

Mesenchymal Stem Cell Exosomes as Nanotherapeutic Agents for Neurodegenerative Diseases

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Abstract. Neurodegenerative diseases are systemic diseases with high heterogeneity and complicated etiology dependent on proper interneuronal communication, resulting in severe syndromes including cognitive impairment and dementia. The blood-brain barrier (BBB) remains Central nervous system (CNS) therapeutic delivery, a significant challenge without effective vivo therapeutic methods in clinical practice. Mesenchymal stem cells (MSC) with multi-directional differentiation potential have the characteristics of low immunogenicity, strong proliferation ability, immune regulation, and multi-directional differentiation potential. The repair effects have been identified mediated by transplanted MSCs paracrine factors, including exosomes and nanometer-sized cell communication mediators, to reduce tissue injury and enhance repair, growth, and regeneration. MSC-derived exosomes have become an attractive vehicle by passing through the blood-brain barrier (BBB), delivering therapeutic agents targeting the brain for treating autoimmune and neurodegenerative diseases. Safeties, convenience, and the effectiveness of MSC-derived exosomes have been demonstrated mainly through mechanistic clinical and preclinical evidence of potential nanotherapeutic agents for further prevalent use. Thus, we want to investigate the clinical applications of MSC-derived exosomes to reveal their regenerative treatment capacity from direct and indirect neuron repairment effect, reduced neuroinflammation, and nanotherapeutic agent advantage. This paper discusses the potential and practicality of using this novel cell-free entity of mesenchymal stem cell derivatives such as exosomes in vivo administration as a therapeutic modality for treating degenerative disease and pathologies and innovation and emerging trends in the field.

Keywords: Exosomes; mesenchymal stem cell, regenerative medicine; drug delivery; blood-brain barrier; neurodegenerative disease; inflammation; immune system; neural cells.

1. Introduction

Neurodegenerative disease is a type of disease that causes dysfunction, damage of neurons and their accessory dendrites, axons, synapses, and glial cells. The primary manifestation is the gradual loss of neuron structure and function, which affects the patient's memory, movement, language, and intelligence. Different brain damage areas lead to different types of diseases, among which Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Huntington's disease are the four most common disease types severely impacting patients' life [1]. Traditional treatments for neurodegenerative diseases often have limitations and poor efficacy. It is necessary to explore innovative treatment methods. Stem cells are defined as a type of cell that can self-renew and differentiate into various cells, organs, and tissues. Currently, data from multiple clinical trials have shown that stem cell-based therapy, especially mesenchymal stem cells (MSCs), can improve the treatment effectiveness of a variety of neurodegenerative diseases through several mechanisms, including paracrine factors involving exosomes [2] and transplantation of MSCs. As MSCs' repairing and regenerative functions are primarily mediated by paracrine factors [3], exosomes are crucial messengers involved in multiple cell signaling transduction [4] with vitro multipotential capacities secretes immunomodulatory and regenerative bioactive factors, upregulated immunosuppressive cytokine indoleamine 2,3-dioxygenase, CD4+, CD25+, FOXP3+ regulatory T cells (Tregs) and down-regulated pro-inflammatory Th1 and Th17 cytokines including IL-6, IL-12p70, IL-17AF, and IL-22 [5]. Cell-derived vesicles as exosomes have become an attractive vehicle for targeting nanotherapeutic agents to the brain as passing through the blood-brain barrier (BBB). Compared to

MSCs, MSC-derived vesicles nanoparticles have lower immunogenicity and side effects, penetrate the blood-brain barrier targeting neurologic diseases, and avoid potential pulmonary embolism related to transplantation of MSCs [6]. These characters shed light on the potential clinical applications, especially in neurodegenerative diseases, including gradual freezing disease (amyotrophic lateral sclerosis), multiple sclerosis, spinocerebellar ataxia [1-5]. The patient's overall condition has significantly improved movement control, sensory information processing, memory, decision-making [3]. Compared with traditional drug treatment alone, patients who receive stem cell transplantation simultaneously during treatment have shown better recovery results in neurodegenerative diseases [6]. With the research progressing, stem cell-related regenerative treatments will bring new hope of recovery for patients with neurological diseases.

2. Mesenchymal Stem Cells Exosomes in Current Treatments

2.1 Direct Therapeutic Effect

2.1.1 Immunogenicity and Immune System Regulation

Mesenchymal stem cells (MSC) are a type of pluripotent stem cells belonging to the mesoderm, mainly found in connective tissue and interstitium of organs such as bone marrow, umbilical cord blood, umbilical cord tissue, placental tissue, adipose tissue [7-9]. They are non-hematopoietic stem cells with multi-directional differentiation potential, such as osteoblasts, chondrogenic and adipogenic cells, or non-mesenchymal series cells with unique cytokine secretion functions. In 2002, two teams respectively discovered that mesenchymal stem cells have the strong immunosuppressive ability with low immunogenicity without immune rejection even in cross-species [7]. Dr. LeBlanc published an article on the clinical study of MSCs in treating graft-versus-host disease (GVHD) in 2004 [7]. It reported the success of the haploidentical allogeneic incompatible mesenchymal stem cell transplantation for GVHD treatment [10]. This is the first clinical research article using the immunosuppressive ability of MSCs to exert therapeutic effects. The extensive immunosuppressive function is the unique function of MSCs, inhibiting the activation of a variety of immune cells, making it a great candidate for regulating immune inflammation. For Treg cells with similar functions, the scope of inhibition is limited to T lymphocytes [11]. Like MSC immune regulation function, MSC-derived exosomes inhibit the proliferation of T cells and their immune response through the interaction between cells, thus exerting the function of immune reconstruction. Low immunogenicity with the non-obvious surface antigen results in light allogeneic transplantation rejection and low matching requirements of mesenchymal stem cells [12]. The discovery of the immunosuppressive function of MSCs has also significantly promoted the clinical research of MSCs for the treatment of immunoreactive diseases as the US FDA has approved nearly 60 clinical trials in MSC treatment in autoimmune, inflammatory, neurological, orthopedic conditions, and traumatic injuries, including Crohn's disease, Multiple Sclerosis, Lupus, COPD, and stroke recovery, etc. [13]. According to preliminary clinical reports, mesenchymal stem cells have noticeable curative effects in treating these diseases [11-13].

2.1.2 The Paracrine Effect of MSC

MSC has been demonstrated to promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons through the Paracrine system [14]. Therapies will capitalize on the innate trophic support from MSCs or augmented growth factor support, such as delivering brain-derived neurotrophic factor or glial-derived neurotrophic factor into the brain to support injured neurons, using MSC exosomes as the delivery vehicles [15]. Clinically tested human MSCs are the most prolific producers for exosomes therapeutic in animal models of disease and exhibit immunosuppressive activity, one of the most commonly employed cell types, cell-based therapy for treating human diseases. Recently, several mechanisms have been identified regarding the therapeutic potential of MSCs, discovering new treatment methods with the exosomes and microvesicles packaging various molecules transfer [16]. The therapeutic potential of mesenchymal

stromal cells (MSCs) may be primarily mediated by paracrine factors contained in vesicles [17]. Extracellular vehicles (EVs) from many cell sources have now been recognized as essential messengers in intercellular communication via the transfer of bioactive lipids, proteins, and RNAs [16, 17]. The increasing pandemic diseases COVID-19 have stimulated MSC-derived exosome products as therapeutic agents in neuronal smell and taste loss, continuing to widen the clinical application of MSC exosomes immunomodulation [18]. Therefore, MSC-derived exosomes are promising neurodegenerative disease therapeutic agents.

2.1.3 Repair and Regeneration in Nerve

The therapeutic effect of MSC is mainly divided into two aspects: The directional differentiation of MSC to replace and repair damaged tissues [19]. MSCs have the potential to regenerate nerve tissue. The research directions are mainly concentrated in two areas: the first direction is nerve damage caused by severe trauma or ischemia; the second direction is neurological diseases such as multiple sclerosis, frostbite syndrome, ischemic stroke, Parkinson's disease, etc. [20-22]. By releasing cytokines and active molecules TGF- β , which regulates damage and repair processes, MSCs can regulate immune responses and protect neuronal structures [22]. In addition to the differentiation potential of MSCs, the structure and function of tissues and organs can be reconstructed without immune rejection in immune-related diseases and degenerative diseases [23]. With the feature of MSC low immunogenicity, allogeneic MSC transplantation does not cause obvious immune rejection. MSC does not express costimulatory molecules B7-1, B7-2, CD40, and CD40L related to recognizing human leukocyte antigen (HLA) and major histocompatibility conformant class II molecules (MHC-II), such as HLA-DR antigen [24]. It lacks the costimulatory channel in signal transduction that activates the immune response, immune tolerance, and low immunogenicity [25]. MSC also has significant immunoregulatory properties, which can inhibit the immune rejection induced by allogeneic cell or tissue transplantation, systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune reactions, so it has a good application prospect for the treatment of immune injury and degenerative neural diseases, brain damage, and repair of peripheral nerves. Preclinical studies have confirmed that MSCs can promote the recovery of nervous system function [25-28].

Exogenous infusion of MSCs can be recruited to the injury site and participate in the immune regulation of the injury microenvironment [26]. The tissue repair ability of MSCs depends on strong inflammatory stimuli, which promotes MSCs to secrete a large number of growth factors, immunoregulatory factors, chemokines and promote tissue repair [27]. MSCs release cytokines through paracrine methods and still have multi-directional differentiation potential after continuous subculture and cryopreservation [28]. They can be used as ideal seed cells to repair tissue and organ damage caused by aging and disease. It is a promising application Source of stem cells for disease treatment. However, a particular problem in MSC treatment is that after systemic transplantation, MSCs are quickly retained in the pulmonary vascular system due to their large size. Although less than 1% of MSCs can reach the target site, the therapeutic effect is often observed. In the present research, the therapeutic function of MSC is mainly attributed to the paracrine effect [28, 29]. Extracellular vehicles (EVs) and exosomes produced by orthotopic transplantation of cells in the body may be the mechanism by which stem cells promote tissue remodeling and regeneration at remote sites. Several recent studies have shown that MSC-derived exosomes can regulate the immune system through various mechanisms with therapeutic functions [30].

2.2 Indirect Therapeutic Effect

2.2.1 Exosomes

The primary cell regeneration function of MSCs mainly relies on proteological exosomes with biologically active morphology using miRNA, which participates in the exchange of signals between cells communicating in the cellular environment [30]. In recent years, there has been more and more research on the biological origin, biological function, and biological characteristics of exosomes

focusing on the diagnostic and therapeutic roles of different signal molecules in disease evolution through exosomes. Exosomes are an essential part of the transfer of material and information between cells, which transfer biological molecules such as lipids, carbohydrates, proteins, mRNA, miRNA, and DNA from one cell to another, thereby exchanging genetic information and reprogramming [30, 31]. The host cell communicates between cells. It is the carrier of information and material exchange between cells. In addition, studies have confirmed that exosomes usually also have the biological characteristics of the cells from which they originate. For example, stem cell exosomes also have the features of differentiation and regeneration, low immunogenicity, and high survival rate after transplantation [31].

Exosomes in cerebrospinal fluid (CSF) contributed to signal transduction between neural cells and hematopoietic cells. The association and regeneration of EVs have been reported in the peripheral nervous system and the central nervous system, significantly involved in the pathogenesis of neurodegenerative disease through EV-mediated communication between neural and glial cells. [32] have become potential carriers for neurodegenerative therapy due to their nano-size and flexibility enable them to cross major biological barriers, such as the blood-brain barrier (BBB), extensively. In contrast to liposome preparations, exosomes are naturally occurring secretory membrane vesicles with low toxicity [32]. From their ubiquity in the body, it can be inferred that they are well tolerated in the body. In addition, the inherent homing ability of exosomes suggests their potential utility in drug delivery. For example, exosomes derived from melanoma preferentially enter sentinel lymph nodes, and this homing ability can be used for targeted drug delivery [33].

2.2.2 Mechanism of MSC-derived exosomes

Recently, the mechanism of exosomes in MSCs is being widely concerned and studied. Its contents can participate in information transmission and signal transduction between cells, change cell or tissue metabolism in the body, affect the body's damage and repair, and participate in tumors. The exosomes of MSCs contain cytokines, growth factors, proteins, signal lipids, various mRNAs, and regulatory miRNAs [34]. In addition, the miRNA in the exosomes of mesenchymal stem cells can regulate gene expression. The ratio is higher than that of cells, such as miR-155, let-7f, miR-199a, miR-221, miR-125b-5p, and miR-22 and so on, enabling it to participate in a variety of physiological and pathological processes and play an intervening role in multiple clinical diseases [35, 36]. Due to its 40-150 nm small nanometer size and the characteristics of the outer layer lipid, it is easy to reach the injured site through the blood circulation after it is injected. At present, exosomes derived from MSCs have been used in many studies, such as animal models of organ damage, tumor suppression, and immune response regulation models [37].

Studies have shown that mesenchymal stem cell exosomes play an important role in resisting cardiomyocyte apoptosis, resisting cardiomyocyte damage, promoting neovascularization, and anti-inflammatory [38]. It can effectively prevent acute myocardial infarction, hypertension, heart failure, and cardiomyopathy. Intramyocardial delivery of MSCs exosomes can reduce the area of myocardial infarction and effectively improve the survival rate of myocardial cells. Exosomes of induced pluripotent stem (iPS) cells can transmit protective signals to cardiomyocytes when they are affected by acute myocardial ischemia (MIR) [39, 40]. In addition, mesenchymal stem cell exosomes have also been applied to clinical treatment research for diseases including new coronary pneumonia, Alzheimer's disease, and endometrial injury [40].

2.2.3 Exosomes in Neurodegenerative disease

Alvarez-Erviti et al. found that DC-derived exosomes can deliver siRNA to the brain of mice and inferred that drugs or siRNA loaded with exosomes can pass through the BBB. They first transduced DC to express the exosome membrane protein LAMP2B, which can be fused with neuron-specific RVG peptides [41]. The purified DC exosomes contained exogenous siRNA targeting BACE1, a crucial regulatory gene in the pathogenesis of Alzheimer's disease; these exosomes were injected intravenously into mice [42]. These exosomes specifically enter the neurons, microglia, and oligodendrocytes in the brain, leading to the knockdown of the BACE1 gene in the mouse model.

This study proves the feasibility of specific systemic delivery of exosomes. More importantly, the study shows that exosomes can pass through biological barriers, indicating the possibility of RNAi-based treatment of new brain tumors and brain metastases [41-43].

Injecting MSC-derived exosomes secreted into TBI rats intravenously observed that the exosomes secreted by MSCs could reduce the activation of astrocytes and microglia in the brain of TBI rats. Which represents the ability to inhibit inflammation and promote the recovery of nerve function [43]. A significant obstacle in treating neuroinflammatory diseases is no effective carrier to transport drugs across the blood-brain barrier. Exosomes can become ideal drug delivery vehicles of neuroinflammatory illnesses due to low immunity, innate stability, high delivery efficiency, and crossing the blood-brain barrier. The research results show that encapsulating curcumin into exosomes can increase curcumin's solubility, stability, and bioavailability compared with curcumin encapsulated in liposomes alone [44].

Zhang's research showed exosome-encapsulated curcumin intranasally to interfere with lipopolysaccharide-induced rat brain inflammation model and myelin oligodendrocyte glycoprotein-induced rat experimental autoimmune brain inflammation. The results showed that curcumin encapsulated by exosomes could also be rapidly transported to the rat brain by nasal administration and induced apoptosis of activated microglia, delaying the progress of experimental autoimmune encephalitis in rats [44, 45]. This also provides a new idea for the medication and treatment of inflammatory diseases of the nervous system. In addition to delivering drugs, exosomes are also used to load small interfering RNAs to target specific genes in the brain. Alvarez-Erviti et al. confirmed that exosomes secreted by dendritic cells could transfer small interfering RNA to inhibit target genes in brain neurons, microglia, and oligodendrocytes [45]. On the other hand, exosomes encapsulate μ receptor small interfering RNA technology to improve the symptoms of morphine addiction in mice by inhibiting the levels of μ receptor mRNA and protein in the mouse brain. The study of exosomes as biomarkers and future therapeutic targets will also provide a new perspective for further understanding of the occurrence and development of inflammatory diseases of the nervous system [46].

3. Exosomes as Novel Drug Delivery Method

Exosomes are naturally suitable for transporting proteins, mRNA, miRNA, various non-coding RNAs, mitochondrial DNA, and genomic DNA, making them useful for delivering interfering RNA and other therapeutic substances such as small lipophilic molecules [47]. Anti-inflammatory agents' curcumin, anti-cancer agents' doxorubicin, and paclitaxel are loaded into exosome vesicles to treat related diseases. Exosome-based drug delivery systems have significant advantages in treating neurodegenerative diseases due to their endogenous nature, minimizing immunogenicity and toxicity [48]. For example, some studies have shown that the therapeutic efficacy of doxorubicin-loaded into exosomes is greatly enhanced compared with other delivery systems. The adverse effects on major organ systems, especially the heart and brain, are significantly reduced.

These MSC-derived exosomes can also effectively promote the healing of fractures, protect and repair kidney damage, treat myocardial weakness and hind limb ischemia, and regulate and promote muscle regeneration. In animal experiments, the application of MSC exosomes has played a significant role in treating ovarian cell senescence [47, 48]. In vitro cell experiments have also verified that exosomes secreted by human umbilical cord mesenchymal stem cells can effectively inhibit the apoptosis of rat ovarian granulosa cells induced by cisplatin and improve the survival rate of the cells. [49] However, some problems still need to be solved if the exosomes are actually put into clinical use, including further improving the specificity of targeted delivery; determine the frequency of administration. It is necessary to understand the bioavailability and half-life of the drug encapsulated in the exosomes; and the choice of the site of exosome administration; with the potential toxicity of exosome drugs to non-target effects [49, 50].

Unlike traditional stem cell therapy, exosomes are not living cells without the potential to develop into cancer cells after being injected into the injured part of the body. Therefore, exosomes may be

rapidly gaining attention as a new strategy to replace stem cell therapy, and they can overcome many risks and difficulties in cell therapy [50].

4. Summary

MSC-derived exosomes have been confirmed and have immeasurable application value in the field of modern medicine. Mesenchymal stem cells exert their therapeutic effects through communicating secretion factors and supporting matrix function by stable and balanced tissue microenvironment enable cells in the tissue to recover, repair, and regenerate. MSC exosomes Compared with stem cells, stem cell exosomes are more flexible and easier to modify with the versatility to interact with multiple cell types in nearby and targeted areas to trigger appropriate cellular responses. Therefore, stem cell exosomes will undoubtedly become one of the promising applications and research directions in the era of cell therapy. However, the exact regenerative mechanism of exogenous exosomes in vivo action, biodistribution, and targeted delivery has not been fully elucidated. There are still many works waiting for us to complete on the road of future research, including further clarifying the signal molecules related to stem cell proliferation, differentiation, and integration; exploring the internal environment in the host brain that is most suitable for transplanted stem cells to survive, proliferate and repair the damage, and so on. New technologies and innovations may help fill this knowledge gap and further promote the therapeutic clinical application of exosome-based regenerative therapies.

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