

Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy

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Abstract

Mesenchymal stromal/stem cells (MSCs) are involved in tissue homeostasis through direct cell-to-cell interaction, as well as secretion of soluble factors. Exosomes are the sort of soluble biological mediators that obtained from MSCs cultured media *in vitro*. MSC-derived exosomes (MSC-DEs) which produced under physiological or pathological conditions are central mediators of intercellular communications by conveying proteins, lipids, mRNAs, siRNA, ribosomal RNAs and miRNAs to the neighbor or distant cells. MSC-DEs have been tested in various disease models, and the results have revealed that their functions are similar to those of MSCs. They have the supportive functions in organisms such as repairing tissue damages, suppressing inflammatory responses, and modulating the immune system. MSC-DEs are of great interest in the scope of regenerative medicine because of their unique capacity to the regeneration of the damaged tissues, and the present paper aims to introduce MSC-DEs as a novel hope in cell-free therapy.

Introduction

Mesenchymal stromal/stem cells (MSCs) as non-hematopoietic stem cells resided in the stroma of the bone marrow^{1,2} and comprise 0.001%–0.01% of the total nucleated bone marrow cells.^{3,4} Although MSCs are isolated from human adipose tissue, liver, spleen, thymus, umbilical cord blood, placenta, Wharton's jelly, brain, lung, dental pulp, palatine tonsils, peripheral blood and other sources,⁵⁻¹¹ but they are mainly present in the bone marrow.^{12,13}

MSCs don't have identical markers due to species diversity, various tissue sources and culture conditions probably.¹⁴ MSCs derived from different sources are similar in phenotype, but are different in functions.⁸ The international society for cellular therapy (ISCT) has suggested some criteria for characterizing human MSCs which summarized in 1) Plastic adherence property in standard culture conditions, 2) Expression of CD105, CD90, and CD73, and lack expression of CD34, CD45, CD14 or CD11b, CD79a or CD19 and HLA-DR markers and 3) Differentiation potency to adipocytes, chondroblasts, and osteoblasts *in vitro*.¹⁵

MSCs exert their roles in the bone marrow via direct cell-to-cell cross-talk as well as secretion of broad-spectrum soluble factors.¹⁶ MSCs can migrate to injured tissues and because of their differentiation capability into various cell lineages and through secretion of soluble molecules can regenerate those injured tissues.^{17,18} MSCs augment angiogenesis and inhibit fibrosis via angiogenic and antifibrotic factors, respectively.¹⁹ In addition to,

MSCs have neuroprotective and immunosuppression effects.^{7,20-24}

MSCs which recognized as main components of stromal cell niches support hematopoietic stem cells (HSCs) homing, proliferation, self-renewal, and differentiation in the bone marrow.^{16,25-30} In addition, MSCs suppress HSCs apoptosis.^{29,31} Major soluble mediators which produced by MSCs contain cytokines, various growth factors, microRNAs, and exosomes which may affect the differentiation capacities of MSCs and promote tissue repairs.^{19,32,33}

The search for MSC-derived exosomes (MSC-DEs) is an attractive scope of the investigation because they are the paracrine effectors of MSCs and involved in cell-to-cell interactions. In this paper, we have summarized characteristics, properties, and isolation of the exosomes secreted by MSCs, also its applications in regenerative medicine as cell-free therapy.

Extracellular vesicles

Extracellular vesicles (EVs) are a general term for different types of membranous components in the 20–1000 nm diameter which released by various cell types in cultured media include stem cells, B and T lymphocytes, dendritic cells, mast cells, adipocytes, neurons, platelets, endothelial and epithelial cells.³⁴⁻³⁶ In addition, EVs isolated from many body fluids such as urine, serum, amniotic fluid, saliva, cerebrospinal fluid, breast milk, and nasal secretions.³⁷⁻⁴¹ Cancer cells secrete exosomes

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in greater levels than normal cells that play a vital role in diagnosis, development, and treatment of some cancers.^{40,42-44}

According to the size and source of production, EVs are divided into three general types: 1) Ectosomes or microvesicles with 200–1000 nm that derives from the plasma membrane, 2) Exosomes (40–100 nm) that originate from the inside budding of the late endosomal membrane, and 3) Apoptotic bodies with 50-500 nm that release from apoptotic cells.^{34,45-48} Among all of them, exosomes have attracted much interest in the last decades.

Exosomes

Exosomes have 1.10-1.21 g/mL flotation density in a sucrose gradient that can be precipitated by centrifugation at 100,000 ×g force.^{34,49} Because of endosomal origin of the exosomes, all of them include membrane-associated proteins, such as tetraspanins (e.g. CD9, CD63, CD81 and CD82), MHC-I and MHC-II, heat-shock proteins (e.g. Hspa8, Hsp60, Hsp70, Hsp90), GTPases (EEF1A1, EEF2) and proteins involved in multivesicular body biogenesis (Alix and TSG101).^{35,37,49,50} Moreover, metabolic enzymes (e.g. GAPDH, LDHA, PGK1, aldolase, PKM), cytoskeletal proteins (e.g. actin, moesin, syntenin), and the carrier proteins such as albumin were identified in exosomes.⁴⁹

The certain protein components rely on the origin of exosomes and may undulate according to physiological variations. Apart from the common surface markers of exosomes (CD9 and CD81), MSC-DEs express several molecules of MSCs, such as CD29, CD44, CD90 and CD73.^{51,52}

In addition to proteins, exosomes are enriched with a collection of cytokines, certain lipid rafts such as phosphoglycerides, cholesterol, ceramide, fatty-acyl chains as well as mRNAs, miRNAs, non-coding RNAs, tRNAs, rRNAs and rarely DNA.^{35,53-55} The comprehensive content of exosomes is accessible freely online at <http://exocarta.org> as well as <http://microvesicles.org> (vesiclepedia) databases.

Exosomes originate from the fusion of multivesicular bodies with the plasma membrane. This discharge relies on different chemical, environmental and mechanical stimulants, such as gamma-irradiation, calcium ionophores, heparanase, statins, hypoxia (low O₂), acidosis conditions and matrix detachment that all of them increased exosome secretions.^{35,53,56} Besides, cross-link activation of TCR/CD3 in T lymphocytes,⁵⁷ low O₂ in placental MSC culture media,⁵⁸ K⁺-dependent depolarization of neural cells⁵⁹ causes the induction of exosome secretions.

Isolation and Storage of Exosomes

The basic and common method for exosome isolation and purification from cell culture supernatants and in different biological fluids is ultracentrifugation that is often combined with sucrose density gradients.³⁶ Consecutive centrifuge forces cause to cells and larger

particle's removal and exosomes precipitation by centrifugation at least 100,000 ×g force.⁶⁰ Other procedures for exosome isolations include high-performance liquid chromatography (HPLC), ultrafiltration, exosome precipitation by volume excluding polymers, e.g. Polyethylene glycols (PEGs), affinity purification with specific antibodies against CD9, CD63, CD81, and CD82.^{60,61} In addition, today exosome isolation kits are commercially available that are based on efficient techniques and provide convenient separation. Conditions with low PH increase isolation and existence of exosomes.⁶² After separation, exosome identities are determined by at least two of these methods include atomic force microscopy (AFM), scanning electron microscopy (SEM), dynamic light scattering (DLS), flow cytometry (FCM), western blotting, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) or ELISA.⁶³⁻⁶⁵

After the isolation and characterization, exosomes for *in vivo* or *in vitro* applications must be frozen because they are unstable at room temperature and 37 °C. Exosomes can be stored for 6 months at -20 °C without cryopreservative agents.⁶⁶ Sokolova et al. have examined the stability of exosomes during storage at -20 °C, 4 °C and 37 °C. They reported that at 4 °C and 37 °C the size of the exosomes decreased and also degradations or structural changes occur. Several freeze and thawing cycles (up to -20 °C) and ultracentrifugation did not change the size of exosomes.⁶⁵ Hence, -20 °C or lower temperatures are suitable for exosome storage without changes in the size and structure of the exosomes.

Therapeutic effects of MSC-Derived Exosomes

Mesenchymal stem cells improve repair of injured tissues, also modulation of immune responses. These effects of mesenchymal stem cells are widely mediated by differentiations of MSCs, paracrine signals, and several secreted molecules such as microvesicles.^{67,68} MSC-DEs investigated largely in many activities of these cells and its effects on other cells. These exosomes probably to participate in many physiological and pathological processes because they carry trophic factors, which can be delivered to recipient cells.^{35,69} Therefore, the isolation and identification of exosomes from MSCs cultured media have made them a popular choice for cell-free therapy in research and clinical trials that could have clinical applications in the near future.

The intravenous injection of exosomes secreted from the human umbilical cord-MSC (huc-MSC) is tolerable in animal models because they had supportive effects on weight loss and had no harmful effects on renal or liver function.⁷⁰ MSC-DEs through recovery, repair, and regeneration of the tissue play an important role in maintaining tissue homeostasis⁷¹ and have cardioprotective effects through exciting proliferation, apoptosis prevention, angiogenesis induction, and oxidative stress suppression.⁴ These exosomes (MSC-DEs) also have anti-apoptosis and anti-inflammatory effects, anti-cardiac remodeling, cardiac regeneration,

neovascularization and anti-vascular remodeling effects in cardiovascular system.⁷² MSC-EVs keep cardiac tissue from ischemic injury through angiogenesis-promoting effects.⁷³ In addition, MSC-derived exosomes decrease myocardial ischemia/reperfusion (MI/R) injury in mouse models.^{74,75}

MSC-derived exosomes by activation of PI3K/Akt pathway, increase in ATP levels, reduce oxidative stress promote the myocardial viability and cardiac function MI/R injury; therefore, MSC-DEs can be a potential adjuvant for reperfusion.⁷⁶

The exosomes derived from BM-MSC keeps kidney against ischemia reperfusion damages with diminished inflammatory responses and apoptosis in rats.⁵¹ In addition, exosomes increase renal epithelial cell proliferation *in vitro*.⁷⁷ It has proved that in the mouse models BM-MSC-derived exosomes keep the intestines from necrotizing enterocolitis (NEC).⁷⁸

MicroRNAs are a type of small non-coding RNAs (~18-24 nucleotides) which regulate proliferation, differentiation, development, and cell death.⁷⁹ Due to exosomes enriched with microRNAs,^{53,80} probably play a crucial role in cellular functions such as tissue homeostasis and hematopoiesis. Several miRNAs in adult MSC-derived exosomes, including *miR-191*, *miR-222*, *miR-21*, and *let-7a* adjust cell proliferation, *miR-222*, *miR-21*, and *let-7f* induce angiogenesis and *miR-6087* causes promotion of endothelial cell differentiation.⁸¹

MSC-derived exosomes accelerate muscle regeneration via promoting myogenesis and angiogenesis, which mediated by miRNAs (e.g. *miR-494*) to be dependent on the effect of cytokines present in exosomes.⁸² One study at 2013 reported that MSC-DEs due to enriched with *miR-16*, suppress tumor progression and angiogenesis via down-regulation of the expression of vascular endothelial growth factor (VEGF) in tumor cells⁸³ and another study at 2012 was reported that MSC-DEs promoted tumor growth in *in vivo* through the increasing VEGF expression by activating extracellular signal-regulated kinase1/2 (ERK1/2) pathway in tumor cells.⁸⁴

Xin et al. showed that intravenous infusion of MSC-derived exosomes after stroke improves neurogenesis, neurite remodeling and angiogenesis.⁸⁵ Exosomes have multimodal neuroprotective effects because they can pass over the blood-brain barrier in spite of most drugs.⁸⁶ In addition, MSC-exosomes induce axonal development,⁸⁷ so this can make a new window in treatment of the neurodegenerative disorders.

Exosomes derived from huc-MSC have the immunomodulatory effects through an increase in the percentage of T-regulatory cells (CD4+ CD25+ FoxP3+) and dissuasion the proliferation of T CD4+ and T CD8+ cells.⁸⁸ MSC-DEs improve the survival of allogenic skin graft in mice and delay the occurrence of GVHD for two days by the shift of activated T CD4+ cells to T-regulatory cells.²⁴

Conclusions and Future Directions

There have been some attracting therapeutic effects of MSC-derived exosomes in various animal models. Exosomes are ideal vehicles for drug or gene delivery because they enriched with trophic factors. Over the past decades, some studies have been conducted on MSC-DEs showed that exosomes have the capacity in repairing tissue damages, suppressing inflammatory responses, and modulating the immune system but their effects on tumor progression remain controversial and require further studies. Exosome secretions and also its compositions rely on types of sources and environmental conditions; therefore, optimization of the exosome collection procedures from various sources of MSCs gives promising confidence to establish cell-free therapy based exosomes in future. MSCs have immunosuppressive capacities through inhibition of proliferation and maturation of most immune cells, also increasing regulatory T-cells.^{7,89} In addition, MSCs are good vectors for *Mycoplasma hyorhinis* infection that had anti-proliferative effects on lymphocytes and MSCs; also, it increases the risk of graft versus host disease (GVHD) in hematopoietic stem cell transplantation (HSCT).⁹⁰ MSC-DEs have low immunogenicity capacity rather than MSCs^{66,91} and have no infection risks. In addition, there is the risk of ectopic differentiation of MSCs after systemic infusion;⁹² hence, exosome-based therapy can be a good replacement for mesenchymal stem cell-based therapy soon. Understanding the exact cellular and molecular mechanisms involved in the effect of MSC-DEs on tissue regeneration requires further investigations.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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