Chinese Medical Journal®

Free-cell therapeutics and mechanism of exosomes from adipose-derived stem cells in promoting wound healing: current understanding and future applications

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Adipose stem cells (ADSCs) have been reported to have multidirectional differentiation potential, and can promote cell proliferation and growth, and nerve regeneration function, which has become a promising treatment regimen for wound repair. Exosomes derived from ADSCs (ADSCs-Exos) have excellent tissue repair functions, including high vascular endothelial growth factor A, fibroblast growth factor 2, hepatocyte growth factor, platelet-derived growth factor subunit BB, and other important wound healing factors.[1] Supplementary Figure 1, http://links.lww.com/CM9/A828 is a simplified diagram of the exosome formation and release model.

Wound healing refers to a series of pathophysiological processes in which local tissues undergo regeneration, repair, and reconstruction after tissue loss caused by injury factors. It involves inflammation, angiogenesis, proliferation, tissue remodeling, and scar repair. In recent years, with the increasing research on ADSCs-Exos, their potential applications in promoting or supporting wound healing have become research hotspots. In contrast to traditional therapy, it achieves healing effect by activating the wound healing mechanism. Herein, we discuss in detail the roles of ADSC-Exos in various stages of wound healing [Supplementary Figure 2, http://links.lww.com/CM9/A829].

In the early stage of wound healing, inflammatory phenomena such as hyperemia, white blood cell infiltration, and local redness and swelling are observed. The anti-inflammatory function of ADSC-Exos is mainly related to its protein and RNA. In addition, macrophages play a crucial role in the inflammatory phase of wound healing. In both normal and diabetic mice, the accumulation of monocytes or macrophages in the wound could significantly accelerate the speed of wound healing. ADSC-Exos could be transferred into macrophages. Argininase-1 is activated by the active signal transducer and activator of transcription 3 (STAT3) carried by exosomes, inducing macrophages to polarize to the M2 phenotype, thereby reducing the inflammatory response. Li *et al*[2] reported the effect of ADSC-Exos overexpressing nuclear factor-E2-related factor 2 (Nrf2) for the treatment of diabetic rats, and found that ADSC-Exos overexpressing Nrf2 can significantly reduce the levels of inflammatory cytokines such as interleukin- 1β, interleukin-6, and tumor necrosis factor-α.

Angiogenesis is a key step in wound healing. It is a complex and highly regulated process involving the expression of various angiogenic factors. Yang *et al*[3] explored the effect of ADSC-Exos on cerebral vascular remodeling. The results of the capillary network formation experiment showed that exosomal microRNA-181b-5p upregulated vascular endothelial growth factor (VEGF), and accelerated the migration and formation rates of cerebral vascular endothelial cells after hypoxia and glucose treatments. Moreover, miR-21 overexpression of ADSC-Exos was found to significantly promote the angiogenesis of human umbilical vein endothelial cells. ADSC-Exos overexpressed by miR-21 could promote angiogenesis by activating the Akt, also called protein kinase B, and extra-cellular regulated protein kinases (ERK) pathways, and the expressions of hypoxia inducible factor-1α (HIF-1α) and stromal cell derived factor-1.[4] ADSC-Exos contain vascular growth factors such as thrombopoietin, milk fat globule epidermal growth factor 8, and angiopoietin-like protein 1. Matrix metalloproteinases carried by exosomes can promote the activation of

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001857

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Chinese Medical Journal 2022;135(15)

Received: 29-06-2021; **Online:** 30-05-2022 **Edited by:** Yuanyuan Ji

angiogenic factors, thus greatly increasing the activity of angiogenesis.[5] Lu *et al*[6] found that ADSC-Exos increased miR-486-5p level, which obviously promoted angiogenesis of human mammary epithelial cells. Notably, this effect was realized by miR-486-5p through its target gene *Sp5*. Xu *et al*[7] systematically studied the effect of microRNAs on angiogenesis. The results of real-time polymerase chain reaction showed that miR-423-5p and miR-21-5p had the maximum enrichment in ADSC-Exos. The authors also verified that miR-423-5p was the critical and most abundant miRNA in ADSC-Exos for promoting angiogenesis.

The proliferation and differentiation of fibroblasts mark the beginning of new tissue formation. Zhang *et al*[8] isolated normal human dermal fibroblasts and ADSC-Exos from patient dermal and adipose tissue. Real-time cell analysis, cell counting kit-8 analysis, and cell scraping experiments showed that proliferation and migration of fibroblasts treated with exosomes were significantly increased in a dose-dependent manner compared with untreated cells. After exosome stimulation, the mRNA and protein levels of type I collagen, type III collagen, transforming growth factor beta 1, and basic fibroblast growth factor in fibroblasts were increased. Notably, exogenous ADSC-Exos can promote collagen expression only in the early stage of wound repair, while in the later stage of wound repair, collagen production is inhibited.

The signaling pathway of wound healing is complex, and involves various overlapping stages of wound repair. Zhang *et al*[8] evaluated the effect of ADSC-Exos on wound repair using a mouse model of incision wound and found that ADSC-Exos could facilitate the proliferation and migration of human fibroblasts and increase collagen formation through the phosphatidylinositol 3-kinases/Akt (PI3K/Akt) signaling pathway. Growth factors such as PDGF, epidermal growth factor, TGF-β, VEGF, and insulin like growth factor-1 play a role by activating this signaling pathway. Ma *et al*[9] treated human immortalized keratinocytes (HaCaT) cells with hydrogen peroxide to study its therapeutic effect on skin diseases. The results showed that ADSC-Exos could reduce apoptosis by promoting the proliferation and migration of HaCaT cells. In addition, the experiment also showed that ADSC-Exos may promote skin wound healing by activating the Wnt/β-catenin signal pathway. Zhang *et al*[10] showed that ADSC-Exos can up-regulate AKT phosphorylation level and HIF-1α expression in HaCaT cells. ADSC-Exos treated with hypoxia can activate the protein kinase A signaling pathway and promote the expression of VEGF to accelerate wound healing. Studies have shown that ADSC-Exos significantly increased the expression of miR-19b in recipient cells, and miR-19b targets chemokine cc-motif ligand 1 to regulate the TGF-β pathway thereby accelerating skin wound healing.[11]

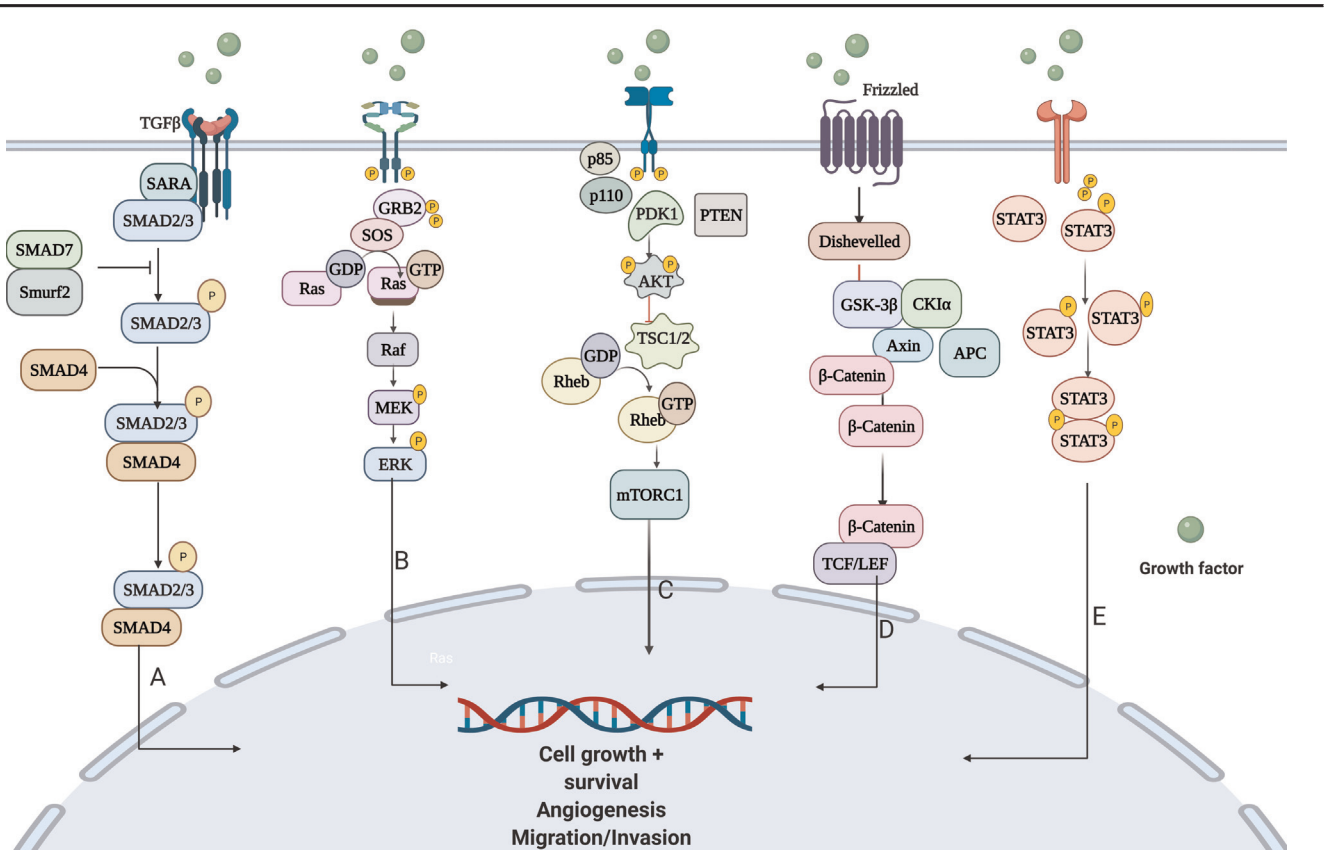

Figure 1: Signaling pathway diagram of ADSC-Exos promoting wound healing. A: TGF-β signaling pathway; B: ERK/MAPK signaling pathway; C: PI3K/Akt signaling pathway; D: Wnt/β-catenin signaling pathway; E: JAK-STAT signaling pathway. ADSC-Exos: exosomes derived from adipose stem cell; TGFβ: transforming growth factor beta; ERK: extracellular regulated protein kinases; MAPK: mitogen-activated protein kinases; PI3K: phosphatidylinositol 3-kinases; JAK: Janus kinase; STAT: signal transducer and activator of transcription; SARA: subacute ruminal acidosis; GRB: Growth factor receptor-bound protein; SOS: son of sevenless factor.

The signaling pathways of ADSC-Exos that are known to promote wound healing include TGF-β, ERK/mitogen-activated protein kinases (ERK/MAPK), PI3K/Akt, Wnt/β-catenin, Janus kinase-STAT (JAK-STAT), which are involved in regulating cell proliferation, differentiation, inflammation, and apoptosis. They are different but interrelated signaling pathways [Figure 1].

The TGF-β signaling pathway. The TGF-β family ligand dimer forms complexes with the corresponding type II and type I receptors on the membrane [Figure 1]. The type I receptor is phosphorylated by the type II receptor and activates its activity. Thereafter, the type I receptor recruits and activates the downstream Smad protein. Smad2/3 is phosphorylated and binds to Smad4, and after translocation into the nucleus, it acts as a nuclear factor to participate in the transcriptional regulation of target genes. The ERK/MAPK signaling pathway [Figure 1]. It involves the sequential activation of rat sarcoma, rapidly accelerated fibrosarcoma, mitogen-activated protein kinase, and ERK. Activated ERK further activates downstream pathways, and these kinases are activated sequentially to jointly regulate cell proliferation and differentiation, stress inflammation, and other multiple responses. The PI3K/Akt signaling pathway [Figure 1]. PI3K is a dimer composed of the regulatory subunit p85 and the catalytic subunit p110. When it binds to growth factor receptors (such as epidermal growth factor receptor), it activates Akt, which is phosphorylated and subsequently activates downstream mammalian target of rapamycin (mTOR) targets. Activated Rheb directly regulates mTORC1 and regulates cell proliferation, differentiation, apoptosis, and migration phenotypes. The Wnt/β-catenin signaling pathway [Figure 1]. When WNT binds to the membrane receptor Frizzled, the intra-cellular protein dishevelled is activated and stabilizes the free β-catenin protein in the cytoplasm. β-Catenin then enters the nucleus and binds to T-cell factor/lymphoid enhancing factor transcription factors, thereby activating downstream target gene transcription. The JAK-STAT signaling pathway [Figure 1]. Various cytokines/growth factors bind to receptors and can be phosphorylated to activate JAK, phosphorylate tyrosine residues of downstream target proteins, and recruit and phosphorylate transcription factor STAT, which then enters the nucleus as a dimer. STAT binds to target genes, regulates the transcription of downstream genes as well as cell proliferation, differentiation, and apoptosis. There are few studies on the mechanisms of ADSC-Exos in promoting wound healing, and extensive research is needed in the future.

Our future research will focus on using ADSC-Exos in combination with other biological materials to promote wound healing or use it after co-cultivation with other types of mesenchymal stem cell exosomes with the same function. Recent studies have shown that gene overexpression can also promote wound healing. A promising way to accelerate wound healing is to use clustered regularly interspaced short palindromic repeats gene editing technology. Implanting the edited gene after overexpression or structural modification into the human body, and interacting with exosomes may produce positive effects. In summary, the research on effects of ADSC-Exos on wound healing is in its infancy, and numerous experimental studies are needed to confirm and optimize this new cell-free treatment strategy.

Funding

This work was supported by the grants from the National Nature Science Foundation of China (Nos. 81873939 and 31970374).

Conflicts of interest

None.

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How to cite this article: Liu N, Xie Y, Zhen Y, Shang Y, Wang G, Zhu L, An Y. Free-cell therapeutics and mechanism of exosomes from adipose-derived stem cells in promoting wound healing: current understanding and future applications. Chin Med J 2022;135:1803–1805. doi: 10.1097/CM9.0000000000001857