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MSC-exosome: A novel cell-free therapy for cutaneous regeneration

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Abstract

Cutaneous regeneration is a dynamic and complex process that requires a series of coordinated interactions involving epidermal cells, dermal cells, growth factors, the extracellular matrix (ECM), nerves and blood vessels at a damaged site. Mesenchymal stromal cells (MSCs) have been reported to participate in all afore-mentioned stages. Exosomes are one of the key secretory products of MSCs, resembling the effect of parental MSCs. They can shuttle various proteins, messenger RNA (mRNA) and microRNAs (miRNAs) to modulate the activity of recipient cells, and play important roles in cutaneous wound healing. Compared with MSCs, exosomes are more convenient to store and transport. Moreover, they avoid many risks associated with cell transplantation. Therefore, MSC-exosome–mediated therapy may be more safe and efficient. In this review, we summarize the latest studies and observations on the role of MSC-exosome in the acute and chronic wound model and provide a comprehensive understanding of the role of exosomes in wound healing. This review can assist investigators in exploring new therapeutic strategies for enhancing the efficacy of MSC-exosome for cutaneous repair and regeneration.

Key Words: *angiogenesis, cutaneous regeneration, exosomes, mesenchymal stromal cell, wound healing*

Introduction

The skin is frequently damaged as a result of acute and chronic wounds such as extensive burns, trauma or diabetic ulcers. These risks not only destroy the barrier function of the skin but also alter the perceptions of temperature, pain and touch [1]. Patients with such cutaneous wounds experience physical and mental health problems, and these wounds also bring a huge socioeconomic burden [2]. Therefore, identifying an effective approach to accelerate cutaneous regeneration and restore the function of the damaged skin is urgently required. In recent years, mesenchymal stromal cells (MSCs) have gained much attention in cutaneous repair and regeneration.

MSCs are a population of undifferentiated adult stem cells that can self-renew and differentiate into multiple lineages [3–6]. MSC-based therapy has achieved positive effects in various animal models of diseases and several human clinical trials; MSCs have been clearly demonstrated to have favorable therapeutic effects in diseases, such as osteogenesis imperfecta [7], bone fracture [8], traumatic brain injury [9], stroke [10] and myocardial infarction [11,12]. Over the last decades, numerous basic and clinical studies have indicated that MSCs are a promising strategy for cutaneous regeneration because they are easily isolated, with low immunogenicity, and create a favorable environment for tissue regeneration [13]. Our previous studies have also shown that MSCs can home to injured tissues and undergo subsequent transdifferentiation to repair and replace damaged cells, thereby facilitating tissue repair [14,15]. In addition, MSCs have been reported to accelerate cutaneous regeneration by modulating the inflammatory response, promoting the formation of a well-vascularized granulation matrix, increasing the proliferation and migration of skin cells and inhibiting apoptosis [16]. However, emerging evidence has shown that transplanted MSCs have some risks. Vulliet *et al.* showed that intra-arterial injection of MSCs resulted in the occurrence of myocardial micro-infarction [17]. These

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results indicate that developing cell-free treatment with a similar action of MSCs is necessary.

Currently, it is believed that MSCs achieve a therapeutic effect *in vivo* mainly through paracrine signaling; MSCs can release biologically active molecules that affect the proliferation, migration and survival of the neighboring cells [18–20]. Several studies have reported that MSC-derived conditioned medium promotes cutaneous regeneration [21,22]. Moreover, Kim *et al.* showed that adipose-derived MSCs significantly promote wound re-epithelialization and reduce the wound size through paracrine effects *in vivo* [23]. Exosomes are the key bioactive vesicles responsible for the paracrine effects of MSCs; exosomes regulate many physiological and pathological processes by affecting the survival, proliferation, migration and gene expression of recipient cells and by reprograming targeted cell behaviors [24]. Exosomes derived from human umbilical cord MSCs (hucMSC-exosome) [25], human adipose MSCs (hASC-exosome) [26], and human induced pluripotent stem cell–derived MSCs (hiPS-MSC-exosome) [27] can facilitate cutaneous wound healing by delivering various functional proteins, RNAs and soluble cytokines [28–30]. Our recent results confirmed that HucMSC-exosomes enhance the wound healing of skin burn injury by delivering Wnt4 and 14-3-3ζ protein [25,31,32]. In this review, we discuss the roles of MSC-exosomes in cutaneous regeneration and summarize the underlying molecular mechanisms.

Biogenesis of exosomes

According to the size and source of extracellular vesicles (EVs), EVs can be divided into three types. Apoptotic bodies are the largest group of extracellular vesicles (≥1000 nm in diameter) that released by blebbing of plasma membranes of dying cells. However, microvesicles (100–1000 nm in diameter) are slightly larger extracellular vesicles generated by outward budding from the plasma membrane [33]. Exosomes (50–100 nm in diameter) are small membrane vesicles that originate from the inside budding of the late endosomal membrane. In addition, exosomes also have several unique characteristics: classic type of spherical or dish morphology, lipid bilayer, density of 1.13–1.19 g/mL and certain enriched protein markers (tetraspanins,TSG101, Hsp70) [34]. Exosomes are secreted by almost all of the living cells that have been examined so far, including normal epithelial cells [35], tumor cells [36], B cells [37], mast cells [38], dendritic cells [39] and T cells [40]. In addition, exosomes have been found widely present in a variety of body fluids such as blood [41], urine [42], saliva [43] and breast milk [44].

Isolation and characterization of exosomes

There are a number of methods for the identification of exosomes isolated from cell culture supernatant or biological fluids. The basic and most commonly used method for exosome isolation and purification is ultracentrifugation, combined with sucrose density gradients. Other procedures for exosome isolation include high-performance liquid chromatography (HPLC), ultrafiltration, the immune-bead isolation and many commercial kits [45].

According to exosomal biochemical properties (including size, morphology and protein markers), there are different ways that are frequently used to characterize the isolated exosomes. The morphology of the exosomes is usually identified using transmission electron microscopy (TEM) [46], scanning electron microscopy (SEM) [47] and immunoelectron microscopy (IME) [48]. In addition, atomic force microscopy (AFM) can enable us to clearly observe single three-dimensional structures of exosomes [49]. Nanoparticle tracking analysis (NTA) is used to detect the size distribution and counts of exosomes [50]. Enzyme-linked immunosorbent assay (ELISA) and Western blotting are convenient to analyze the protein contents of exosomes and molecular profiles, frequently used for detected exosomal biomarkers (including CD9, CD81, CD63, TSG101 and ALIX) [49,51].

Positive effects of MSC-exosomes in cutaneous regeneration

The cutaneous regeneration process can be summarized as three overlapping stages: inflammation phase, proliferation phase (cell proliferation and re-epithelization) and remodeling phase [52]. In this section, we briefly summarize the important roles of MSC-exosomes in each phase of the cutaneous regeneration process, generalize the current research achievements and discuss the underlying mechanism (Table I).

Mechanism of MSC-exosome behavior on immune-regulation of damaged tissues

Inflammation is a self-defense mechanism of the body in response to harmful stimuli, and inflammation aims to restore the homeostatic balance in organisms; the inflammation phase begins immediately when the body suffers damage or after pathogen invasion [53]. An acute and well-regulated inflammatory response is beneficial for normal wound healing [54]. By contrast, a chronic and inappropriate inflammatory response may lead to delayed wound healing, including fibrosis, excessive scar formation or re-epithelialization inhibition [55]. At 24–36 hours after skin injury, neutrophils, as the predominant cell type, are initially recruited to the