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Mesenchymal Stem Cell-derived Exosomes: Applications in Cell-free Therapy

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중간엽줄기세포유래 엑소좀: 비세포치료제로서의 활용

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Mesenchymal stem cells (MSCs) are an attractive resource for refractory patients because of their anti-inflammatory/immunomodulatory capability and multi-lineage differentiation potential. The transplantation of MSCs has led to positive results in preclinical and clinical application to various diseases, including autoimmune disease, cardiovascular disease, cancer, liver cirrhosis, and ischemic stroke. On the other hand, studies have shown that paracrine factors, not direct cell replacement for damaged cells or tissue, are the main contributors in MSC-based therapy. More recently, evidence has indicated that MSC-derived exosomes play crucial roles in regulating the paracrine factors that can mediate tissue regeneration via transferring nucleic acids, proteins, and lipids to the local microenvironment and cell-to-cell communication. The use of these exosomes is likely to be beneficial for the therapeutic application of MSCs because their use can avoid harmful effects, such as tumor formation involved in cell transplantation. Therefore, therapeutic applications using MSC-derived exosomes might be safe and efficient strategies for regenerative medicine and tissue engineering. This review summarizes the recent advances and provides a comprehensive understanding of the role of MSC-derived exosomes as a therapeutic agent.

Key words: Exosome, Mesenchymal stem cell, Stem cell, Therapy

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INTRODUCTION

Mesenchymal stem cells (MSCs) are one of the most extensively employed somatic stem cells for cell-based therapy because of their excellent immunomodulatory and multi-lineage properties [1, 2]. Approximately 800 clinical trials utilizing MSCs, listed at www.clinicaltrials.gov, support that MSCs are a very promising cell source for the treatment of various human diseases. Over the last decade, the potential of MSC-based cell therapy has been demonstrated by its successful application in clinical trials for a wide variety of diseases [3]. However, the precise therapeutic mechanisms of MSCs in human patients have not been clearly elucidated. Accumulative evidence has shown that MSCs exert therapeutic impact through paracrine-mediated effects but not direct cell replacement [4]. Previous studies have also demonstrated that transplanted MSCs have very low survival rates, indicating

that their mechanisms of action are not through the MSCs themselves [5].

More recently, MSC-derived exosomes have been described as essential mediators in the therapeutic functions of MSCs [6]. Several studies have revealed that exosomes secreted by MSCs perform diverse functions, and they have been successfully applied in graft-versus-host disease, allogenic skin grafts, and myocardial ischemia/reperfusion injury [7-9]. Although the exact mechanisms of action of exosomes are not fully understood, exosomes have been described as more stable and reservable cell-derived vesicles compared with cells. MSC-derived exosomes play a critical role in exerting beneficial effects because they pose no risk of immune rejection and aneuploidy [6]. For these reasons, exosomes may provide an ideal alternative therapy for diverse diseases.

Based on recent advances, the therapeutic mechanisms of MSCs can be divided into two types: 1) a paracrine factor-mediated mechanism involving cytokines and hormones and 2) an exosome-mediated mechanism containing RNA and other molecules. We herein summarize the therapeutic potential of MSC-mediated exosomes to better understand them as cell-free agents.
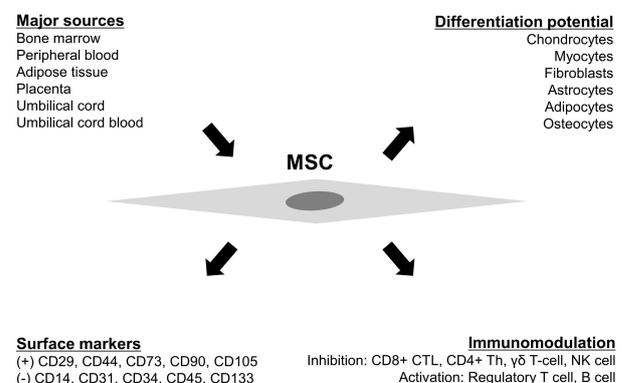
MAIN ISSUE

1. Mesenchymal stem cell

In 1966, MSCs were first reported by Friedenstein et al [10]. At the beginning, they were isolated from rat bone marrow, but since then, many investigators have isolated MSCs from various tissues, including amniotic fluid, cartilage, tonsil, synovial tissue, skin, tooth, peripheral blood, umbilical cord blood, liver, lung, spleen, adipose tissue, and placenta (Figure 1). It is easier to obtain MSCs from these sources, and cells with higher quality than those derived from bone marrow could be isolated [11, 12]. MSCs are generally positive for CD29 (β_1-integrin), CD44 (hyaluronate receptor), CD73 (SH-3), CD90 (Thy-1), and CD105 (SH-2), while it is negative for hematopoietic surface markers (CD14, CD34, CD45), CD133 (AC133), and CD31 (Figure 1) [13]. It was also reported that MSCs

express major histocompatibility complex (MHC) class I, whereas they do not express human leukocyte antigen class II antigens [14]. The described surface proteins could be expressed differently depending on tissue source, species, and method of isolation and cultivation. MSCs possess high proliferative and self-renewal capacity when maintained in classical culture conditions, but there are variations depending on their surroundings or cell donors, as MSCs are highly sensitive to external environments or stimuli, especially in *in vitro* culture.

MSCs with multi-differentiation potential are maintained in an undifferentiated state under proper culture conditions. However, MSCs could be easily differentiated into osteoblasts, adipocytes, and chondrocytes when placed under certain environments (Figure 1). Differentiation capacity is another index for the identification of MSCs in addition to morphological observation and surface phenotyping [15]. It has been previously reported that mesoderm-derived MSCs could be trans-differentiated into endodermal and ectodermal lineages, suggesting that

Major sources
Bone marrow
Peripheral blood
Adipose tissue
Placenta
Umbilical cord
Umbilical cord blood

Differentiation potential
Chondrocytes
Myocytes
Fibroblasts
Astrocytes
Adipocytes
Osteocytes

MSC

Surface markers
(+) CD29, CD44, CD73, CD90, CD105
(-) CD14, CD31, CD34, CD45, CD133

Immunomodulation
Inhibition: CD8+ CTL, CD4+ Th, γδ T-cell, NK cell
Activation: Regulatory T cell, B cell

Figure 1. Schematic representation of mesenchymal stem cell (MSC). MSCs can be obtained from various sources in the body, including bone marrow, peripheral blood, adipose tissue, placenta, umbilical cord, and umbilical cord blood. They have the ability to differentiate into myocytes, chondrocytes, fibroblasts, astrocytes, adipocytes, and osteocytes under specific *in vitro* conditions. In general, MSCs are positive for CD29 (β_1-integrin), CD44 (HCAM), CD73 (5'-nucleotidase), CD90 (Thy-1), and CD105 (endoglin), but negative for CD14, CD31 (PECAM-1), CD34, CD45, and CD133 (prominin-1). Soluble factors secreted from MSCs including transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF), nitric oxide (NO), prostaglandin E2 (PGE2), and indoleamine 2,3-dioxygenase (IDO) affect various subsets of lymphocytes including CD8+ cytotoxic T-lymphocytes (CTL), regulatory T-lymphocytes (Treg), B-lymphocytes, natural killer (NK) cells, gamma delta T-cells, and CD4+ helper T-lymphocytes (Th).

MSCs might be widely utilized and could improve the functions of damaged tissues or organs [16].

MSCs express a number of surface proteins but are negative for the co-stimulatory surface proteins CD40, CD80, CD86, and MHC II. The immunophenotypic characteristics of MSCs enable their successful transplantation because of the absence of immunorejection. MSCs can also modulate and inhibit the functions of immune cells such as dendritic cells and B cells (Figure 1) [17]. These features make MSCs an attractive candidate for cell-based therapy. Therefore, application of non-engineered MSCs to various diseases such as autoimmune disorders may potentially be suitable for enhanced treatment.

2. Clinical application of mesenchymal stem cells

Stem cell therapy is the medical treatment of patients with various disorders using autologous or allogeneic stem cells through local or systemic administration depending on the patient's conditions or damaged tissues. A number of experiments have been performed to verify the effects of infused MSCs using various animal models. From these studies, it has been proposed that primary MSCs can be effectively used as organ grafts in mice, while cultivated MSCs *in vitro* lost homing property easily because of the absence of adhesion molecules [18]. These results indicated that infused MSCs are difficult to engraft on host cells or tissues [19].

Nevertheless, other studies have demonstrated the beneficial therapeutic effects of MSCs since the first clinical trial was performed in 1995 [20]. Recently, data from clinical trials have shown numerous clinical applications of MSCs for various diseases, including acute myocardial ischemia, lung fibrosis, spinal cord injury, Alzheimer's disease, stroke, liver cirrhosis, and amyotrophic lateral sclerosis [21]. Previous studies have shown that hepatic regeneration was promoted in a carbon tetrachloride-injured liver model by human placenta-derived MSCs [22]. More recently, a meta-analysis of 950 patients demonstrated that MSCs can be beneficial in enhancing the heart function of patients with myocardial infarction [23]. These data support the use of MSCs in human applications.

Therapies using MSCs are novel emerging strategies that are undergoing rapid development, even though MSC-based therapies have adverse effects such as tumorigenicity and utilization of animal-derived reagents. Thus, researchers are aiming to find more optimal, advanced, and safe conditions or methods for MSC therapy in the future.

3. MSC-derived exosomes

Exosomes, one of several types of cell-derived vesicles, were first discovered in mammalian reticulocytes [24]. They are lipid bilayer vesicles that can be characterized based on their morphology (cup- or saucer-shaped) and size (30~100 nm) by electron microscopy [25]. Exosomes can be confirmed by surface markers such as CD9, CD63, CD81 of tetraspanins, heat-shock proteins such as HSP60, HSP70, HSP90, Alix, and tumor susceptibility gene 101, which distinguish them from microvesicles and apoptotic bodies of various extracellular vesicles [26]. Exosomes are secreted directly from the plasma membrane or from multi-vesicular bodies fused with the plasma membrane (Figure 2) [27] and can be isolated by various methods such as ultracentrifugation, chromatography, and commercial
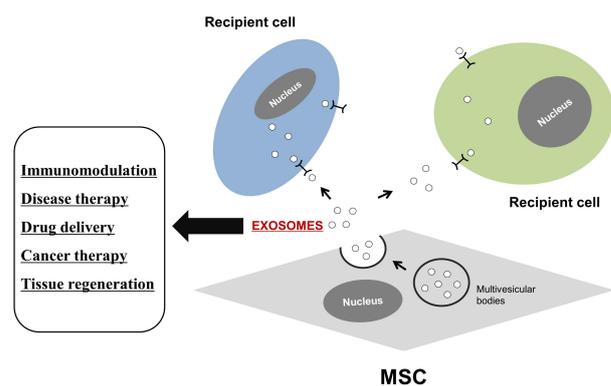
Figure 2. The biogenesis and function of MSC-derived exosomes. Secreted exosomes through fusion of multi-vesicular bodies with the cell membrane are mediators of paracrine effects. Exosomes can elicit cell-to-cell communication through intracellular uptake or membrane receptors. Exosomes, which are bilipid membrane vesicles with diverse proteins and RNAs, have the potential to exert various effects such as immunomodulation, disease therapy, and tissue repair in local recipient cells.

kits based on polymer precipitation. Purification by ultracentrifugation at 100,000×g is the most widely established method [25].

Exosomes released from various types of cells including stem cells, B cells, mast cells, platelets, cancer cells, dendritic cells, T cells, and Schwann cells as well as physiological fluids including media of cultivated cells, plasma, urine, and bronchial fluid can travel through biological fluids to diverse tissues or organs [28]. Although the exact functions of exosomes are not well known, increasing evidence has suggested that secreted exosomes can communicate with recipient cells in nearby or remote areas by signaling through transferring proteins, RNAs, and other molecules, resulting in the alteration of various signaling activities in affected cells (Figure 2). In this regard, there is growing interest in their use as a therapeutic target or diagnostic marker.

Recently, the use of MSC-derived exosomes has been suggested as a novel strategy in regenerative medicine and tissue engineering because there is no immune rejection and tumor formation based on MSC properties [29]. Furthermore, the use of exosomes is appropriate in terms of manufacturing as undifferentiated MSCs have a long lifespan compared to that of other types of cells [30]. Generally, MSC-derived exosomes carry complex biologically active molecules including proteins, mRNAs, and microRNAs. The exosomal contents are associated with diverse cellular and biochemical processes, such as cell-to-cell communication, tissue regeneration, metabolism, and immune modulation (Figure 2). Thus, MSC-derived exosomes have been considered as a promising tool to detect various molecules resulting from various cellular responses via interaction with diverse cell types. Moreover, MSC-derived exosomes play a crucial role in maintaining and restoring homeostasis within the body by mediating the function of MSCs. Particularly, exosomes have a key role in maintaining normal tissues when the homeostasis of the microenvironment is impaired by internal/external stimuli, injury, or disease [31].

4. Therapy using MSC-derived exosomes

Direct transplantation of viable MSCs poses risks such as tumor formation *in vivo*, resulting in adverse terminal outcomes. Transplantation of MSCs is relatively safe in terms of immune rejection, but the large size of MSCs has led to occlusion in the distal vasculature after intravascular administration [32]. Furthermore, some studies have reported potential risks of MSC transplantation due to calcification and/or ossification [33]. Exosomes can circumvent the risks involved in cell transplantation because cultured MSCs themselves are not used in clinical application.

Membrane molecules of exosomes such as lactadherin, transferrin receptors, tetraspanin proteins, tumor necrosis factor receptors, and integrins play key roles in the homing of exosomes to specific cells or tissues. In addition, the contents of exosomes, including RNA and proteins, can be protected from toxic chemicals or internal/external stimuli as exosomes bear a bilipid membrane [34, 35]. Exosomes containing biological molecules have the potential to participate in diverse biochemical and cellular interactions and alter the activities of cells via various signaling pathways.

Several studies have applied MSC-derived exosomes to reduce cardiac fibrosis in ischemic heart disease, inhibit cancer cell growth, and enhance functional recovery in stroke [36-38]. Amarnath et al [39] also reported that MSC-derived exosomes effectively inhibited graft-versus-host disease by repressing Th1 cells, which resulted in promoted adenosine-based immune suppression, suggesting that MSC-derived exosomes are an ideal therapeutic tool to treat a variety of conditions, especially untreatable diseases.

1) MSC-derived exosomes in autoimmune diseases

Exosomes derived from MSCs have been shown to mediate immune response in autoimmune models [40]. The immunological potential of exosomes affects certain processes in innate and adaptive immunity, including antigen presentation, immune modulation such as T cell

activation, and anti-inflammation, as demonstrated by several studies showing the crucial involvement of exosomes in immunoregulation [41]. Recent studies have reported that MSC-derived exosomes have potential novel therapeutic effects on autoimmune uveitis by inhibiting the migration of inflammatory cells [42]. Moreover, Stella Cosenza et al showed that MSC-derived exosomes are efficient in suppressing inflammation and exhibited an anti-inflammatory role by decreasing the percentage of CD4 and CD8 T cells and increasing regulatory T cell populations [43]. Therefore, it is suggested that MSC-derived exosomes could be used as new and safe therapeutic agents for a variety of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, type 1 diabetes mellitus, Hashimoto's thyroiditis, and Graves' disease.

2) MSC-derived exosomes in cardiovascular diseases

The favorable effects of exosomes have been demonstrated in animal models relevant to cardiac disorders in human. In an acute myocardial infarction model, exosomes improved cardiac repair by increasing cardiomyocyte growth, inhibiting fibrosis, and reducing apoptosis [44]. Studies have also shown that exosomes collected from MSCs significantly reduced infarct size and protected cardiac tissues from ischemic injury by enhancing blood vessel formation in a rat myocardial infarction model [45, 46]. Furthermore, Feng et al demonstrated that miRNAs such as miR-22 in exosomes secreted from MSCs played a role in reducing infarct size and cardiac fibrosis via anti-apoptotic effects in a myocardial infarction model [47]. Recent data have also demonstrated that MSC-derived exosomes markedly enhanced cell survival, improved cardiac protective effects, reduced cardiac fibrosis, and restored cardiac function after myocardial infarction in a rat model [48], proposing that exosomes derived from MSCs could be potential therapeutic agents for the treatment of cardiovascular diseases.

3) MSC-derived exosomes in liver diseases

The therapeutic effects of MSC-derived exosomes have been reported in preclinical data, demonstrating that the exosomes can be applied for regenerative and reparative medicine in liver diseases, including liver fibrosis and acute liver injury [49, 50]. MSC-derived exosomes were found to promote hepatic regeneration in drug-induced liver injury models and suppress hepatocellular carcinoma growth in a rat model [51, 52]. A recent study has also shown that MSC-derived exosomes containing miR-125b suppressed the activation of hedgehog signaling, which reduced fibrosis and suggested that microRNAs in exosomes released from MSCs contributed to liver regeneration [53]. In a mouse model of acute liver injury that investigated the functions of MSC-derived exosomes, the exosomes evoked hepatoprotective effects by promoting high cell viability and inducing an increase in hepatocyte proliferation [51]. The beneficial effects of exosomes led to high expression of anti-apoptotic and proliferation genes, which eventually upregulated genes and proteins involved in liver regeneration and reduced aspartate aminotransferase, alanine aminotransferase, and proinflammatory cytokines such as interleukin-6, interleukin-1β, and interferon-γ, tumor necrosis factor-α. These results imply that exosome-mediated therapy offers a novel strategy for treating diverse types of liver diseases.

CONCLUSION

Over the last decades, paracrine effects have been found to be the therapeutic mechanism of MSCs, but not cell replacement or differentiation in damaged cells or tissues. Exosomes secreted from MSCs are crucial mediators and messengers of paracrine signaling action and delivery. In other words, exosomes from MSCs have the potential ability to modify the function of damaged cells and tissues back to the normal state via signaling pathways based on the transfer of microRNAs, proteins, and mRNAs. One of the advantages exosome-based therapy is that cells themselves are not used, as large MSCs do not circulate easily, while small exosomes readily

circulate if a high dose is administered in the body. Although exosomes have many potential advantages as therapeutic or diagnostic targets in clinical or research settings as mentioned above, some limitations and disadvantages have also been reported. One of the major limitations is that there is no standardized method to characterize heterogeneous MSC-derived exosomes. For this reason, effective and standardized methods of isolating exosomes from different MSCs should be developed in the future. Recently, clinical trials using exosomes are under way, but issues on the effectiveness and safety of exosomes still exist. First, mass production of exosomes should be possible at good manufacturing practice grade for safe and efficient clinical applications. Moreover, efficient guided delivery of exosomes into targeted cells or tissues is critical for successful therapeutic efficacy. Understanding cell-to-cell communication via microvesicles including exosomes is also crucial to maximize the effects of exosome-mediated approaches in the field of stem cells and regenerative medicine. Accumulating technology and experience through clinical testing using MSC-based exosomes have advanced one step closer to more rapid development of stem cell-based therapies. In conclusion, the therapeutic potential of MSC-derived exosomes presents promising new stem cell-based cell-free agents for many intractable and rare diseases.

요 약

중간엽줄기세포는 항염증능, 면역조절능 뿐만 아니라 다계 통으로의 분화능 때문에 난치성 환자 치료를 위한 매력적인 대 안적 치료방법으로 알려져 왔다. 지금까지 중간엽줄기세포의 이식 치료법은 면역질환, 심혈관질환, 암, 간질환 및 뇌졸중을 비롯한 다양한 질병의 전임상 및 임상적용에 긍정적인 결과를 가져왔다. 여러 연구들에 의하면, 중간엽줄기세포를 이용한 치 료는 손상된 세포나 조직에 중간엽줄기세포가 이동하여 직접 세포를 대체하거나 분화시키는 작용이 아니라 중간엽줄기세포 에서 분비하는 여러 인자들 즉, 주변분비 효과(paracrine effect)에 의한 것으로 확인되고 있다. 최근에 중간엽줄기세포 유래 엑소좀은 핵산, 단백질, 지질 등을 손상된 세포나 조직의

국소 미세환경으로 전달함으로써 세포간 상호작용을 통해 조직 재생을 중재할 수 있는 중요한 역할을 하는 것으로 알려졌다. 엑 소좀의 이용은 세포이식으로부터 발생할 수 있는 종양형성과 같은 다양한 위험성을 피할 수 있으므로 줄기세포 기반 치료 적 용에 유용성이 매우 높다. 이러한 이유에서 중간엽줄기세포 유 래 엑소좀은 재생의학 및 조직공학에서 안전하고 효율적인 치 료적 도구(tool)가 될 수 있다. 여기에서 우리는 치료제로서의 중간엽줄기세포 유래 엑소좀의 정의와 역할에 대한 최신 지견 과 함께 포괄적인 이해를 제공하고자 한다.

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