Human bone marrow mesenchymal stem cells-derived exosomes stimulates cutaneous wound healing through TGF- β /Smad signaling

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Abstract

Background: Cutaneous wound healing represents a morphogenetic response to injury, and it designed to restore anatomic and physiological function. Human bone marrow mesenchymal stem cells-derived exosomes (hBM-MSCs-Ex) is a promising source for cell-free therapy and skin regeneration.

Methods: In this study, we investigated the therapeutic effects and underlying mechanism of hBM-MSCs-Ex on cutaneous wound healing in rats. We assessment of the role of hBM-MSCs-Ex in the two type of skin cell: human keratinocytes (HaCaT) and human dermal fibroblasts (HDFs). proliferation in vitro . Furthermore, we used a full-thickness skin wounds to evaluate the effects of hBM-MSCs-Ex on cutaneous wound healing in vivo.

Results: Our results demonstrated that hBM-MSCs-Ex promote both two type of skin cell growth effectively and accelerate the cutaneous wound healing ($p < 0.01$). Then, we found that hBM-MSCs-Ex significantly down-regulated TGF- β 1, Smad2, Smad3, and Smad4 expression, while up-regulated TGF- β 3 and Smad7 expression ($p < 0.05$).

Conclusions: In conclusion, our findings indicated that hBM-MSCs-Ex effectively promote the cutaneous wound healing through inhibiting the TGF- β /Smad signal pathway, providing a new sight for the therapeutic strategy of hBM-MSCs-Ex for the treatment of cutaneous wounds.

Introduction

Cutaneous wound healing is characterized by repairing of damaged tissue, and the tissue regeneration orchestrated by multiple cells to re-establish protective barrier[1]. The crucial goals of skin wounds treatment are rapid wound closure and scar-less healing. Previous studies have shown that mesenchymal stem cells (MSCs) capable of self-renewal and multipotential differentiation, which have great therapeutic potential for tissue regeneration and skin function recovery[2, 3]. However, MSC transplantation may induce little immunoreactivity to the host[4]. To avoid immunoreactivity issues, MSC-derived exosomes have received considerable attention for modulating wound repair[5–7]. Studied has shown that MSC-derived exosomes accelerated the wound healing through increased re-epithelialization, angiogenesis, and tissue granulation[6]. In our previously published research shown that hBM-MSCs-Ex treatment significantly reduced liver fibrosis in rats[8]. Therefore, we used the promising exosomes type, hBM-MSCs-Ex, to investigate the paracrine effect on wound healing process, and to investigate the associated signal pathway with this process.

TGF- β /Smad signal pathway is an evolutionarily conserved pathway with numerous functions ascribed[9]. Transforming growth factor-beta (TGF- β) is considered as one of the essential growth factors in wound healing[10]. Working through the Smad pathway, it is the main inducer of fibroblast differentiation which is essential for wound healing[11]. TGF- β activates downstream mediators Smad2 and Smad3 which result in the differentiation of fibroblasts into alpha smooth muscle expressing (α -SMA) myofibroblasts[12]. The phosphorylated Smad2/Smad3 activate Smad7 promoter to up-regulate

Smad7 expression, and then Smad7 inhibits TGF- β 1 expression for negative feedback regulation[13]. Increased studies have demonstrated that the TGF- β /Smad signal plays an important role in cutaneous wound healing via regulating the proliferation and migration of keratinocytes, dermal fibroblasts, and other skin cells which participate in wound healing process[14, 15]. Furthermore, TGF- β /Smad signaling can regulate the tissue fibrosis and scar formation[16]. In this study, we hypothesized that hBM-MSCs-Ex can promote cutaneous wound healing via regulating the TGF- β /Smad signaling pathway.

Materials And Methods

Cell culture

HaCaT and human dermal fibroblasts HDFs was purchased from Chinese Academy of Medical Sciences, China. Human bone marrow mesenchymal stem cells (hBM-MSCs) were generously provided by Dr. Yi Wang (Jilin University, Changchun, China), P3-5 lines of hBM-MSCs were used in the experiments. Cells cultured in DMEM (Gibco, Grand island, USA) supplemented with 10% FBS (Gibco, Grand island, USA), humidified 5% CO₂ atmosphere at 37 °C.

Cell Proliferation Assay

HaCaT and HDFs were cultured until cells grew to 70 ~ 80% confluence, then seeded in 96-well plates at a density of 4,000 cells per well. Then, we prepared hBM-MSCs-Ex which purification and characterization in our previous published paper[8]. Supplemented the cells with 100 μ L hBM-MSCs-Ex (25 μ g/mL) or PBS (Invitrogen, Shanghai, China), then incubated at 37 °C with 5% CO₂ for 5 days. The cell viability was examined by CCK-8 (Sigma, San Francisco, U.S.), and corresponding OD value measured at the 490 nm wavelength.

Immunofluorescence Staining (IF)

HaCaT and HDFs cultured by hBM-MSCs-Ex (25 μ g/mL) or PBS were incubated in 24-well plate for 24 h. When cells reached 60 ~ 70% confluence, incubated with 4% paraformaldehyde for 10 minutes, and incubated with 1% bovine serum albumin (BSA; Biosharp, Hefei, China) for 30 minutes. Then, cells were incubated with antibodies against PCNA (1:100 dilution, BD Biosciences, Franklin Lakes, NJ, U.S.), and isotype-matched rabbit or mouse IgG/IgM (1:100 dilution, Abcam, Cambridge, UK) served as the negative controls. Next, incubated with the secondary antibody (anti-rabbit IgG, 1:500 dilution, Abcam, Cambridge, UK) for 2 h, and the nuclei were labeled with DAPI (Thermo Scientific, Waltham, U.S.) for 5 min. Lastly, the intensity was examined by a fluorescence microscopy (EVOS, Thermo Scientific, Waltham, U.S.), and the PCNA positive cells were analyzed in ten random optical fields.

Animals And Treatments

The 8-week old, female Sprague-Dawley (200 g) rats were purchased from Jilin Biotechnology Co., Ltd. (Changchun, China). All animal experiments were performed in accordance with the guidelines of the Animal Experiment Ethic Committee of Jilin University. The animal model was made according to previously published methods[17]. Briefly, rats were anesthesia and shaved the dorsal hair. Then, we made one full-thickness skin excisional wounds of 10 mm in diameter circular holes in each rats. The rats were randomly divided into three groups (n = 8/group): PBS group; hBM-MSCs group (intravenous injection with 1×10^6 cells/ rat); hBM-MSCs-Ex group (250 mg, harvested from 1×10^6 hBM-MSCs). The skin damage was treatment and recorded photographically every four days. The wound area was calculated out using Adobe Photoshop CS6, lasso tool was used to trace and circle the edge of wound on photograph, then calculate the circled area based on the pixels of that area. Rats were euthanized on day 16 and collected the healed tissue.

Histological Examination

Skin tissue sections were cut at 4 μ m thickness and used for histological examination. Hematoxylin and eosin (H&E) staining was performed following the manufacturer's standardized protocols (Sigma, San Francisco, U.S.). Immunohistochemistry (IHC) was carried using the Kit (Maixin KIT-9710, Fuzhou, China) following the manufacturer's instructions. Briefly, the sections were deparaffinized, rehydrated and incubated in a 99 °C water bath for 15 min, 3% H_2O_2 was added for 15 min, and blocked with 10% normal goat serum for 1 h at 37 °C. Next, the sections were incubated with primary antibody anti- α -SMA with 1:500 dilution (Abcam, Cambridge, UK) overnight at 4 °C. Next, these sections were incubated with biotinylated goat-anti-rabbit IgG antibody for 2 h. Then, we used diaminobenzidine solution as the chromogenic agent at 37 °C for 15 min, and incubated with avidin peroxidase reagent sequentially. Lastly, we used hematoxylin was for counterstaining. These sections were photographed using a microscope (EVOS, Thermo Scientific, Waltham, U.S.).

Western Blot

Proteins were extracted from the skin healed tissue in the lysis buffer. The protein samples in SDS sample buffer were heated to 95 °C for 10 min, and separated on SDS-polyacrylamide gels. Resolved proteins were then electro blotted onto nitrocellulose membranes and probed with antibody against TGF- β 1, TGF- β 3, Smad2, Smad3, Smad4, Smad7 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 dilution, Abcam, Cambridge, UK) overnight at 4 °C. Then, the samples followed by secondary antibodies HRP-conjugated goat anti-rabbit IgG (1:1000 dilution, Abcam, Cambridge, UK), and visualized by chemiluminescent detection according to the manufacturer's instructions (Immobilon western chemiluminescent HRP substrate, Millipore).

Real-time PCR Assay

Total RNA from skin healed tissue was extracted with Trizol (Invitrogen, Shanghai, China) according to the manufacturer's protocol. SYBR Green I dye was used for reverse transcription in an ABI 7500 fluorescence quantitative PCR instrument, and the mRNA levels of TGF- β 1, TGF- β 3, Smad2, Smad3, Smad4, Smad7 and GAPDH were measured, and the primers were added in supplementary Table S1. The thermocycler conditions as follow: initial step at 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Expression levels were recorded as cycle threshold (Ct). Data were acquired using the 7500 Software (Applied Biosystems Life Technologies, Foster City, CA, U.S.). All reactions were performed in triplicate and the data were analyzed using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Statistical analysis was performed using Prism 6 (Graph Pad software) and Image J. One-way ANOVA with Dunnett's multiple comparisons test was used to test for statistically significant differences. All quantitative data were given as the mean $\pm$ SD. for at least three independent experiments, and $p < 0.05$ was considered to be statistically significant.

Results

Assessment of the role of hBM-MSCs-Ex in the two type of skin cell proliferation

In vitro, we investigated whether hBM-MSCs-Ex stimulate two type of skin cell proliferation, both HaCaT and HDFs proliferation was measured for 5-day. As shown in the Fig. 1a and 1b, the growth rate of hBM-MSCs-Ex treatment group significantly increased both in HaCaT and HDFs, compared of PBS group ($p < 0.05$ and $p < 0.01$). Based on the CCK-8 results, we conducted immunofluorescent staining analysis to confirm the proliferative role of the above results. As shown in Fig. 1c and 1d, the intensity of PCNA⁺ in hBM-MSCs-Ex treatment group was significantly higher than that of PBS group (28.2% vs 62.1% in HaCaT and 34.8% vs 71.3% in HDFs) both in the two type of skin cells ($p < 0.01$). In vitro experiment, the results demonstrated that hBM-MSCs-Ex promote both two type of skin cell (HaCaT and HDFs) growth effectively.

hBM-MSCs-Ex Promote Cutaneous Wound Healing In Vivo

We established a full-thickness skin wounds injury mode in rats to investigate the roles of hBM-MSCs-Ex in wound healing (Fig. 2a). As shown in Fig. 2b and 2c, the wound area of hBM-MSCs-Ex treatment group was significantly reduced from 4 to 16 days after surgery, compared with PBS and hBM-MSCs group ($p < 0.05$, $p < 0.01$). It indicating that hBM-MSCs-Ex treatment is not only much better than PBS group (negative control), but also better than hBM-MSCs group (positive control). The wound in hBM-MSCs-Ex group healed completely on the 16 ± 2.3 day after surgery. Taken together, the results showed that hBM-MSCs-Ex effectively accelerated the cutaneous wound healing.

Next, we assessed the effects of the hBM-MSCs-Ex on the wound healing quality. H&E staining results indicated that cutaneous appendages including hair follicles and sebaceous glands in hBM-MSCs-Ex group (21.3 ± 5.4 /filed) were much more than in PBS group (1.2 ± 2.8 /filed, $p < 0.001$) and hBM-MSCs group (15.4 ± 4.1 /filed, $p < 0.001$), as shown in Fig. 3a and 3b. α -SMA is an important indicator of angiogenesis. IHC results showed that the percentage of α -SMA positive area was significantly increased in hBM-MSCs-Ex group ($8.1\% \pm 1.2$), when compared with the PBS group ($1.3\% \pm 0.5$ / HPF, $p < 0.001$) as well as hBM-MSCs group $5.6\% \pm 0.9$, $p < 0.01$). It indicated that hBM-MSCs-Ex enhanced skin function and angiogenesis.

hBM-MSCs-Ex Regulate The TGF- β /Smad Signal Pathway

To investigate the underlying mechanism of the effect of hBM-MSCs-Ex and on skin healed tissue, the expression level of TGF- β 1, TGF- β 3, Smad2, Smad3, Smad4, and Smad7 (components of the TGF- β /Smad signaling pathway) were analyzed by Western blot and RT-qPCR. Both Western blot and RT-qPCR results showed the significantly decreased expression of TGF- β 1, Smad2, Smad3, and Smad4 in hBM-MSCs-Ex treatment group, compared the other two control groups ($p < 0.05$, $p < 0.01$), which illustrated the inhibition of TGF- β /Smad signaling in the presence of hBM-MSCs-Ex (Fig. 4a and 4b). To find how the TGF- β /Smad signaling was inhibited, we detected the Smad7 was significantly increased in hBM-MSCs-Ex treatment group, compared the other two control groups (Fig. 4a and 4b, $p < 0.05$, $p < 0.01$). Inhibitory Smad7, which are activated by the binding of the TGF- β super family to the cell surface receptors. TGF- β 1 is fibrotic isoform, while TGF- β 3 is the anti-fibrotic isoform. Interestingly, the hBM-MSCs-Ex treatment group significantly up-regulated the expression of TGF- β 3, compared the other two control groups (Fig. 4a and 4b, $p < 0.05$, $p < 0.01$). These results suggest that hBM-MSCs-Ex effectively promote the cutaneous wound healing through inhibiting the TGF- β /Smad signal pathway (Fig. 5).

Discussion

In this study, our results indicate that hBM-MSCs-Ex stimulates cutaneous wound healing both in vitro and in vivo. In vitro, hBM-MSCs-Ex hBM-MSCs-Ex promote both two type of skin cell (HaCaT and HDFs) proliferation effectively. In vivo, hBM-MSCs-Ex accelerate cutaneous wound healing, via inhibiting the TGF- β /Smad signal pathway.

Recently, studies have shown that the prospective application of MSCs-derived exosomes promoted cutaneous wound healing[5, 7, 18]. The possible roles of MSCs-derived exosomes in wound healing through promotion of cell proliferation, migration, differentiation, angiogenesis and matrix reconstruction[6]. HaCaT and HDFs are the two main skin cells of participating in cutaneous the wound healing[19]. In our study, we found that hBM-MSCs-Ex promote HaCaT and HDFs growth effectively (Fig. 1). It indicated that hBM-MSCs-Ex may promote HaCaT and HDFs proliferation to participate the process of the wound healing. In addition, fibroblasts are key players for exosomes in wound healing, and are the main cell types that synthesize, secrete, and deposit ECM collagen and elastic fibers[20].

MSCs-derived exosomes accelerate wound healing through promoting angiogenesis and restoration of skin function[21]. Previous study has proved that stem cell conditioned medium may contains many pro-angiogenesis factors to promoted wound healing in skin injury[22, 23]. Our results demonstrate that hBM-MSCs-Ex significantly accelerate wound healing (Fig. 2b and 2c), and promoted α -SMA expression which is an important indicator of angiogenesis (Fig. 3a and 3c). It due to that MSCs-derived exosome contains various growth factors to play an important role in cutaneous regeneration and repair[6, 18]. Interestingly, we also found there are many cutaneous appendages regeneration, such as hair follicles and sebaceous glands (Fig. 3a and 3b). This provides favorable conditions for the restoration of skin function.

TGF- β 1/Smad pathway is an important pathogenic mechanism in wound healing[9]. TGF- β 1 is considered to be a key mediator in tissues scarring, and mostly by activating its downstream mothers to against decapentaplegic (Smad) signaling[13]. It have prove that TGF- β 1 exerts its biological effects by activating downstream mediators including Smad2 and Smad3[15]. The phosphorylates cytoplasmic mediators, Smad2 and/or Smad3, and a heterotrimeric complex is formed with Smad4 that translocates into the nucleus, binds a consensus sequence, and regulates gene transcription[14]. While these activity is negatively regulated by Smad7 expression[16]. In our study, we found hBM-MSCs-Ex significantly down-regulate of TGF- β 1, Smad2, Smad3, and Smad4 expression, and up-regulate of Smad7 expression (Fig. 4). It demonstrated that hBM-MSCs-Ex may accelerate cutaneous wound healing through inhibiting the TGF- β /Smad signal pathway (Fig. 5). TGF- β 1 are associated with fibrosis, while TGF- β 3 has been associated with anti-fibrotic or scar-less in wound healing activity, and they have been observed to play an ssential role in regulating epidermal and dermal cell movement during wound repair[10]. In our study, we found hBM-MSCs-Ex decreased of TGF- β 1 expression and increased TGF- β 3 expression (Fig. 4). This may be one of the crucial reasons to promotes the skin scar-less wound healing.

In conclusion, we successfully investigated the role of hBM-MSCs-Ex on cutaneous wound healing. Our results demonstrated that hBM-MSCs-Ex can exert promoting effect of cutaneous wound healing via inhibiting the TGF- β /Smad signal pathway.

Abbreviations

hBM-MSCs: Human bone marrow-derived mesenchymal stem cells; hBM-MSCs-Ex: Human bone marrow mesenchymal stem cells-derived exosomes; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HSCs: Hepatic stellate cells; Hyp: Hydroxyproline; MDA: Malonaldehyde; qRT-PCR: Quantitative real-time PCR; HaCaT: human keratinocytes; HDFs: human dermal fibroblasts; TBIL: Total bilirubin; TP: Total protein; α -SMA: Alpha-smooth muscle actin; γ -GT: Gamma glutamyl transpeptidase

Declarations

Ethics approval and consent to participate

All the protocols and procedures were approved by the Animal Experiment Ethics Committee of the Jilin University, China (approval No. YXA2019-0136).

Consent for publication

Not applicable.

Availability of supporting data

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' Contributions

T.J. carried out the molecular genetic, animal studies, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. Z.W. carried out the WB and animal studies. J.S. participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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References

1. Singer AJ, Clark RA: **Cutaneous wound healing**. *The New England journal of medicine* 1999, **341**(10):738-746.
2. McFarlin K, Gao X, Liu YB, Dulchavsky DS, Kwon D, Arbab AS, Bansal M, Li Y, Chopp M, Dulchavsky SA *et al*: **Bone marrow-derived mesenchymal stromal cells accelerate wound healing in the rat**. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society* 2006, **14**(4):471-478.
3. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS: **Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts**. *Journal of dermatological science* 2007, **48**(1):15-24.

4. Francois S, Mouiseddine M, Mathieu N, Semont A, Monti P, Dudoignon N, Sache A, Boutarfa A, Thierry D, Gourmelon P *et al*: **Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in a xenogenic transplant model.** *Annals of hematology* 2007, **86**(1):1-8.
5. Wang X, Jiao Y, Pan Y, Zhang L, Gong H, Qi Y, Wang M, Gong H, Shao M, Wang X *et al*: **Fetal Dermal Mesenchymal Stem Cell-Derived Exosomes Accelerate Cutaneous Wound Healing by Activating Notch Signaling.** *Stem Cells Int* 2019, **2019**:2402916.
6. Kim S, Lee SK, Kim H, Kim TM: **Exosomes Secreted from Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Accelerate Skin Cell Proliferation.** *International journal of molecular sciences* 2018, **19**(10).
7. Qiu H, Liu S, Wu K, Zhao R, Cao L, Wang H: **Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: A review.** *Journal of cosmetic dermatology* 2019.
8. Rong X, Liu J, Yao X, Jiang T, Wang Y, Xie F: **Human bone marrow mesenchymal stem cells-derived exosomes alleviate liver fibrosis through the Wnt/beta-catenin pathway.** *Stem Cell Res Ther* 2019, **10**(1):98.
9. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND, Zhao YY: **New insights into TGF-beta/Smad signaling in tissue fibrosis.** *Chemico-biological interactions* 2018, **292**:76-83.
10. Lichtman MK, Otero-Vinas M, Falanga V: **Transforming growth factor beta (TGF-beta) isoforms in wound healing and fibrosis.** *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society* 2016, **24**(2):215-222.
11. Walraven M, Gouverneur M, Middelkoop E, Beelen RH, Ulrich MM: **Altered TGF-beta signaling in fetal fibroblasts: what is known about the underlying mechanisms?** *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society* 2014, **22**(1):3-13.
12. Lindert S, Wickert L, Sawitza I, Wiercinska E, Gressner AM, Dooley S, Breitkopf K: **Transdifferentiation-dependent expression of alpha-SMA in hepatic stellate cells does not involve TGF-beta pathways leading to coinduction of collagen type I and thrombospondin-2.** *Matrix Biol* 2005, **24**(3):198-207.
13. Mokoena D, Dhilip Kumar SS, Houreld NN, Abrahamse H: **Role of photobiomodulation on the activation of the Smad pathway via TGF-beta in wound healing.** *Journal of photochemistry and photobiology B, Biology* 2018, **189**:138-144.
14. Guo J, Lin Q, Shao Y, Rong L, Zhang D: **miR-29b promotes skin wound healing and reduces excessive scar formation by inhibition of the TGF-beta1/Smad/CTGF signaling pathway.** *Canadian journal of physiology and pharmacology* 2017, **95**(4):437-442.
15. Walton KL, Johnson KE, Harrison CA: **Targeting TGF-beta Mediated SMAD Signaling for the Prevention of Fibrosis.** *Frontiers in pharmacology* 2017, **8**:461.
16. Jun EK, Zhang Q, Yoon BS, Moon JH, Lee G, Park G, Kang PJ, Lee JH, Kim A, You S: **Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-beta/SMAD2 and PI3K/Akt pathways.** *International journal of molecular sciences* 2014, **15**(1):605-628.

17. Rong X, Chu W, Zhang H, Wang Y, Qi X, Zhang G, Wang Y, Li C: **Antler stem cell-conditioned medium stimulates regenerative wound healing in rats.** *Stem Cell Res Ther* 2019, **10**(1):326.
18. Zhao B, Zhang Y, Han S, Zhang W, Zhou Q, Guan H, Liu J, Shi J, Su L, Hu D: **Exosomes derived from human amniotic epithelial cells accelerate wound healing and inhibit scar formation.** *Journal of molecular histology* 2017, **48**(2):121-132.
19. Gabbott CM, Sun T: **Comparison of Human Dermal Fibroblasts and HaCat Cells Cultured in Medium with or without Serum via a Generic Tissue Engineering Research Platform.** *International journal of molecular sciences* 2018, **19**(2).
20. Darby IA, Laverdet B, Bonte F, Desmouliere A: **Fibroblasts and myofibroblasts in wound healing.** *Clinical, cosmetic and investigational dermatology* 2014, **7**:301-311.
21. McBride JD, Rodriguez-Menocal L, Guzman W, Candanedo A, Garcia-Contreras M, Badiavas EV: **Bone Marrow Mesenchymal Stem Cell-Derived CD63(+) Exosomes Transport Wnt3a Exteriorly and Enhance Dermal Fibroblast Proliferation, Migration, and Angiogenesis In Vitro.** *Stem cells and development* 2017, **26**(19):1384-1398.
22. Lee CY, Kang JY, Lim S, Ham O, Chang W, Jang DH: **Hypoxic conditioned medium from mesenchymal stem cells promotes lymphangiogenesis by regulation of mitochondrial-related proteins.** *Stem Cell Res Ther* 2016, **7**:38.
23. An Y, Liu WJ, Xue P, Ma Y, Zhang LQ, Zhu B, Qi M, Li LY, Zhang YJ, Wang QT *et al*: **Autophagy promotes MSC-mediated vascularization in cutaneous wound healing via regulation of VEGF secretion.** *Cell death & disease* 2018, **9**(2):58.

Figures

Figure 1

hBM-MSCs-Ex promoted the two type of skin cells (HaCaT and HDFs) proliferation. a,b The proliferation curve of HaCaT and HDFs after treated with hBM-MSCs-Ex. c Immunofluorescent staining of HaCaT and HDFs using PCNA antibody. d Percentages of PCNA positive cells in HaCaT and HDFs. Note that HaCaT, human keratinocytes ; HDFs, human dermal fibroblasts; bar = 100 μ m, * p < 0.05, ** p < 0.01, n = 3; data are reported as mean $\pm$ SD.

Figure 2

hBM-MSCs-Ex accelerate cutaneous wound healing in vivo. a The plan of experiment in vivo. b The representative photos of dorsal full-thickness wound healing in rats. c Quantitative analysis of wound area (n=8/group, bar= 5mm). *p <0.05, ** p <0.01 compared to PBS group, ##p <0.01 compared to hBM-MSCs group, data are reported as mean ±SD.

Figure 3

hBM-MSCs-Ex enhanced the cutaneous wound healing quality. a The representative H&E stain and IHC of PCNA images of the wound at 16 days after treatment. b The number of cutaneous appendages including hair follicles and sebaceous glands/field (40×) in the healing tissue. c The percentage of α-SMA positive area. Note that blue arrow, sebaceous gland; purple arrow, α-SMA positive area, bar= 1mm, *p < 0.05, ** p < 0.01, *** p < 0.001, n=8/group, data are reported as mean ± SD.

Figure 4

hBM-MSCs-Ex regulate the TGF- β /Smad signal pathway. a Western blot analysis of key TGF- β /Smad signaling-related protein levels in skin healed tissue treated with hBM-MSCs-Ex. b Relative mRNA expression levels of key TGF- β /Smad signaling-related gene in skin healed tissue treated with hBM-MSCs-Ex. * $p < 0.05$, ** $p < 0.01$, $n=3$, data are reported as mean $\pm$ SD.

Figure 5

hBM-MSCs-Ex stimulates cutaneous wound healing by regulating the TGF-β/Smad signal pathway. hBM-MSCs-Ex inhibited TGF-β1 and activated TGF-β3 expression; TGF-β isoforms and activins stimulate intracellular signaling via Smad-2/3 transcription factors; Phosphorylates Smad-2 and Smad-3, which bind to Smad-4 leading to the transcription and expression of α-SMA; Inhibitory Smad7, which are activated by the binding of the TGF-β super family to the cell surface receptors.

Supplementary Files

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