Journal of Dermatological Science xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

LETTER TO THE EDITOR

Hair growth-promoting effects of adipose tissue-derived stem cells

To the Editor,

Adipose tissue-derived stem cells (ADSCs) can display multilineage plasticity and share similar characteristics with bone marrow-derived mesenchymal stem cells [1]. Moreover, ADSCs have various cytokine-secreting properties and beneficial paracrine effects on surrounding cells or tissues [2]. Recently, paracrine function is considered one of the most important therapeutic benefits of therapy using mesenchymal stem cells [3,4].

Regarding hair biology, several reports have demonstrated that growth factors from the surroundings stimulate hair growth in *ex vivo* and animal models [5,6]. We have identified secretory factors derived from ADSCs by ELISA and proteomics previously, including IGF binding protein precursors, PDGF, KGF, HGF, VEGF, and fibronectin [7,8]. These factors are well-documented to be related to hair growth stimulation [6]. To determine whether these paracrine effects of ADSCs can promote hair growth, we investigated the effects of ADSCs on hair.

Human ADSCs were cultured as previously described [9]. Subcutaneous adipose aspirates ($n = 5$, females, mean age = 43.8 years) were acquired during liposuction. ADSCs were isolated from the aspirates by 0.075% collagenase type I (Sigma–Aldrich, St. Louis, MO, USA) for 45 min at 37 °C, filtered through 70 μm mesh filters, and mixed with α-modified Eagle medium (α MEM; Invitrogen, Carlsbad, CA, USA), and then centrifuged. The ADSC fraction (pellet) was washed, centrifuged at 1200 × *g* for 5 min, and then resuspended in α-MEM with 10% FBS and cultured in 5% CO_2 at 37 °C. Adipogenic differentiation from ADSC toward adipocyte was induced by insulin, dexamethasone, isobutyl-methyl-xanthine, and indomethacin for 9–15 days.

For harvesting the conditioned media (CM), ADSCs (5×10^5 cells) and mature adipocytes were cultured in α-MEM serum-free medium. Conditioned medium of ADSCs (ADSC-CM) and adipocytes (adipocyte-CM) were collected after 48 h, centrifuged, and filtered using a 0.22 μm syringe filter.

As hair cycle changes involve rapid remodeling of both epithelial and dermal components, proliferation of cultured human dermal papilla cells (hDPCs) and immortalized keratinocytes, HaCaT cells, were studied. Human dermal papilla cells (hDPCs) and HaCaT cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen-Gibco-BRL) with 10% FBS. The cell proliferation after ADSC-CM treatment was determined by MTT assay using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide. We used different sources of ADSCs for independent experiments.

ADSC-CM treatment significantly enhanced proliferation of hDPCs up to 130% at a concentration of 25, 50 and 75%, at 48 h, while adipocyte-CM inhibited proliferation (Fig. 1A).

To clarify the underlying mechanism, signaling proteins related to proliferation were analyzed by Western blot. Primary antibodies against phospho-Akt (Ser473; Cell Signaling Tech., Inc., MA), Akt (Cell Signaling Tech., Inc.), and phospho-Erk (Thr202/Tyr204; Cell Signaling Tech., Inc.) were used. The level of pAkt was increased 24 and 48 h after ADSC-CM treatment compared to that of total Akt (Fig. 1B). Moreover, the expression of pErk was clearly increased with ADSC-CM, while total Erk remained unchanged.

Next, cell cycle analysis of ADSC-CM-treated hDPCs was performed using a FACScan flow cytometer (Beckton-Dickinson, CA). hDPCs (3.2×10^5 cells/dish) were starved to G1 arrest. Then, media was replaced with serum-free media or 50% ADSC-CM for 48 h. ADSC-CM decreased the phase of cell growth (G1), while the phases of DNA synthesis (S) and mitosis (G2-M) were increased (Fig. 1C upper right panel). Subsequent Western blotting revealed that expression of p21, p27, and CDK4 were not changed (not shown), while cyclin D1 (Serotec, UK) and CDK2 (Santa Cruz Biotechnology), cell cycle-related key molecules, were clearly up-regulated by ADSC-CM after 24 h (Fig. 1C).

From the results presented here indicated that ADSC-CM led to the increase in proliferation of hDPCs through the modulation of the cell cycle. In addition, ADSC-CM activated both Erk and Akt signaling pathways, which indicated the enhancement of survival and proliferation of DPCs. DP size is reported to be well-correlated with hair growth cycle, and the cell number of DP is increased in anagen phase [10].

ADSC-CM also promoted the proliferation of HaCaT cells significantly at a concentration of 10% to 30% (Fig. 1D). Since ADSC-CM showed marked mitogenic effects on cells, we studied the effects of ADSC-CM on hair shaft elongation. A total of 370 anagen hair follicles from 5 male volunteers (mean age = 34.8 years, 70–80 follicles per subject) were cultured in Williams E medium (Gibco BRL, Gaithersburg, MD, USA) with 10 ng/mL hydrocortisone, 10 g/mL insulin, 2 mM L-glutamine, and 100 U/mL penicillin at 37 °C in a 5% CO_2. After the addition of 12.5%, 25%, 37.5%, or 50% ADSC-CM to William's E medium, the length of hair follicles in 12.5% ADSC-CM-treated group significantly elongated by 40%, as much as that in positive control treated with 1 mM of minoxidil, compared to the control after 6 days (Fig. 2A).

The back skin of 7-week-old male C3H/HeN nude mice (Orient Bio, Korea) was shaved ($n = 48$) and their hair follicles were synchronized in telogen stage. ADSCs (5×10^5 cells/50 mL PBS) or PBS were subcutaneously injected into dorsal skin every 3rd day for 9 days. Concurrently, ADSC-CM (1 mL) or control medium was topically applied on the back of the other C3H mice. After intradermal injection of ADSCs on the back of C3H/HeN mice at the age of 7 weeks, the conversion of telogen to anagen was induced earlier than in controls (Fig. 2B upper panel). We also found accelerated hair growths after topical application of ADSC-CM (Fig. 2B lower panel). Histologically, the back skin of ADSC-CM-treated mice showed increased number of hair follicles (Fig. 2C).

0923-1811/$36.00 © 2009 Published by Elsevier Ireland Ltd on behalf of Japanese Society for Investigative Dermatology.
doi:10.1016/j.jdermsci.2009.10.013

Please cite this article in press as: Won CH. Hair growth-promoting effects of adipose tissue-derived stem cells. J Dermatol Sci (2009), doi:10.1016/j.jdermsci.2009.10.013

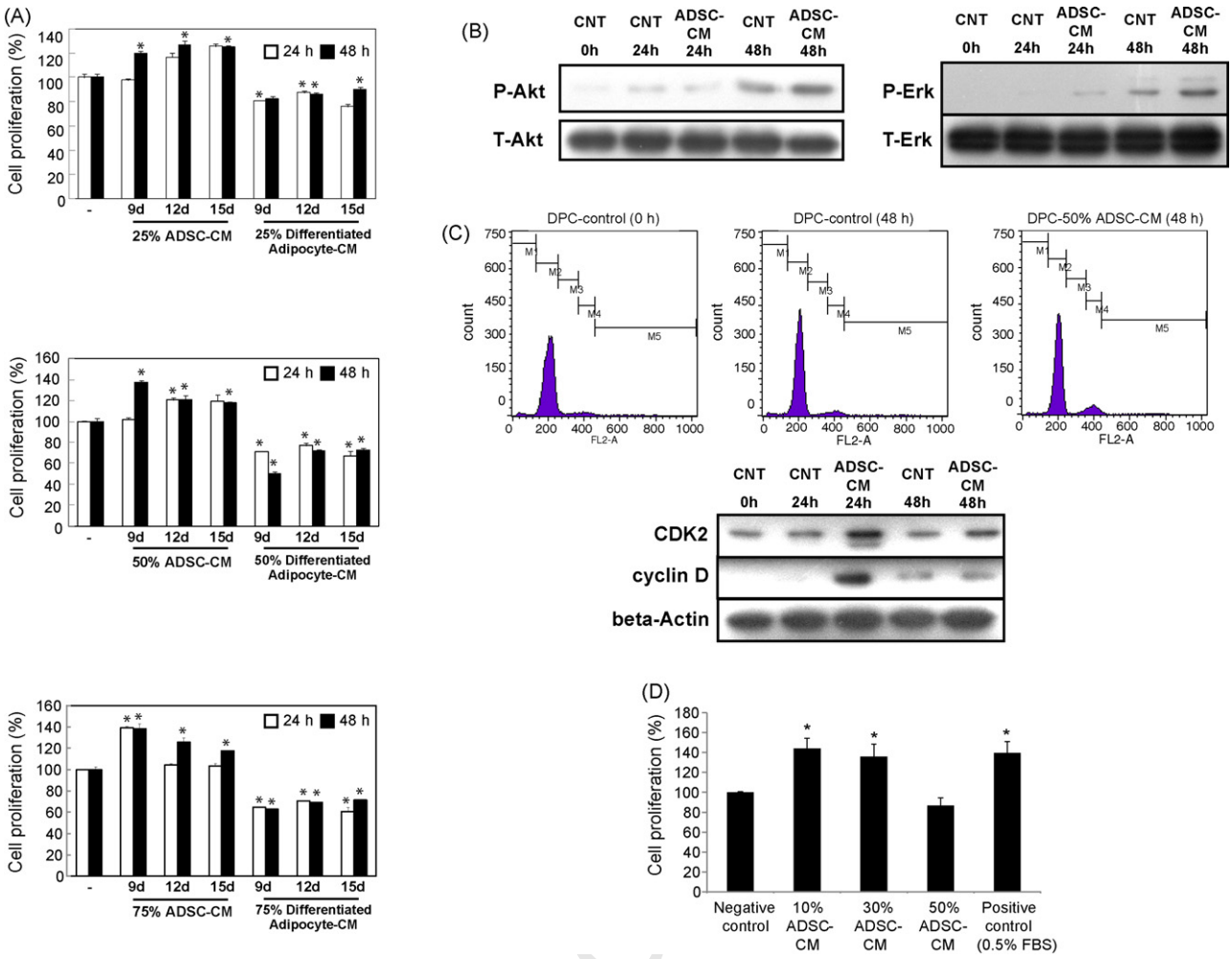

Fig. 1. The effects of conditioned medium of adipose tissue-derived stem cells (ADSC-CM) on human follicular DPCs. (A) The separated ADSCs were maintained in α-MEM for 9, 12, and 15 days. Adipogenic differentiation from ADSC toward adipocyte was induced by adipogenic differentiation medium (insulin, dexamethasone, isobutyl-methyl-xanthine, and indomethacin) for 9, 12, and 15 days. The conditioned media (CM) from cultured ADSCs and differentiated adipocytes in serum-free conditions were prepared after 48 h incubation, respectively. Human DPCs were incubated with ADSC-CM and adipocytes-CM and proliferation was measured by MTT assay after 24 h and 48 h. ADSC-CM treatment for 48 h significantly enhanced proliferation of cultured DPCs, while mature adipocytes inhibited proliferation. * $p < 0.05$ vs. negative control. Student's t test was used for statistical analysis. All results are the means of experiments performed at least in three or more independent experiments. We used different source of ADSC for MTT assay of independent experiments. A "d" in this figure indicates the number of the days for induction toward mature adipocytes from ADSCs with adipogenic medium or the days for maintenance of ADSCs. (B) The level of phosphorylated Akt was slightly increased in DPCs after ADSC-CM treatment (left panel). The expression of phosphorylated Erk was clearly increased in DPCs with ADSC-CM incubation (right panel). (C) ADSC-CM led to the increase in proliferation of DPCs through the modulation of the cell cycle (upper panel). ADSC-CM treatment on cultured human DPCs for 48 h led to the increase in S phase and reduction in G1 arrest (upper right) compared with control (middle). Expression of cyclin D and CDK2 was clearly up-regulated by ADSC-CM treatment after 24 h (lower panel, Western blot). (CNT: control, ADSC-CM: ADSC-conditioned media treated human dermal papilla cells). (D) HaCaT cells were incubated with ADSC-CM for 48 after 24 h' starvation and proliferation was measured by MTT assay as well. ADSC-CM promoted the proliferation of HaCaT cells significantly at a concentration of 10% to 30%. *$p < 0.05$ vs. negative control. All results are the means of experiments performed at least in three or more independent experiments. We used different source of ADSC for MTT assay of independent experiments. Student's t test was used for statistical analysis.

Please cite this article in press as: Won CH. Hair growth-promoting effects of adipose tissue-derived stem cells. J Dermatol Sci (2009), doi:10.1016/j.jdermsci.2009.10.013

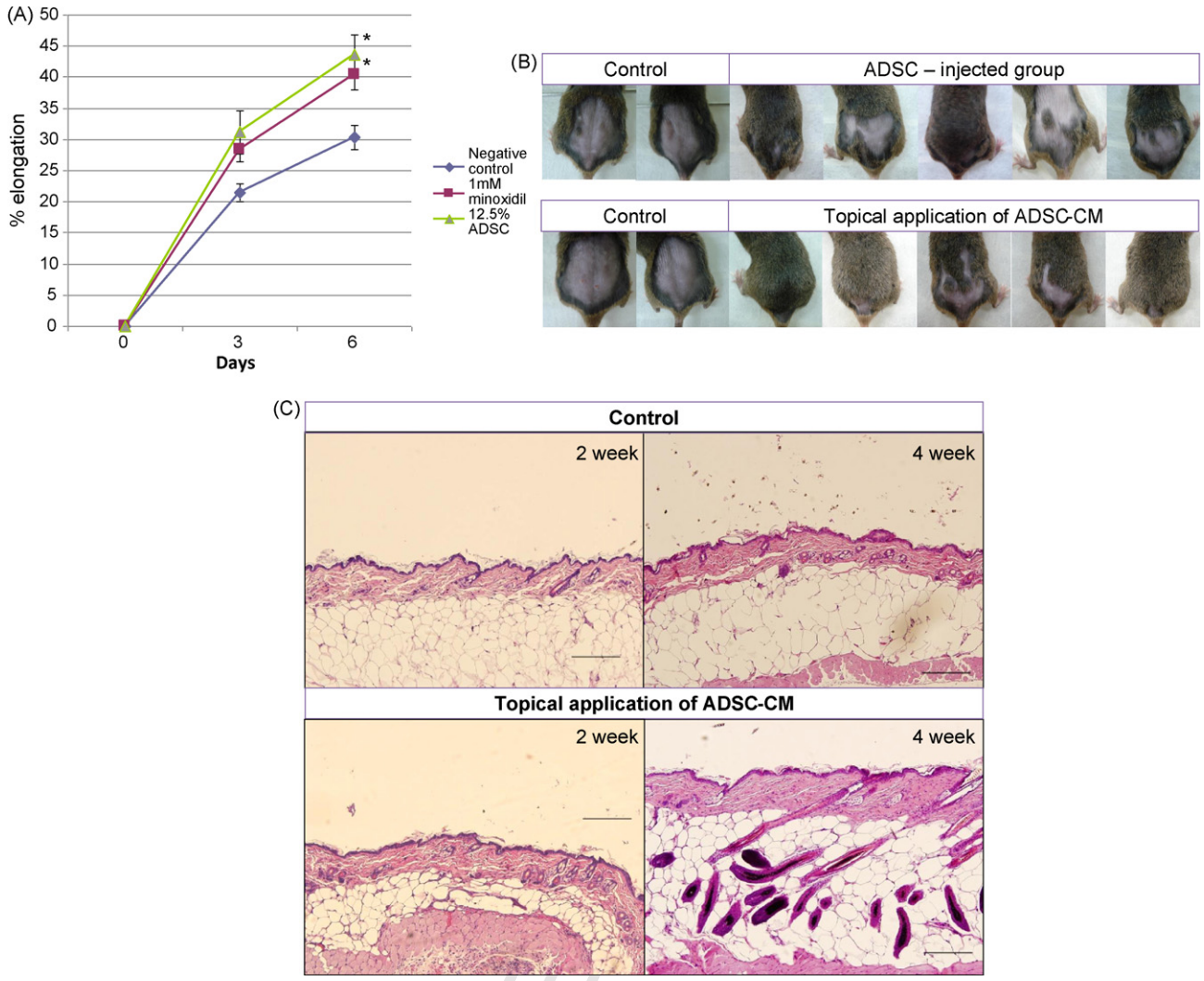

Fig. 2. The effects of adipose tissue-derived stem cells (ADSCs) on hair growth. (A) The effect of ADSC-conditioned medium (CM) on hair growth in *ex vivo* human anagen hair organ cultures (n = 5, male occiput, mean age = 34.8 years). 12.5% ADSC-CM was added to the basal Williams E medium. One-half volume of α-MEM was mixed with Williams E media as a negative control and minoxidil was added at 1 mM with Williams E as a positive control. We observed significant hair shaft elongation when 12.5% ADSC-CM was added to conventional William's E medium. Values were obtained after cumulative growth of more than 15 hair follicles per each growth condition for 6 days and shown as the means ± SEM. $^*p < 0.05$ vs. negative control. Student's t test was used for statistical analysis. Each of the five conditioned media of different ADSC was randomly applied to each of scalp samples from different volunteers. (B) Hair growth stimulation by ADSCs in C3H/HeN mice. The induced hair growth was studied in 7-week-old C3H/HeN mice and hair follicles were synchronously matched in telogen stage. ADSCs (5×10^5 cells) and PBS for control were intradermally injected into the dorsal skin of mice every 3rd day for 9 days. Hair growth was observed for 12 weeks after the first injection compared with the PBS-treated control. Enhanced hair growth was observed in the ADSC-treated group vs. the PBS-treated control after 12 weeks. Representative photos of ADSC injected mice (upper panel). For topical application group, to increase the skin absorption, a 0.2-mm mesoroller (Moohan, Korea) was used 10 times prior to treatment for treatment group and control. Hair growth stimulating effect was evaluated by the darkening of skin color. After 12 weeks, enhanced hair growth was also observed in the ADSC-CM topically applied group vs. control (lower panel). (C) Representative H&E stained sections of dorsal skin of control and ADSC-CM treated mice. Histologic evaluation was performed on the back skin of mice, at 2 and 4 weeks after the first topical application of ADSCs-CM. The back skin of ADSC-CM-treated mice showed increased number of hair follicles after 4 weeks (lower panel).

These findings indicated that locally injected ADSCs and ADSC-CM might stimulate hair growth *in vivo*. Although we are not sure this phenomenon really exists in physiologic conditions, some secretory factors from ADSCs might have more or less therapeutic potential for hair growth. The results presented here suggest that ADSCs promote hair growth by increasing the proliferation of DPCs, and possibly epithelial cells, through modulation of cell cycle, and activating anagen phase in hair cycles. Therefore, the rational manipulation of ADSCs might be a promising tool for hair growth promotion.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (R01-2007-000-10491-0).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2009.10.013.

References

[1] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–95.
[2] Cai L, Johnstone BH, Cook TG, Liang Z, Traktuev D, Cornetta K, et al. Suppression of hepatocyte growth factor production impairs the ability of adipose-derived stem cells to promote ischemic tissue revascularization. Stem Cells 2007;25:3234–43.
[3] Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367–8.

Please cite this article in press as: Won CH. Hair growth-promoting effects of adipose tissue-derived stem cells. J Dermatol Sci (2009), doi:10.1016/j.jdermsci.2009.10.013

[4] Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004;94:678–85.
[5] Danilenko DM, Ring BD, Pierce GF. Growth factors and cytokines in hair follicle development and cycling: recent insights from animal models and the potentials for clinical therapy. Mol Med Today 1996;2:460–7.
[6] Limat A, Hunziker T, Waelti ER, Inaebnit SP, Wiesmann U, Braathen LR. Soluble factors from human hair papilla cells and dermal fibroblasts dramatically increase the clonal growth of outer root sheath cells. Arch Dermatol Res 1993;285:205–10.
[7] Kim WS, Park BS, Kim HK, Park JS, Kim KJ, Choi JS, et al. Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J Dermatol Sci 2008;49:133–42.
[8] Park BS, Jang KA, Sung JH, Park JS, Kwon YH, Kim KJ, et al. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol Surg 2008.
[9] Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem 2004;14:311–24.
[10] Elliott K, Stephenson TJ, Messenger AG. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses. J Invest Dermatol 1999;113:873–7.

Chong Hyun Won[1]
Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

Hyeon Gyeong Yoo[ab1]
Oh Sang Kwon[ab]
Mi Young Sung[ab]
Yong Jung Kang[ab]
Jin Ho Chung[ab]
[a]*Department of Dermatology, Seoul National University College of Medicine, Seoul, Republic of Korea*
[b]*Laboratory of Cutaneous Aging and Hair Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science, Seoul National University, Seoul, Republic of Korea*

Byung soon Park[ab]
[a]*Leaders Clinic, Seoul, Republic of Korea*
[b]*Division of Stem Cell Research, Prostemics Research Institute, Seoul, Republic of Korea*

Jong Hyuk Sung
Department of Biomedical Science, CHA University, Seoul, Republic of Korea

Won Serk Kim
Department of Dermatology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Kyu Han Kim[ab*]
[a]*Department of Dermatology, Seoul National University College of Medicine, Seoul, Republic of Korea*
[b]*Laboratory of Cutaneous Aging and Hair Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science, Seoul National University, Seoul, Republic of Korea*

[*]Corresponding author at: Department of Dermatology, Seoul National University College of Medicine, Yongon-Dong 28, Chongno-Gu, 110-744 Seoul, Republic of Korea.
Tel.: +82 2 2072 3643; fax: +82 2 742 7344
E-mail address: kyuhkim@snu.ac.kr
(W.S. Kim)

[1]These authors contributed equally to this paper.

23 April 2009

Please cite this article in press as: Won CH. Hair growth-promoting effects of adipose tissue-derived stem cells. J Dermatol Sci (2009), doi:10.1016/j.jdermsci.2009.10.013