into clinical trial soon. However, the extraction process of secreted MSC-Exo is relatively complicated and the components wrapped by MSC-Exo are sensitive to the original cells and environmental conditions. For clinical use of MSC-Exo, the primary task might be to simplify and standardize the MSC-Exo extraction to get MSC-Exo with stable properties.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

MSC-Exo: mesenchymal stem cell derived exosomes

BBB: blood brain barrier

MSCs: mesenchymal stem cells

NSCs: neural stem cells