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

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Research

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Abstract

Background: Cutaneous wound healing represents a morphogenetic response to injury, and is 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 cell regeneration effects and its underlying mechanism of hBM-MSCs-Ex on cutaneous wound healing in rats. *In vitro studies,* we evaluated the role of hBM-MSCs-Ex in the two types of skin cells: human keratinocytes (HaCaT) and human dermal fibroblasts (HDFs) for the proliferation. For *in vivo studies*, we used a full-thickness skin wound model to evaluate the effects of hBM-MSCs-Ex on cutaneous wound healing *in vivo*.

Results: The results demonstrated that hBM-MSCs-Ex promote both two types of skin cells growth effectively and accelerate the cutaneous wound healing. Interestingly, we found that hBM-MSCs-Ex significantly down-regulated TGF-β1, Smad2, Smad3, and Smad4 expression, while up-regulated TGF-β3 and Smad7 expression in the TGF-β/Smad signaling pathway.

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

Introduction

Cutaneous wound healing is characterized by repairing damaged tissue and the tissue regeneration orchestrated by multiple cells to re-establish a protective barrier [1]. The primary goals of skin wounds treatment considering rapid wound closure and scar-less healing process. Previous studies have shown that mesenchymal stem cells (MSCs) capable of self-renewal and multipotential differentiation capabilities, which shows promising therapeutic potential for tissue regeneration and skin function recovery [2, 3]. However, MSCs transplantation may induce immunoreactivity to the host [4]. To avoid host-immunoreactivity, MSC-derived exosomes shows a alternative therapeutic attention for regenerative wound repair [5-7]. The current study has been demonstrated that MSC-derived exosomes accelerated the wound healing through increased re-epithelialization, angiogenesis, and tissue granulation [6]. In our previous publication, we have demonstrated that hBM-MSCs-Ex treatment significantly reduced liver fibrosis in rats [8]. In this current study, we used the promising exosomes type, i.e. hBM-MSCs-Ex, to investigate the wound healing process, and to investigate the associated signal pathway mechanism.

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 the natural wound healing process by TGF-β/Smad pathway [10]. Smad is well known as the major inducer of fibroblast differentiation, which is an essential factor for wound healing [11]. During this process, TGF-β activates downstream mediators such as Smad2 and Smad3, which result in the linage differentiation of fibroblasts into alpha-smooth muscle expressing (α-SMA) myofibroblasts [12]. The phosphorylated

Smad2/Smad3 activates Smad7, which promoter to up-regulate Smad7 expression in the differentiation process, and thereby Smad7 inhibits TGF-β1 expression for negative feedback regulation [13]. Recent studies have demonstrated that the TGF-β/Smad signal pathway plays a essential vital role in cutaneous wound healing via regulating the proliferation and migration of keratinocytes, dermal fibroblasts, and other skin cells in the affected areas to participate in the wound healing process [14, 15]. Furthermore, TGF-β/Smad signaling can regulate tissue fibrosis and scar formation [16]. In this current study, we hypothesized that hBM-MSCs-Ex could promote the cutaneous wound healing process via regulating the TGF-β/Smad signaling pathway.

Materials And Methods

Cell culture and exosome purification

HaCaT and HDFs purchased from the 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% CO_2 atmosphere at 37 °C. The purification of hBM-MSCs-Ex involves several centrifugation steps, as described previously [17]. Briefly, hBM-MSCs were cultured in serum-free medium (SFM, Gibco, Grand island, USA) for 2 days. Conditioned medium was first filtered using a 0.1-μm filtering unit. The supernatant was concentrated with a 100-kDa molecular weight cutoff (MWCO) hollow fiber membrane (Millipore, Billerica, MA, USA), at 1000g for 30 min. Then, the concentrated supernatant was loaded onto a 30% sucrose/D_2O cushion (5 ml, density 1.210 g/cm^3), and ultra-centrifuged at 100,000g for 3 h. After exosome-enriched fraction was collected, washed three time with fresh PBS by centrifuged at 1500g (30min each wash) with 100-KDa MWCO. Finally, purified exosomes were passed through a 0.22-μm filter and stored at −80°C until further use. The protein concentration of exosomes was measured by bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China).

Cell proliferation assay

HaCaT and HDFs cells were cultured until 70~80% confluence, trypsinized cells were plated in 96-well plates at a density of 4,000 cells per well. Briefly, hBM-MSCs-Ex purified and characterization as per previously published methods [8]. The cells were treated with either hBM-MSCs-Ex (25 μg/mL) or PBS (control) (Invitrogen, Shanghai, China), then followed by incubated at 37°C with 5% CO_2 for 5 days. The cell viability was determined by CCK-8 kit (Sigma, San Francisco, U.S.), and corresponding OD value measured at the 490 nm wavelength. Result expressed as

Immunofluorescence staining (IF)

HaCaT and HDFs cultured by hBM-MSCs-Ex (25 μg/mL) or PBS were incubated in 24-well plate coated coverslip for 24 h. When cells reached 60~70% confluence, plate were washed with PBS and incubated with 4% paraformaldehyde for 10 minutes (RT). The processed cells blocked with 1% bovine serum

albumin (BSA; Biosharp, Hefei, China) for 30 minutes. The cells were incubated with primary antibodies against Rb anti-PCNA (1:100 dilution, BD Biosciences, Franklin Lakes, NJ, U.S.), and isotype-matched rabbit IgG/IgM (1:100 dilution, Abcam, Cambridge, UK) served as the negative controls. Anti-rb-FITC-488 secondary antibody (1:500 dilution, Abcam, Cambridge, UK) for 2h, and the nuclei were labeled with DAPI (Thermo Scientific, Waltham, U.S.) for 5min. Images have acquired by a fluorescence microscopy (EVOS, Thermo Scientific, Waltham, U.S.), and the PCNA positive cells were counted in ten random optical fields by using ImageJ software.

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 Ethics Committee of Jilin University. The animal model was generated according to previously published methods [18]. Briefly, rats were anesthesia and shaved the dorsal hair, followed by full-thickness skin excisional wound were made of about the size of 10mm in diameter circular holes in rat. 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 (250μg, multi-directional subcutaneous injection). The recovery of skin damage were recorded photographically every four days for 16 days. The wound area was measured using lasso tool (Adobe Photoshop CS6), The wounded area were trace and circle the edge of wound on photograph, then calculate the circled area based on the pixels of that area. End of the study, the rats were euthanized on 16^{th} day to collect the healed and unhealed tissue area in the different treatment groups.

Histological examination

Skin tissue were collected from the mechanical injury region on 16 days, and samples were fixed in 10% formalin in PBS and embedded in paraffin. The skin tissue were sectioned at 4-μm thickness to perform Hematoxylin and eosin (H&E) staining. The staining were performed following the manufacturer's protocols (Sigma, San Francisco, U.S.). The sections were processed for immunohistochemistry (IHC) using the Kit (Maixin KIT-9710, Fuzhou, China) following the manufacturer's instructions. Briefly, the sections were deparaffinized, antigen retrieval were performed by immersion slides in 0.01M sodium citrate buffer solution for 15 min. Quenching of endogenous peroxidase by processing the sections in 3% H_2O_2 for 15 min, followed by blocked sections with 10% normal goat serum for 1 h at 37 °C. The sections were incubated with primary antibody anti-α-SMA or anti-VEGF with 1:500 dilution (Abcam, Cambridge, UK) for overnight at 4 °C. Next day, these sections were incubated with biotinylated goat-anti-rabbit IgG antibody for 2 h and incubated with avidin peroxidase reagent sequentially. Then, the sections were incubated with diaminobenzidine solution as the chromogenic agent at 37 °C for 5 min. Finally, we used H&E staining for counterstaining the sections. These sections were photographed using a bright-field microscope (EVOS, Thermo Scientific, Waltham, U.S.). Cutaneous appendage separated by tissue on one microscope sphere was counted as one unit. The α-SMA and VEGF positive area calculation is based on

the ratio of positive area /total area of the observed field. We used 6 random fields per section and 6 sections in total (n=8 rats) for the quantification of IHC images.

Western blot

Proteins were extracted from the skin healed tissue in the in SDS sample lysis buffer. The samples were heated to 95°C for 10 min, and 40 µg protein samples were separated on SDS-polyacrylamide gels (5% stacking gel and 12% separation gel). Resolved proteins were then transfered onto nitrocellulose membranes and blocked in 5% nonfat powdered milk for 1h, and probed with respective antibody against TGF-β1, TGF-β3, Smad2, Smad3, Smad4, Smad7 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as loading control (1:1000 dilution, Abcam, Cambridge, UK) overnight at 4°C. The blots were blocked in secondary HRP-conjugated goat anti-rabbit IgG antibodies (1:1000 dilution, Abcam, Cambridge, UK), and visualized by chemiluminescent detection substrates (Immobilon western chemiluminescent HRP substrate, Millipore). The densitometric quantification were performed on the protein bands by using AlphaEaseFC software (Alpha Innotech).

Real-time PCR assay

Total RNA from skin healed tissue was extracted with Trizol (Invitrogen, Shanghai, China) according to the manufacturer's protocol. Briefly, the first-strand cDNA was synthesized with 1µg of total RNA using SuperScript II (Invitrogen). SYBR Green I dye was used for reverse transcription in an ABI 7500 fluorescence quantitative PCR instrument, and the mRNA levels of TGF-β1, TGF-β3, Smad2, Smad3, Smad4, Smad7, and GAPDH were measured using the respective primers listed in Table (Supplementary Table S)1. The thermocycler conditions as follow: initial step at 95°C for 2min, followed by 40 cycles at 95°C for 15s, and 60°C for 1min. 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 $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

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

Results

Invitro studies: hBM-MSCs-Ex improves skin cell proliferation. .

In vitro, we investigated whether hBM-MSCs-Ex treatment can stimulate the cell proliferation by using HaCaT and HDFs skin cells. After the procedure, the proliferation assay was measured consecutive for 5-

day. In HaCaT and HDFs cells, we observed significant increase in exponential cell proliferation and the growth rate constantly increased from day 2 after the hBM-MSCs-Ex treatment, compared to the PBS group (Fig.1a and 1b, $p<0.05$ and $p<0.01$). Based on the CCK-8 results, we performed immunofluorescent staining analysis on day 5 to confirm whether hBM-MSCs-Ex treatment can induce the proliferative effect in these cell line. As shown in the representative images in Fig.1c and 1d, the we observed an significant increase in the number of PCNA positive cells in hBM-MSCs-Ex treatment group compared to PBS group (62.1% *vs* 28.2% in HaCaT and 71.3% *vs* 34.8% in HDFs in the two types of skin cells ($p<0.01$). *In vitro* experiment, the results demonstrated that treatment of hBM-MSCs-Ex can promote cell proliferation and maintain cell growth effectively in the both skin cells (HaCaT and HDFs).

Invivo studies: hBM-MSCs-Ex improves cutaneous wound healing

To investigate the roles of hBM-MSCs-Ex in wound healing, we established a full-thickness skin wounds injury model in rats, as illustrated in the experimental design in Figure 2a. The area measurements in the wounded region show a significant improvement in the wound closure after the treatment of hBM-MSCs-Ex and hBM-MSCs treatment group (Fig 2b and 2c). We observed a significant reduction in the wound area in the hBM-MSCs treatment group starting from day 4 after the surgery and constantly improved in reduction until day 16^{th} compared with PBS The wound in hBM-MSCs-Ex group completely healed on the 16±2.3 day after surgery. ($p<0.05$, $p<0.01$). These results indicates that hBM-MSCs isolated exosomes are more can effectively accelerated the cutaneous wound healing process as demonstrated in the animal model.

hBM-MSCs-Ex restore the normal skin morphology

To understand the process of restoring the skin morphology regenerated during the healing process, we assessed the effects of the hBM-MSCs-Ex on the wound healing quality. To study the general morphology of skin regeneration, we performed H&E staining. The observation of H&E staining images shows a in indication that regeneration of cutaneous appendages in the affected area, including restoring hair follicles and sebaceous glands in hBM-MSCs-Ex group (21.3 ± 5.4 /filed) were compared to other treatments such as PBS group (1.2 ± 2.8 /filed, $p<0.001$) and hBM-MSCs group (15.4 ± 4.1 /filed, $p<0.001$), (Fig. 3a and 3b). Because of α-SMA and VEGF are both important indicators of angiogenesis. We performed IHC in the wound area samples from the different treatment groups. The α-SMA positive area quantification results showed that the percentage of α-SMA positive area were significantly increased in hBM-MSCs-Ex group (8.1% ± 1.2), when compared with the PBS group (1.3% ± 0.5 / HPF, $p<0.001$) as well as hBM-MSCs group 5.6 %± 0.9, $p<0.01$), (Fig 3a and 3c). Consist with the above results, the percentage of VEGF positive area was significantly increased in hBM-MSCs-Ex group (12.3% ± 2.4), compared to the PBS group (1.6% ± 1.5/ HPF, $p<0.001$) and hBM-MSCs group (5.1%± 1.7, $p<0.001$), (Fig 3a and 3d). The above results indicated that hBM-MSCs-Ex not only enhanced the wound process but also restore skin function and angiogenesis in the affected areas.

hBM-MSCs-Ex regulate the TGF-β/Smad signal pathway

Finally, we investigates the underlying mechanism of hBM-MSCs-Ex induced skin healing process in the affected tissue. The expression level of TGF-β1, TGF-β3, 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 (respective protein and RNA levels) expression of TGF-β1, Smad2, Smad3, and Smad4 in hBM-MSCs-Ex treatment group, compared the other two control groups (p <0.05, p <0.01). The alteration of signaling molecule during the healing the process illustrated that hBM-MSCs-Ex treatment plays a major mechanism in inhibiting TGF-β/Smad signaling pathway (Fig.4a and 4b).

To understand the mechanism how the TGF-β/Smad signaling was inhibited. We study the role of Smad7 (downstream molecules) in the TGF-β signaling pathway, interestingly, 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-β1 is fibrotic isoform, while TGF-β3 is the anti-fibrotic isoform. The hBM-MSCs-Ex treatment group significantly up-regulated the expression of TGF-β3, 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 (Fig5).

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 promotes both two types of skin cells (HaCaT and HDFs) proliferation effectively. *In vivo,* hBM-MSCs-Ex accelerates 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, 19]. 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 major skin cells types which participating in cutaneous wound healing [20]. In our study, we found that hBM-MSCs-Ex promote HaCaT and HDFs growth effectively (Fig1). It indicated that hBM-MSCs-Ex promote HaCaT and HDFs cell proliferation to participate in the process of wound healing. In addition, fibroblasts are critical players for exosomes in wound healing and are the main cell types that synthesize, secrete, and deposit ECM collagen and elastic fibers[21]. Studies have shown that MSCs exert their therapeutic effect via secretion of soluble factors, included the exosomes [5, 6]. There is substantial evidence that exosomes have the ability to promoted skin regeneration when applied topically or injected systemically [22, 23]. In this study, we have administration subcutaneous injection of exosomes at multi-directional to delay its clearance in the body. In addition, hBM-MSCs appear to be limited because of poor cell retention at the wound site [24], To overcomes this issue we adopted intravenous injection routes to maximize the effect of treating wounds in our treatment groups.

MSCs-derived exosomes accelerate wound healing process through promoting angiogenesis and restoration of skin function [25]. Previous studies have proved that stem cell-conditioned medium may contain exosomes that contain pro-angiogenesis factors to promoted wound healing in skin injury[26, 27]. Our results demonstrate that hBM-MSCs-Ex significantly accelerate wound healing (Fig2b and 2c) and modulate α-SMA expression which is an important indicator of angiogenesis (Fig3a and 3c). Due to MSCs-derived exosome contains various growth factors to play an important role in cutaneous regeneration and repair [6, 19]. Interestingly, we also found there are many cutaneous appendages regeneration, such as hair follicles and sebaceous glands (Fig3a and 3b). During the normal healing process in addition if provides hBM-MSCs-Ex which favor conditions for the restoration of skin function much quicker than normal healing process.

Recently studies, indicated that different types of MSCs therapy or combined therapies with some unique biotechnology factors can stimulate cutaneous wound healing[28-31]. Current studies have report that a new combination therapeutical approach shows effective combinational treatment composed of adipose derived mesenchymal stem cells (AD-MSCs) platelet-rich plasma (PRP) and hyaluronic acid (HA) dressing which shown to stimulate the wounds healing and regeneration process [30-32]. The complete closure of wound is inducing a new neodermis and stimulating regeneration and a protected environment in a humid environment [33-35]. Many supporting evidence indicates that adipose-derived stem cells (ASCs) and adipocyte-secreted exosomal microRNA promote wound repair [36]. Another study has demonstrated that hBMSCs on gelatin scaffold with poly N-isopropylacrylamide (pNIPAAm) as transplanted grafts for improving skin regeneration [42]. More recent clinical trials shown to improve the hair density by administration human follicle stem cells (HFSCs) [37-39]. In addition, autologous fat grafting it is better approach to consider for the correction of wound scars in the affected regions [40, 41]. However, in our current study, we administration directly hBM-MSCs-Ex on mechanical damaged skin area, which is more clinically transferable, more safe and effective cell-free reagents compared with their parents cells skin regeneration therapies. Moreover, we have demonstrated that, hBM-MSCs-Ex treatment is more effective than of hBM-MSCs in wound healing, such as some indicators of wound area and cutaneous appendages.

TGF-β1/Smad pathway is an important pathogenic mechanism in wound healing [9]. TGF-β1 is considered to be a key mediator in tissues scarring and mostly by activating its downstream against decapentaplegic (Smad) signaling [13]. It has proven that TGF-β1 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 translocate into the nucleus, binds a consensus sequence, and regulates gene transcription [14]. While these activities is negatively regulated by Smad7 expression [16]. In our study, we found hBM-MSCs-Ex significantly down-regulate of TGF-β1, Smad2, Smad3, and Smad4 expression, and up-regulate of Smad7 expression (Fig4). It demonstrated that hBM-MSCs-Ex might accelerate cutaneous wound healing through inhibiting the TGF-β/Smad signal pathway (Fig5). TGF-β1 are associated with fibrosis, while TGF-β3 has been associated with anti-fibrotic or scar-less wound healing activity, and they have been observed to play an essential role in regulating epidermal and dermal cell movement during wound repair[10]. In our study, we

found hBM-MSCs-Ex decreased of TGF-β1 expression and increased TGF-β3 expression (Fig4). 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 could exert promoting effect of cutaneous wound healing via inhibiting the TGF-β/Smad signal pathway. The current approach provides a better knowledge in the wound healing process and new therapeutic strategy for the treatment of cutaneous wounds.

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; VEGF: Vascular endothelial growth factor; γ- GT: Gamma glutamyl transpeptidase; AD-MSCs: adipose derived mesenchymal stem cells; PRP: combined treatment composed of platelet-rich plasma; HA: hyaluronic acid; pNIPAAm: N-isopropylacrylamide; HFSCs:Human follicle stem cells

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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Figures

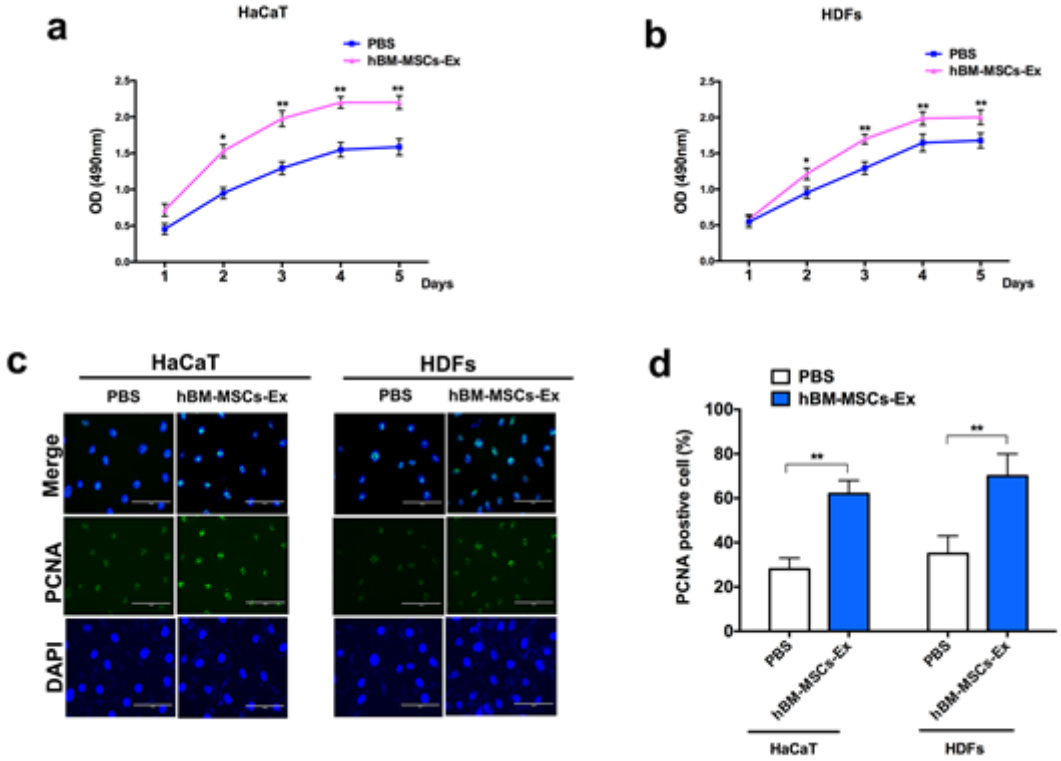

Figure 1

hBM-MSCs-Ex promotes proliferation in HaCaT and HDFs skin cells. The cell proliferation curve were shown from respective HaCaT and HDFs skin cells after treated with hBM-MSCs-Ex (a,b). Immunofluorescent staining were performed in HaCaT and HDFs for PCNA positive cells and representative images were shown (c). The quantification of PCNA positive cells (in percentages) in HaCaT and HDFs were determined of (d). Note that HaCaT, human keratinocytes; HDFs, human dermal fibroblasts; bar = 100 μm, *p <0.05, **p <0.01, n = 3; data are reported as mean ±SD.

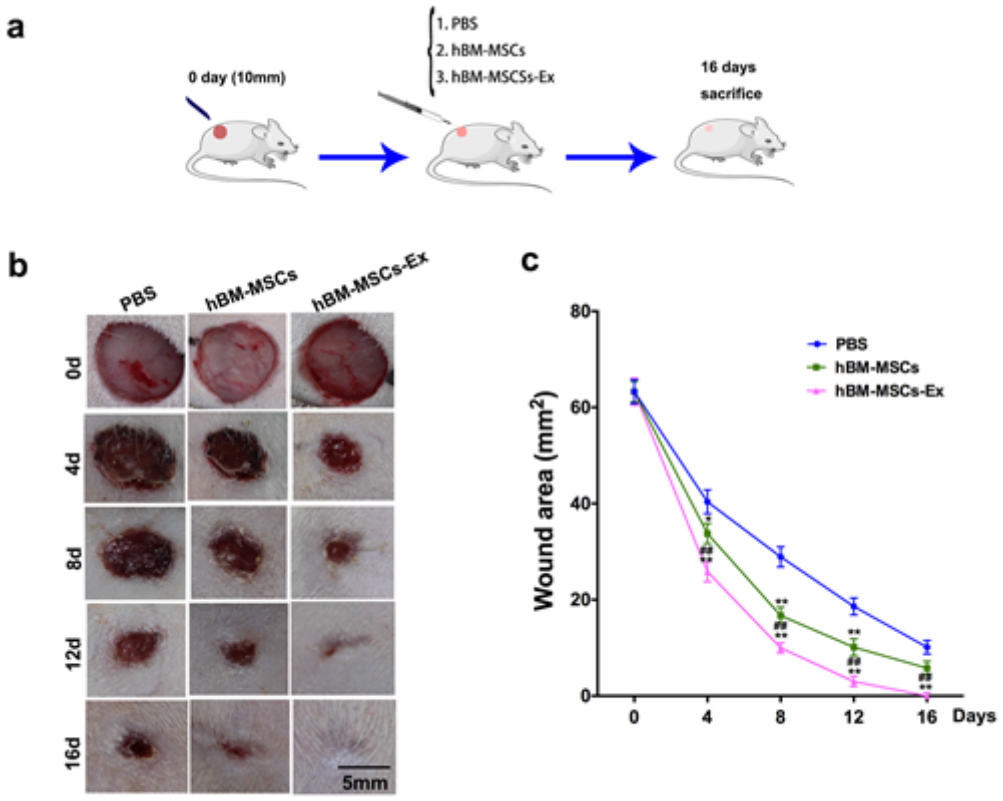

Figure 2

hBM-MSCs-Ex treatments accelerate cutaneous wound healing process in vivo. The illustration of experimental design and plan of experiment performed in vivo (a). The representative photos shown in the dorsal full-thickness wound area of the rat (b). Quantitative analysis of wound area in the respective treatment groups (c). n=8/group, scale 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.

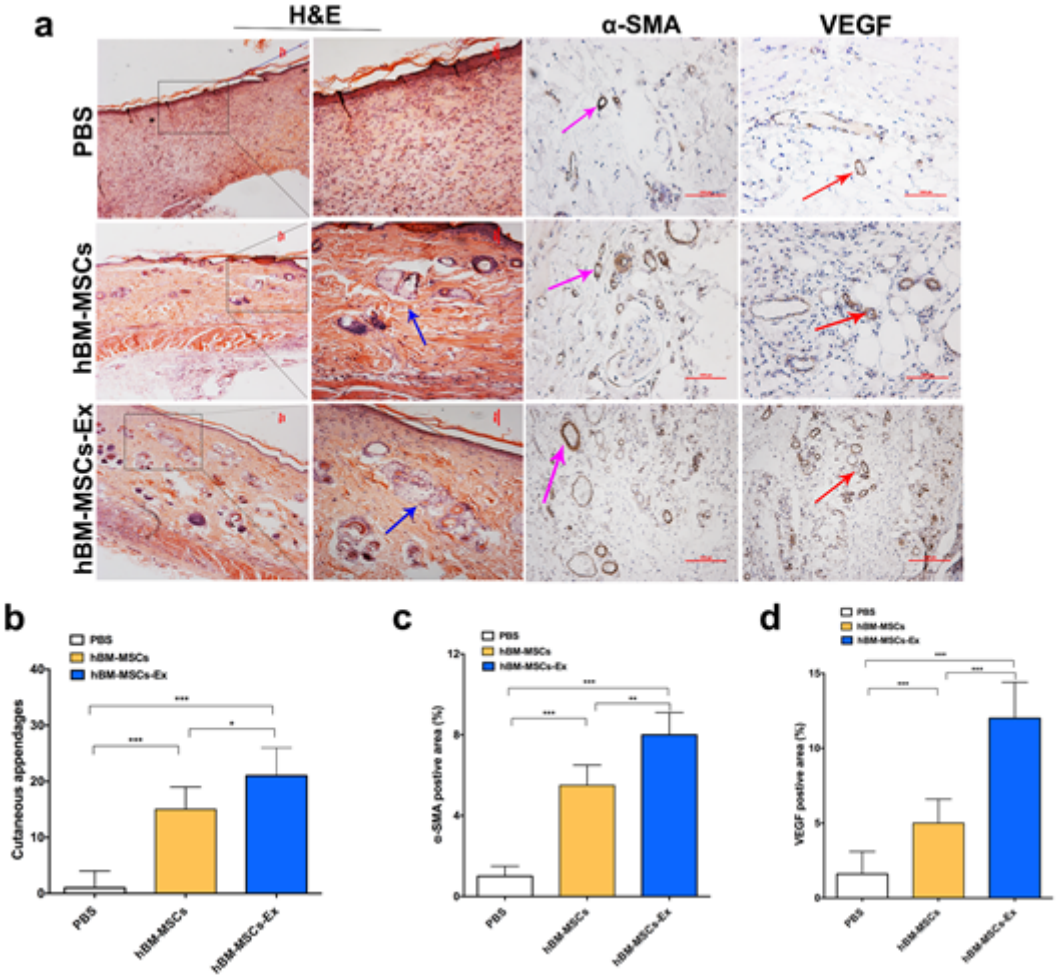

Figure 3

hBM-MSCs-Ex enhanced the cutaneous wound healing quality. The representative images of H&E staining in the different treatment groups (a). The image quantification of number of cutaneous appendages including hair follicles and sebaceous glands/field (40×) in the healing tissue (b). The blue arrow indicates, the presence of sebaceous gland. Immunostaining for α-SMA and VEGF were shown respective images at 16 days after treatment (a). The percentage quantification of α-SMA and VEGF positive area (c, d). The purple arrow indicates the α-SMA positive cells, red arrow indicates VEGF positive cells. Scale bar= 1mm, *p <0.05, **p <0.01, *** p <0.001, n=8/group, data are reported as mean ±SD.

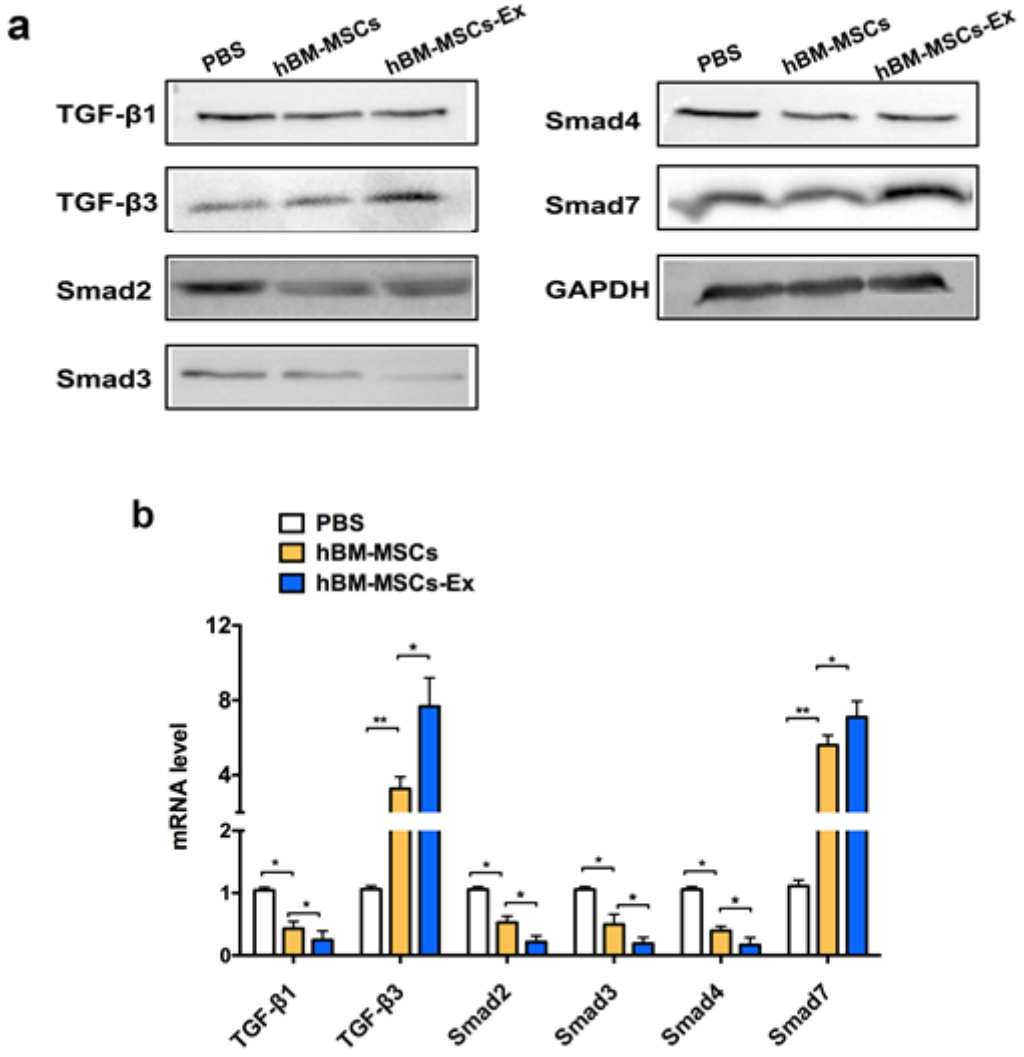

Figure 4

hBM-MSCs-Ex regulate the TGF-β/Smad signal pathway. Representative Western blot of key TGF-β/Smad signaling-related protein levels in skin tissue treated with hBM-MSCs-Ex (a). The quantification of relative mRNA expression levels of major TGF-β/Smad signaling-related gene in skin healed tissue treated with hBM-MSCs-Ex (b). *p <0.05, **p <0.01, n=3, data are reported as mean ±SD.

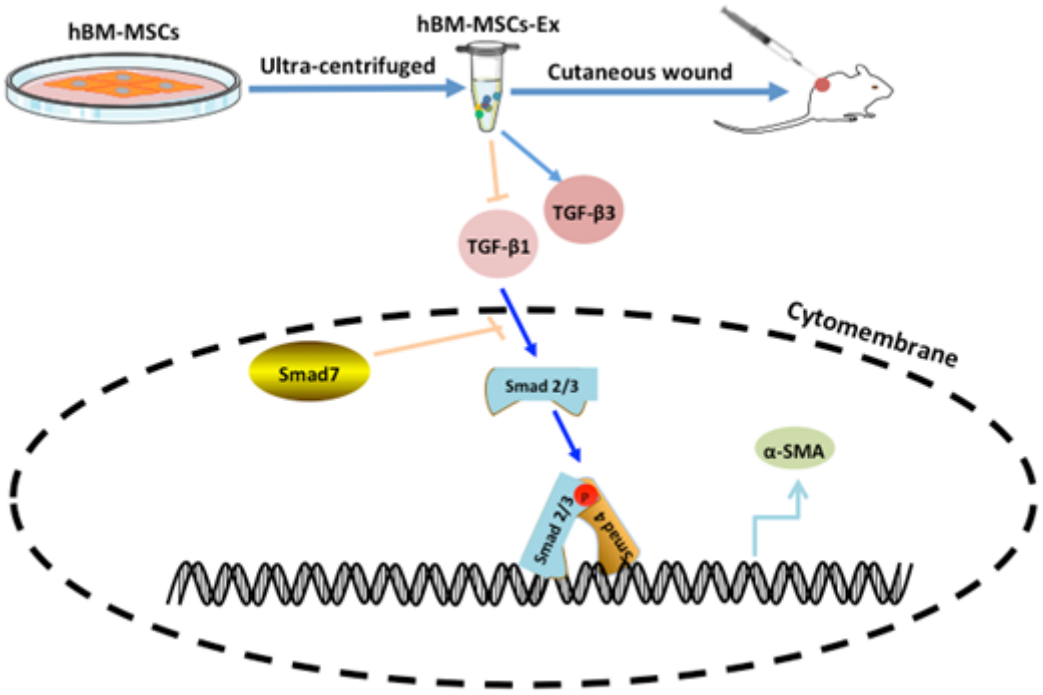

Figure 5

Illustration of 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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- SupplementaryMaterial.docx