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WORD COUNT	4009	TIME SUBMITTED	09-JUN-2022 04:40PM
		PAPER ID	87050178

Name of Journal: *World Journal of Stem Cells*

Manuscript NO: 74752

Manuscript Type: ORIGINAL ARTICLE

Basic Study

Application of BM-MSC-Derived Extracellular Vesicles Promotes Hair Growth through Activation of Human Dermal Cells and Augmentation of Hair Shaft Elongation

Human MSC-EVs Promotes Hair Growth

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Abstract

BACKGROUND

Dermal papillae (DP) and outer root sheath (ORS) cells play important roles in hair growth and regeneration by regulating the activity of hair follicular cells.

AIM

Extracellular vesicles (EVs) are known to regulate various cellular functions; however, the effects of human mesenchymal stem cell-derived EVs (hMSC- EVs) on hair growth, particularly on the human derived hair follicle cells (DP and ORS cells), and the possible mechanism of these effects are unknown. Hence, we investigated the effects of hMSC- EVs on DP and ORS cells and hair follicles.

METHODS

hMSC-EVs were isolated and characterized by transmission electron microscopy, nanoparticle tracking analysis, western blotting, and flow cytometry. The activation of DP and ORS cells were analyzed using cellular proliferation, migration, western blotting, and real-time polymerase chain reaction. Hair follicle growth was evaluated using *ex vivo* human hair follicles.

RESULTS

Wnt3a was present in a class of hMSC- EVs and was associated with the EV membrane. hMSC- EVs promoted the proliferation of DP and ORS cells. Moreover, they translocated β-catenin into the nucleus of DP cells by increasing the expression of β- catenin target transcription factors (Axin2, EP2, and Lef1) in DP cells. Treatment with hMSC- EVs also promoted the migration of ORS cells and enhanced the expression of keratin differentiation markers (Keratin (K)-6, K16, K17, and K75) in ORS cells. Furthermore, treatment with hMSC- EVs increased hair shaft elongation in cultured human hair follicles.

CONCLUSION

These findings suggest that hMSC-EVs are candidates for further preclinical and clinical studies on hair loss.

Key Words: mesenchymal stem cells; extracellular vesicles; hair growth; dermal papillae; outer root sheath cells

Rajendran RL, Gangadaran P, Kwack MH, Oh JM, Hong CM, Sung Y, Lee J, Ahn BC. Application of BM-MSC-Derived Extracellular Vesicles Promotes Hair Growth through Activation of Human Dermal Cells and Augmentation of Hair Shaft Elongation. *World J Stem Cells* 2022; In press

Core Tip: Alopecia is a common medical problem affecting both males and females. This study found that Wnt3a is enriched in human mesenchymal stem cell-derived extracellular vesicles (hMSC- EVs) and associated with their EVs' surface. hMSC- EVs associated wnt3a can activate the Wnt/β-catenin signaling in recipient dermal papillae cells. hMSC- EVs activate keratin differentiation in recipient outer root sheath cells and increase hair shaft elongation. These findings open up for new hair growth treatment strategies to be developed for alopecia.

INTRODUCTION

Hair loss is a common and progressive condition that affects both males and females. Within hair follicles (HFs), cells and their secretory factors undergo complex and intricate interactions for the progression of the hair follicle cycle from the telogen to the anagen[1,2]. Hair loss can be stopped, and hair regrowth can be improved to a certain extent by minoxidil or finasteride treatment, but complete recovery is not possible. The hair transplant surgery is another option to avoid baldness, not a cure for male pattern baldness and associated with complications such as edema, rarely bleeding, folliculitis, numbness of the scalp, telogen effluvium and infection[3,4]. Dermal papilla (DP) and

outer root sheath (ORS) cells support the regulation of the hair cycle. However, they gradually lose their key hair-inducing properties upon pathological conditions[5]. The restoration of DP and ORS cell functions is required to promote hair regrowth.

Extracellular vesicles (EVs) are basically spherical vesicles released by nearly all cells into the extracellular milieu and are found in body fluids and culture medium. EVs comprise functional lipids, proteins, and nucleic acids and act as the mediators of intercellular communication. EVs are classified into exosomes or small EVs, microvesicles, and apoptotic bodies. Exosomes are released by cellular multivesicular bodies, whereas microvesicles are formed from the outward budding of the plasma membrane; both are secreted under normal cellular conditions. By contrast, apoptotic bodies are formed during cell death[6,7].

In recent years, EVs have been emerging as potential therapeutic candidates for various diseases, including ischemic disease, wound healing, and hair regrowth, by delivering their cargoes to target cells[8–12]. Recently several studies have showed a potential therapeutical effects of EVs or nanovesicles from DP cells[13–17], fibroblasts[18,19], stem cells[11,20], macrophage[21,22] and neural progenitor cells[23] on hair growth. Nearly half of these studies reported enhanced hair regrowth using DP cells as source cells, which showed potential as therapeutic candidates for hair re-growth. However, the clinical translation of EVs derived from DP cells is limited because HFs are not readily available for isolating DP cells and DP cells gradually lose key hair-inducing properties upon *in vitro* culture[13,24]. Stem cells have been used for regenerative therapies for the last few decades, including hair regeneration[25–27], because they can be readily isolated from the bone marrow, adipose tissue, and umbilical cord and can be generated using induced pluripotent stem cells[28]. In our previous report, we studied the efficacy of MSC-derived EVs on hair regrowth in addition to the efficacy of mouse bone marrow-derived MSC-EVs on human DP cells using a mouse model[11]. In another study, human MSC-EVs were used in a mouse model[29].

In the present study, we investigated the functions of human bone marrow MSC (hMSC)-derived EVs (hMSC-EVs) on inducing hair growth. In addition, we examined

the possible molecular mechanisms responsible for hair regrowth. Finally, hMSC-EV-treated human DP cells, human ORS cells, and human HFs were examined for the activation of DP and ORS cells and their effects on hair shaft elongation in human HFs.

MATERIALS AND METHODS

Cell Culture

Bone Marrow-Derived Mesenchymal Stem Cells; Normal, Human (PCS-500-012™) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's (DMEM)-F12 (HyClone, Logan, UT, USA) supplemented with 10% EV-depleted fetal bovine serum (FBS; Hyclone; ultracentrifuged at 120,000 ×g for 18 h at 4°C) and antibiotics (1% penicillin–streptomycin) (Gibco, Carlsbad, CA, USA) and maintained at 37°C and 5% CO_2.

Isolation and Culture of Human DP and ORS Cells

During the hair transplantation of male patients with androgenic alopecia, the biopsy specimens from the occipital scalps were obtained after receiving consent. The Medical Ethical Committee of the Kyungpook National University Hospital (Daegu, Korea) approved all of the described studies (IRB No. KNU 2018-0155). The hair follicles were dissected to isolate the DP cells from the bulbs, and the cells were transferred to tissue culture dishes coated with bovine type I collagen and cultured in low-glucose DMEM (HyClone, Logan, UT, USA) supplemented with 1% antibiotic–antimycotic and 20% heat-inactivated FBS at 37°C. The cells were cultured for 7 days with medium replacement every 3 days. The cells were then cultured in low-glucose DMEM supplemented with 10% heat-inactivated FBS in 100-mm culture dishes. Once the cells reached subconfluence, they were harvested using 0.25% trypsin/10 mm EDTA in phosphate-buffered saline (PBS) (split at a 1:5 ratio). Cells from passage number 2 were subsequently used for further experiments[30].

The same hair specimens were used to isolate the ORS cells. The regions of hair shaft and bulb of HFs were removed (to avoid contamination due to other cells). HFs were trimmed and then immersed in DMEM supplemented with 20% FBS in tissue

culture dishes coated with rat collagen type I (Corning, Kennebunk, ME, USA). Cells were cultured for 3 days, and medium was changed with the keratinocyte growth medium, EpiLife medium (Gibco BRL) with 1% antibiotic–antimycotic solution, and 1% EpiLife defined growth supplement medium. After reaching subconfluence, cells were harvested using 0.25% trypsin/10 mm EDTA in PBS (split at a 1:5 ratio) and maintained in EpiLife medium. Cells from passage number 2 were subsequently used for further experiments[18].

Isolation of hMSC-EVs

hMSC-EVs were isolated from culture medium of human BM-MSCs (from Passage 3 to 6) by ultracentrifugation methodology as previously described[10]. In brief, the culture media was centrifuged at 1,500 ×g for 10 min to remove the cells. Next, it was recentrifuged at 4,000 ×g for 20 min to remove cell debris. The collected culture media was filtered through a 0.45-µm syringe filter and ultracentrifuged at 100,000 ×g for 60 min. The collected hMSC-EV pellets were resuspended in PBS and ultracentrifuged at 100,000 ×g for 60 min. hMSC-EVs were then reconstituted in 50–100 µL PBS and stored at −80°C until use. All ultracentrifugation procedures were performed at 4°C using a SW28 rotor (Beckman Coulter, GA, USA). EV concentration was measured using Pierce bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, MA, USA).

Transmission Electron Microscopy (TEM)

The hMSC-EV pellets were resuspended in 100 µL of 2% paraformaldehyde. The samples were then added to Formvar/Carbon TEM grids, and the membranes were air dried for 20 min in a clean environment. The grids were washed with PBS (100 µL) and incubated in 50 µL of 1% glutaraldehyde for 5 min. The grids were then washed with distilled water for 7 × 2 min cycles and observed in HT 7700 transmission electron microscope (Hitachi, Tokyo, Japan) to view the morphology of hMSC-EVs[9].

Nanoparticle Tracking Analysis (NTA)

The measurement of hMSC-EVs was performed by NTA using NanoSight LM10 (Malvern). hMSC-EVs were diluted 1,000-fold with Milli-Q water, and then, a sterile

syringe was used to inject the sample into the chamber while ensuring that no bubbles were present. Measurements (n = 5) were performed and evaluated using NanoSight NTA software. Measurement values corresponded to the measured particle sizes as determined by NanoSight software.

Western Blot Analysis

Western blotting was performed as described previously[31]. In brief, to extract proteins, whole cells and EVs were treated with radio immunoprecipitation assay buffer (Thermo Fisher Scientific) containing a cocktail of protease inhibitors. Total protein concentrations were measured by Pierce BCA protein assay kit (Thermo Fisher Scientific). Equal quantities of proteins (10 µg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Burlington, MA, USA). Blots were first probed with primary antibodies to Alix (Dilution 1:4000; Abcam, Cambridge, MA, USA), cytochrome C (Dilution 1:2500; Abcam), GM130 (Dilution 1:5000; Abcam), Wnt3a (Dilution 1:2500; Abcam), and PCNA (Dilution 1:5000; Cell Signaling Technology, Danvers, MA, USA) and then with an anti-rabbit secondary antibody (Dilution 1:8000; Cell Signaling Technology) conjugated to horseradish peroxidase. The signals were detected using enhanced chemiluminescence (GE Healthcare, Waukesha, WI, USA) as per the manufacturer's protocol. Blot images were cropped and prepared using MS PowerPoint program (Microsoft, CA, USA).

Flow Cytometry

Flow cytometry was performed as described previously[21]. In brief, hMSC-EVs were attached to 4 µm aldehyde/sulfate latex beads (Invitrogen, Carlsbad, CA, USA) by mixing 5 µg of the sample with 10 µL of beads for 15 min. Final volume was made up to 1 mL with PBS and mixed for 2 h in a rotary shaker. The sample reaction was stopped by adding 100 mmol/L glycine (1 mL) and 2% BSA in PBS for 30 min in a rotary shaker. EVs were bound to the beads and incubated overnight at 4°C with Wnt3a. Beads were then incubated for 60 min at 37°C with fluorescein isothiocyanate (FITC)-labeled anti-rabbit antibody. They were then resuspended in 1 mL PBS for flow cytometric analysis

using BD FACS Aria III instrument as per the manufacturer's instructions (BD Biosciences, Franklin Lakes, NJ, USA).

EV Internalization Assay

hMSC-EVs were labeled with DiD dye (hMSC-EVs/DiD) as described previously[9]. In brief, The DP or ORS cells (1×10^4) were cultured on eight-well chamber slides and incubated overnight. The DP were then incubated with non-labeled hMSC-EVs (10 µg/mL) and hMSC-EVs/DiD (5 and 10 µg/mL) for 2 h at 37°C in 5% CO^2. The ORS cells were then incubated with non-labeled hMSC-EVs (5 µg/mL) and hMSC-EVs/DiD (2.5 and 5 µg/mL) for 2 h at 37°C in 5% CO_2. The cells were subsequently fixed in paraformaldehyde and mounted using mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA). The cellular internalization of hMSC-EVs into DP or ORS cells were viewed and captured by a confocal laser scanning microscope (LSM 800 with AiryScan, Zeiss, Oberkochen, Germany).

In Vitro Cell Proliferation Assay

DP or ORS cells were seeded (0.5×10^4/well) in 96-well plates and maintained overnight at 37°C and 5% CO2. Cells treated with hMSC-EVs (DP cells: 2, 4, 6, 8, and 10 µg/mL and ORS cells: 1–5 µg/mL) were maintained for 24 h at 37°C and 5% CO2. Ten microliters of CCK8 (CCK8 assay kit, Dojindo Molecular Technologies, Kyushu, Japan) solution was added to each well, and cellular proliferation was assessed 2 h later by optical density at 450 nm using a spectrophotometer as per the manufacturer's protocol.

β-Catenin Localization in DP Cells by Immunofluorescence Assay

DP cells (1×10^4) were seeded on an eight-well chamber slide and incubated over-night. Next, hMSC-EVs (10 µg/mL) were added and incubated for an additional 24 h. The cells in the chamber were then fixed with 4% paraformaldehyde and probed with primary anti-β-catenin antibody (Dilution 1:200; Cell Signaling Technology) overnight and washed with PBS. Next, fixed cells were incubated with Alexa Fluor FITC-conjugated anti-rabbit antibody for 60 min at room temperature for 45 min. Slides were washed with PBS thrice and mounted using mounting medium with DAPI (Vector

Laboratories). The images were analyzed using confocal microscopy (LSM 5 exciter: Zeiss, Oberkochen, Germany).

β-Catenin Trans-localization in DP Cells by Western Blotting

DP cells (1×10^6) were seeded on a 6-well plate and incubated over-night. Next, hMSC-EVs (5 and 10 µg/mL) were added and incubated for an additional 24 h. The nuclear fraction was isolated using a NE-PER™ Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher Scientific) according to manufacture instructions.

Real-Time Polymerase Chain Reaction (RT-PCR)

Cells were lysed using TRIzol solution (Invitrogen), and total RNA was extracted according to the manufacturer's instructions. Real-time PCR was performed as described previously[21] using SsoAdvancedTM Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) in CFX96 touch-Real-time PCR system (Bio-Rad). PCR primer sequences used in the study are listed in Table S1.

Cell Migration Assay

Migration assays were performed in 24-well cell culture inserts containing transparent PET membranes with 8.0-mm pores (BD Biosciences). Human ORS cells were seeded on the upper chamber insert at 5×10^3/well in 0.5 mL serum-free medium containing 0, 2.5, or 5 µg/mL hMSC-EVs and cultured for 24 h. Medium was supplemented with 10% FBS in the lower chamber as a chemoattractant. After 24 h, cells on the lower surface were fixed with 2% paraformaldehyde, stained with crystal violet, and viewed under phase-contrast microscopy and enumerated.

Hair Shaft Elongation of Human Hair Follicles

Human HFs were isolated and cultured as described previously[32]. HFs were treated with varying concentrations of hMSC-EVs (0, 0.1, 0.5, and 1 µg/mL), and hair shaft elongation was measured on day 6.

Statistical Analysis

All data are expressed as means ± standard deviation. Two-group comparisons were analyzed using Student's t-test in Microsoft Excel (Microsoft, Redmond, WA,

USA) or GraphPad Prism 9 software version 9.0.0(121) (GraphPad Software, San Diego, Inc., CA, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

Characterization of hMSC-EVs and Detection of Wnt3a Associated with EV-Membrane

The morphology of isolated hMSC-EVs was analyzed using TEM. The TEM imaging of hMSC-EVs showed that most hMSC-EVs were spherical in shape, a classical morphology of EVs. Moreover, hMSC-EVs were intact and undamaged by the isolation procedure (Figure 1A). The results of NTA of hMSC-EVs showed that their average diameter was 168.4 ± 78.4 nm (Mode: 144.3 nm) (Figure 1B). The western blotting analysis of biomarkers of EVs revealed that Alix was present in hMSC-EVs. Cytochrome C (a mitochondrial protein) and GM130 (a Golgi apparatus protein), which are negative EV markers, were absent in hMSC-EVs, confirming that hMSC-EVs were not contaminated with other cells or cell organelles. Moreover, the presence and enrichment of Wnt3a was greater in hMSC-EVs than in hMSCs (Figure 1C). Flow cytometry was used to confirm the location of Wnt3a in hMSC-EVs and showed that 34.22% of hMSC-EVs had Wnt3a on their membrane (Figure 1D, E).

hMSC-EVs Promote the Proliferation and Activation of DP Cells.

To examine the interaction and integration of hMSC-EVs with recipient DP cells, hMSC-EVs were labeled with DiD dye and the labeled hMSC-EVs/DiD cells were incubated with DP cells for 4 h. Confocal microscopy showed that hMSC-EVs interact and integrate inside the cells (Figure 2A). The effects of hMSC-EVs on the cellular proliferation of DP cells were examined, and the results showed that hMSC-EV treatment increases the proliferation of DP cells significantly ($p < 0.001$) with 2–6 µg/mL of hMSC-EVs and ($p < 0.01$) with 8–10 µg/mL of hMSC-EVs treatments (Figure 2B). Because hMSC-EVs showed the presence of Wnt3a, we examined the translocation of β-catenin into the nucleus of DP cells after the treatment of hMSC-EVs (10 µg/mL); this experiment revealed a strong signal in the nucleus of DP cells (Figure 2C). In addition to that we also showed dose-dependently elevated β-catenin level in the

nuclear fraction of hMSC-EVs-treated cells compared with that of control-treated cells (Figure 2D). Furthermore, we examined the expression of Wnt/β- catenin target transcription factors (Axin2, EP2, and Lef1). Real- time PCR results showed that there was a significant ($p < 0.001$ or $p < 0.01$) upregulation of Axin2, EP2, and Lef1 expression in DP cells in a dose-dependent manner compared with the control (Figure 2E).

hMSC-EVs Promote the Proliferation and Migration of Human ORS Cells.

Confocal microscopy showed the interaction and integration of hMSC-EVs into ORS cells (Figure 3A). The effect of hMSC-EV on the proliferation of ORS cells was investigated; the results showed that hMSC-EV treatment significantly increases the cellular proliferation of ORS cells ($p < 0.001$) with 1–5 µg/mL (Figure 3B). Because the migration of ORS cells is a hallmark of hair elongation, we examined the migration of ORS cells using hMSC-EVs. After treatment with hMSC-EVs (2.5 and 5 µg/mL), ORS cells showed significantly increased migration at both concentrations ($p < 0.01$ at 2.5 µg/mL and $p < 0.001$ at 5 µg/mL) compared with control in a dose-dependent manner (Figure 3C, D). Furthermore, we examined the expression of keratin differentiation markers (Keratin (K)-6, K16, K17, and K75) in ORS cells after the treatment of hMSC-EVs (2.5 and 5 µg/mL). The real- time PCR results showed a significant upregulation of all keratin mRNAs in a dose-dependent manner compared with the control. K75 showed highest expression ($p < 0.001$), followed by K16 ($p < 0.001$) and K6 ($p < 0.001$) at both concentrations; K17 showed a significant upregulation at 2.5 µg/mL ($p < 0.05$); and hMSC-EV treatment with 5 µg/mL showed no significant difference ($p > 0.05$) compared with control (Figure 3E).

hMSC-EVs Elongate Human Hair Follicles.

To examine the elongation of hair shafts, mini-organ culture was performed with human scalp HFs. HFs were treated with hMSC-EV (0, 0.05, and 0.01 µg/mL) and Wnt inhibitor-XAV939 (5 µM) treatments for 6 days; the results showed that hMSC-EVs increased hair shaft length significantly ($p < 0.01$) at 0.05 µg/mL and ($p < 0.001$) at 0.1 µg/mL compare to control (vehicle), The XAV939 treatment significantly ($p < 0.001$) reduced the hair shaft elongation compare to control (vehicle). Combination treatment

of the hMSC-EV (0.05, and 0.01 µg/mL) and Wnt inhibitor-XAV939 (5 µM) significantly ($p < 0.001$) abolished the hMSC-EV induced hair shaft elongation (Figure 4A, B).

DISCUSSION

EVs were isolated from hMSC culture medium by serial centrifugation, filtration, and ultracentrifugation. Isolated hMSC-EVs displayed intact EV morphology (round) and size distribution. Moreover, hMSC-EVs showed enriched Alix (typical biomarker of EVs) and a lack of cytochrome C (a mitochondrial marker), GM130 (a Golgi marker), and PCNA (a nuclear marker), which confirmed that our hMSC-EVs were not contaminated with cells/cell organelles, consistent with previous reports[9–11]. The Wnt/β-catenin signaling cascade is crucial for the development and maintenance of HFs[13,33]. The presence of Wnt3a in hMSC-EVs was confirmed; Wnt3a was more enriched in EVs than in cells. The enrichment of Wnt proteins into/onto EVs was well reported in several previous studies[34–37]. Furthermore, a significant portion of Wnt3a (34.22%) was associated with the EV membrane. Our previous study with macrophage- and fibroblast-derived EVs also showed that they have >90% (macrophage-derived EVs) or >70% (fibroblast-derived EVs) association with the EV membrane[18,21] and a recent study showed that Wnt3a, Wnt5a and Wnt7a were present on the surface of small EVs isolated from mouse hippocampal cell line (HT-22) and agree with our current study[37].

To exert the therapeutic effects of any EVs, an interaction with target/recipient cells or internalization into target/recipient cells is needed[6,7,13]. Our results revealed that hMSC-EVs actively interact and integrate themselves into DP cells. In the hair growth process, the activation and maintenance of the Wnt/β-catenin signaling cascade in DP cells are crucial[1,11]. In this study, we assessed the effects of hMSC-EVs on the proliferation of DP cells in vitro, which showed increased proliferation. Most studies with various EVs have shown an increase in DP cell proliferation upon treatments[11,13,15,17]. Furthermore, our results revealed that the hMSC-EV treatment of DP cells translocated β-catenin into the nucleus, a requirement for the activation of hair-

inducing transcription factors[38,39]. Our results revealed that hMSC-EVs increased the expression of hair-inducing transcription factors in DP cells (Axin2, EP2, and LEF1), similar results were observed in other studies, which used EVs for treatments[15,18,21].

ORS cells are a putative source of stem cells with therapeutic capacity. Their survival, migration, and differentiation are important for hair follicle maintenance[40,41]. Our results showed that the interaction and integration of hMSC-EVs into ORS cells increased cellular proliferation and migration, which are necessary for hair growth. Furthermore, hMSC-EV treatment increased the expression of differentiation markers (K6, K16, K17, and K75), indicating the differentiation of cultured ORS cells into follicular lineages[42]. Finally, we investigated the hair-inducing properties of hMSC-EVs on human HFs, showing that hMSC-EVs increased hair shaft length, which was abolished by Wnt inhibitor. These findings suggest a potential for the therapeutic effects of hMSC-EVs in human HFs through Wnt/β-catenin signaling. Several other studies using the EVs on HFs have reported to increase hair shaft elongation[15,16,18,21].

In the present study, we showed the enrichment of Wnt3a in hMSC-EVs and some association of Wnt3a with the EV membrane; however, compared with macrophage- and fibroblast-derived EVs, hMSC-EVs showed a lower association of Wnt3a with membrane[18,21,37]. We have not ruled out that other proteins and/or miRNAs may play roles in hair regrowth because a few recent studies showed that miRNA-100, miR-NA-140-5p, and miRNA-218-5p play certain roles in hair regrowth[13,16,23]. Further, a complete proteomic and miRNA analysis is warranted to reveal a more complete understanding of hair growth promoted by hMSC-EV treatment.

CONCLUSION

The present study demonstrates that human bone marrow MSC-EVs enhanced hair growth by activating the hair follicular cells and hair follicles. The hMSC-EVs could be therapeutic candidates for the recovery of hair loss.

ARTICLE HIGHLIGHTS

Research background

Hair loss is one of the most common disorders in both genders. Despite several treatment options available but no definitive treatment method is available. Treatment of extracellular vesicles (EVs) has been proposed as an emerging therapy for hair loss.

Research motivation

Although cell derived EVs treatments have shown reasonable efficacy in studies of hair loss, the molecular mechanism and therapeutic effects are still relatively unknown.

Research objectives

We treated and examined the effects of human bone marrow-derived mesenchymal stem cells derived EVs (hMSC-EVs) in human dermal papillae (DP), outer root sheath (ORS) cells and hair follicles (HF).

Research methods

Various amounts of hMSC-EVs were treated in human DP cells, ORS cells and HFs to investigate the effect of hMSC-EVs on human cells *in vitro* and ex vivo.

Research results

The Wnt3a containing hMSC-EVs treatment increased the proliferation of DP cells, Wnt/β-catenin related cascade signaling and activated the transcription related to hair growth. Similarly, hMSC-EVs treatment increased the proliferation, migration and keratin differentiation in ORS cells. The *ex vivo* treatment of hMSC-EVs increased the human HF shaft elongation.

Research conclusions

To improve hair loss treatment, the hMSC-EVs may become a new strategy for hair loss treatment.

Research perspectives

We demonstrated the effects of hMSC-EVs on hair cells and HFs, which suggests that the success in future experiments may pave way for clinical applications.

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