Whitening Effect of Adipose-Derived Stem Cells: A Critical Role of TGF-β1

Won-Serk Kim,[a] So-Hyun Park,[b] Se-Jin Ahn,[b] Hyung-Ki Kim,[b] Jeong-Soo Park,[c] Ga-Young Lee,[a] Kea-Jeung Kim,[a] Kyu-Kwang Whang,[d] Seung-Hee Kang,[e] Byung-Soon Park,*[,b,e] and Jong-Hyuk Sung*[,b]

[a] *Department of Dermatology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine; Seoul 110–746, Korea:* [b] *Division of Stem Cell Research, Prostemics Research Institute; 60–6 Nonhyun-dong, Kangnam-gu, Seoul 135–010, Korea:* [c] *Department of Biochemistry, College of Medicine, Dankook University; Chunan 330–714, Korea:* [d] *Department of Dermatology, College of Medicine, Ewha Womans University; Seoul 120–750, Korea: and* [e] *Leaders Clinic; Seoul 135–010, Korea.* Received November 3, 2007; accepted January 21, 2008; published online January 22, 2008

It has been demonstrated that adipose-derived stem cells (ADSCs) secrete cytokines and exhibit diverse pharmacological actions. The present study examined the unknown pharmacological action of ADSCs regarding whitening effects. A conditioned medium of ADSCs (ADSC-CM) was harvested and the whitening effect of ADSC-CM was studied in melanoma B16 cells. ADSC-CM treatment inhibited the synthesis of melanin and the activity of tyrosinase in a dose dependent manner. To clarify the underlying mechanisms of the whitening action of ADSCs, protein levels of melanogenic proteins were measured by Western blot. Although expressions of microphthalmia-associated transcription factor and tyrosinase-related protein 2 (TRP2) remained unchanged, those of tyrosinase and TRP1 were down-regulated. Transforming growth factor-β1 (TGF-β1), a potent regulator of melanogenic proteins, was neutralized by the addition of a blocking antibody to ADSC-CM, and down-regulated expression of tyrosinase and TRP1 was almost reversed. Collectively, these results indicate that secretary factors of ADSC inhibit melanin synthesis by down-regulating the expression of tyrosinase and TRP1, which are mainly mediated by TGF-β1.

Key words adipose-derived stem cell; melanin; tyrosinase; tyrosinase-related protein 1 (TRP1); transforming growth factor-β1 (TGF-β1)

Melanin is a main component of human pigmentary system, plays an important role in protecting human skin from harmful effects of UV radiation, and scavenges toxic drugs and chemicals.[1)] The first two steps in the pathway of biosynthesis for melanin are hydroxylation of L-tyrosine to 3-4-dihydroxyphenylalanine (L-dopa) and the oxidation of L-dopa to *o*-dopaquinone. *o*-Quinone is highly reactive and spontaneously forms melanin pigmentation, which causes serious aesthetic problems in humans.[2,3)] Tyrosinase, a multifunctional, glycosylated, copper-containing oxidase with a molecular weight of 60—70 kDa, is the key enzyme in melanin biosynthesis.[4,5)] Therefore, tyrosinase inhibitors are major candidates for skin whitening agents. In the absence of thiolic compounds, dopaquinone evolves spontaneously to L-dopachrome, and then to melanin.[2)] Two other enzymes, tyrosinase-related protein1 (TRP1) and TRP2, have been shown to participate in this processes.

Mesenchymal stem cells within the stromal-vascular fraction of subcutaneous adipose tissue, adipose-derived stem cells (ADSCs), display multi-lineage developmental plasticity and are similar to bone marrow-derived mesenchymal stem cells (BM-MSCs) with respect to surface markers and gene profiling.[6—9)] In addition, BM-MSCs and ADSCs produce various cytokines such as vascular endothelial growth factor, hepatocyte growth factor and transforming growth factor (TGF).[10—12)] Recently, the production and secretion of cytokines has been reported as an essential function of ADSCs.[12—14)] For example, our group demonstrated that ADSCs has an antioxidant effect through the production of diverse antioxidant cytokines.[15)] It has been reported that antioxidants scavenge free radical and usually exhibit whitening action.[1,5)] Antioxidants inhibit the chemical reactions leading to melanin formation, change the type of melanin formed, and interfere with the distribution of pigment and melanosome transfer, so they are good candidates for skin whitening resources.

Cytokines reportedly regulate melanogenesis and are major regulators of tyrosinase and related proteins (TRPs). TGF-β1 inhibits pigment formation and is able to interfere with tyrosine synthesis and possibly with the intracellular stability of the protein itself.[2,16,17)] In addition, interleukin and tumor necrosis factor-α (TNF-α) are able to decrease pigmentation by acting on tyrosinase activity, although they exhibit an inhibitory effect in much higher concentrations.[2,4)] In our previous study, TGF-β1 and interleukine-6 (IL-6) were detected in the conditioned medium of ADSCs (ADSC-CM) by proteomic analysis, implying that ADSC is a good candidate for a skin-whitening agent.[15)] Therefore, we examined the inhibitory effect of ADSC-CM on melanin biosynthesis in B16 melanoma cells. In addition, the underlying molecular mechanisms of the whitening action of ADSC-CM were further investigated in this study.

MATERIALS AND METHODS

Materials Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, and 0.5% trypsin–EDTA were purchased from Gibco-BRL (Invitrogen-Gibco-BRL, Grand Island, NY, U.S.A.). Antibodies against tyrosinase, TRP1, TRP2, and microphthalmia-associated transcription factor (MITF) were obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), and α-melanocyte stimulating hormone (α-MSH), mushroom tyrosinase and L-dopa were purchased from

* To whom correspondence should be addressed. e-mail: prostem@empal.com

Sigma (Sigma-Aldrich, St. Louis, MO, U.S.A.). Horseradish peroxidase-conjugated anti-goat antibody was obtained from Zymed (Zymed, San Francisco, CA, U.S.A.). All other reagents and materials were purchased from Millipore (Millipore, Bedford, MA, U.S.A.).

Isolation and Culture of ADSCs Human subcutaneous adipose tissue samples were obtained from lipoaspiration/liposuction procedures and digested in collagenase type II (Sigma) under gentle agitation for 45 min at 37 °C, filtered with 70 μm mesh filters, and mixed with histopaque-1077, and then centrifuged at 2000 rpm for 10 min. The ADSC fraction was washed with Hank's balanced salt solution (HBSS), centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. The cell pellet was resuspended in DMEM supplemented with 10% FBS and cultured in 5% CO_2 at 37 °C.

Harvest and Preparation of ADSC-CM ADSCs (4×10^5 cells) were cultured in DMEM/F12 (Invitrogen-Gibco-BRL, Grand Island, NY, U.S.A.) serum-free medium. ADSC-CM was collected after 72 h of culturing, centrifuged at 300 ***g*** for 5 min, and filtered through 0.22 μm syringe filter.

Cell Culture and Cell Viability Test of Melanoma B16 Cell Melanoma B16 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 at 37 °C. For cell survival assay, cells (1×10^3 cells/well) were plated in 96-well plates in DMEM/F12 with 0.1% FBS for 24 h to arrest mitosis. After incubating cells with ADSC-CM for 48 h, cells were added with 10 μl of the CCK-8 solution, and incubated for 3 h. The absorbance was measured at 450 nm using a microplate reader (TECAN, Grödig, Austria). OD values of each well were calculated to their relative cell numbers with comparable standard curves.

Measurement of Melanin Content Melanoma B16 cells (1.5×10^4 cells/well) were stimulated with or without α-MSH and pretreated with various concentration (10, 50, 100%) of ADSC-CM for 72 h. After washing with PBS, the cells were dissolved in 100 μl 1 N NaOH containing 50% DMSO at 80 °C and optical density at 492 nm was measured using a microplate reader. The melanin content was calculated by using an authentic standard of synthetic melanin.

Measurement of Tyrosinase Activity in Melanoma B16 Cells Tyrosinase activity was examined in melanoma B16 cells. After incubation with ADSC-CM for 72 h, melanoma B16 cells (1.5×10^4 cells/well) were removed from culture dishes and washed with phosphate-buffered saline. The cell pellet was rendered soluble in 0.1 M sodium phosphate buffer (pH 6.8) containing 0.5% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and centrifuged at 13000 rpm for 20 min at 4 °C. The supernatant was dialyzed against 0.1 M sodium phosphate buffer (pH 6.8) and then used as a source of murine tyrosinase. L-Dopa oxidation activity of tyrosinase was spectrophotometrically determined as described previously.[18] Forty microliters of 25 mM L-dopa, 80 μl 0.1 M sodium phosphate buffer (pH 6.8), and 40 μl of the test sample were added to a 96-well plate. Then, 40 μl of tyrosinase were mixed and incubated at 37 °C. The initial rate of dopachrome formation from the reaction mixture was measured as the increase of absorbance at 492 nm.

Western Blot Analysis Melanoma B16 cells (1×10^5 cells/well) were stimulated with α-MSH and incubated with various concentration of ADSC-CM. The cells were then dissolved in a lysis buffer (50 mM Tris–HCl, 0.15 M NaCl, 1 mM EDTA, 1% Triton X-100, 1% SDS, 50 mM NaF, 1 mM Na_3VO_4, 5 mM dithiothreitol, 1 μg/μl leupeptin and 20 μg/μl PMSF, pH 7.4). Twenty-five micrograms of proteins were separated on an 8% SDS-polyacryamide gel by electrophoresis. The proteins were transferred to polyvinyl difluoride (PVDF) membranes. PVDF membranes were incubated with anti-tyrosinase, MITF, TRP1, TRP2 and α-actin antibody, washed, and incubated with horseradish peroxidase-conjugated anti-goat IgG antibody (1 : 10000 dilution). The blots were reacted with immunobilon western reagent and exposed to X-ray film.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis Melanoma B16 cells (1×10^5 cells/well) were exposed to ADSC-CM with varying concentrations. Total cellular RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.) followed by a reverse transcription with cDNA synthesis kit (Promega, Madison, WI, U.S.A.). cDNA was synthesized from 1 μg of total RNA using 200 U of reverse transcriptase (M-MLV RT) and 20 pM oligodT. The following oligonucleotides were used as primers: tyrosinase (5′- cctcgagcctgtgcctcc-3′ and 5′-gttctcatccccagttag-3′), TRP-1 (5′-ttgtggctcatcatcagg -3′ and 5′-ttgagggtgagttgtgcg-3′), and the internal control β-actin (5′-accctgaagtaccccatcg-3′ and 5′-caccggagtccatcacg-3′). PCRs were performed in a final volume of 20 μl reaction mix that contained 2 μl of the RT reaction mixture, 15 mM $MgCl_2$, 1.25 mM dNTP, 20 pM of each primer and 0.5 U of *Taq* polymerase (Promega, Madison, WI, U.S.A.). Thermal cycling of 30 times, consisted of an initial denaturation at 94 °C for 5 min, then 94 °C for 30 s, 51 °C for 30 s, 72 °C for 30 s, and was terminated by a final extension at 72 °C for 5 min. β-Actin mRNA level was used for sample standardization.

TGF-β1 Blocking by Neutralizing Antibody To clarify the mechanism involving the down-regulation of pigmenting enzymes, TGF-β1 inhibition study using neutralizing antibody (Cambrex, Walkersvill, MD, U.S.A.) was carried out. TGF-β1 (1 μg/ml) antibody was added in 50% (v/v) ADSC-CM, which was incubated with B16 melanoma cells for 24 h. Expression of tyrosinase and TRP1 were measured by Western blot analysis.

Statistical Analysis Data are representative of three or more independent experiments. A one-way ANOVA test, followed by a paired *t*-test, was used for statistical analysis with $p<0.05$ considered to be significant.

RESULTS

Characterization of ADSC In our isolation and culture methods, ADSCs expanded easily *in vitro*, and exhibited a fibroblast-like morphology similar to that of BM-MSCs.[19] In flow cytometry, the expression of the stromal-associated markers, CD73 and CD105, was initially low immediately following isolation, but increased to 97.47% (CD73) and 97.63% (CD105) in ADSCs of passage 3. CD 90, also considered a marker for mesenchymal stem cells, was expressed on more than 80% of freshly isolated ADSCs and highly expressed throughout the passages (96.13% in passage 3). In contrast, the hematopoietic lineage markers CD34 and CD49d (integrin α4) were not observed in expanded ADSCs.

Effect of ADSC-CM on Cell Viability To exclude the possibility that inhibitory effect of ADSC-CM on melanogenesis might be caused by suppression of cell growth, a cell viability test was performed. However, ADSC-CM had no effect on cell viability during this experiment.

Inhibition of Melanin Contents The effect of ADSC-CM on the melanogenic activities of B16 melanoma cells was tested. Treatment of cells with α-MSH markedly increased melanin contents of B16 cells and this was included as a positive control for cell responsiveness. As shown in Fig. 1, ADSC-CM mediated a noticeable decrease of melanin contents in a dose dependent manner.

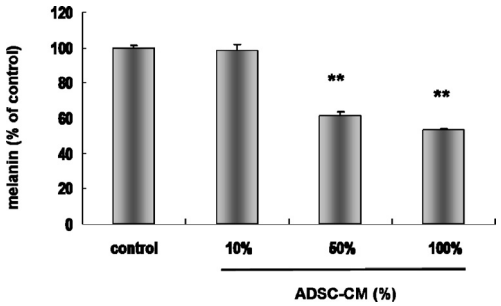

Fig. 1. Effect of ADSC-CM on the Melanin Contents of Melanoma B16 Cells

ADSC-CM inhibited melanin synthesis in a dose-dependent manner. $**p<0.01$.

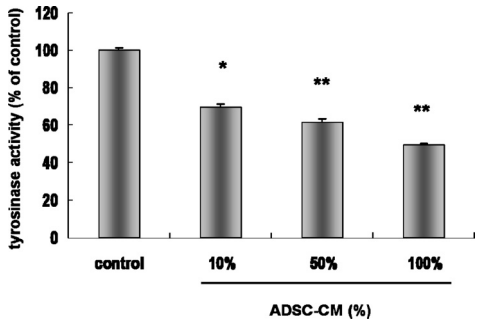

Fig. 2. Effect of ADSC-CM on the Tyrosinase Activity of Melanoma B16 Cells

ADSC-CM inhibited the tyrosinase activity in a dose-dependent manner. $*p<0.05$, $**p<0.01$.

Inhibition of Tyrosinase Activity Treatment of ADSC-CM in the culturing medium of B16 melanoma cells significantly reduced the tyrosinase activity of B16 cells in a dose dependent manner (Fig. 2). To characterize the inhibition mechanism of ADSC-CM, substrate specificity of ADSC-CM on the extracted tyrosinase from mushroom and B16 cells was investigated, but ADSC-CM was not the substrate (inhibitor) of tyrosinase (data not shown). It is reasonable to assume that ADSC-CM does not directly interact with active site of tyrosinase, but rather regulates the expression of tyrosinase. Therefore, the effect of ADSC-CM on the expression of melanogenic enzymes was studied further.

Differential Regulation of Melanogenic Protein Expression by ADSC-CM MITF regulates both melanocyte proliferation and melanogenesis, and is a major regulator of tyrosinase and TRPs. Therefore, expression of MITF was examined after ADSC-CM treatment, but it was not changed (Fig. 3). However, expression of tyrosinase and TRP1 were dramatically down-regulated after 24-h incubation with ADSC-CM in a dose-related manner (Fig. 3). TRP2 expression was also measured, but was not inhibited. Therefore, it can be deduced that the inhibitory effect of ADSC-CM on the melanin synthesis is mediated by down-regulation of tyrosinase and TRP1 in this study (Fig. 4).

mRNA Expression of Tyrosinase and TRP1 To assess ADSC-CM-induced modulations of mRNA expression level, RT-PCR was performed on tyrosinase and TRP1 (Fig. 5). ADSC-CM treatment slightly decreased the mRNA expression of tyrosinase after 72 h, but the effect was negligible. Similarly, mRNA expression of TRP1 remained unchanged.

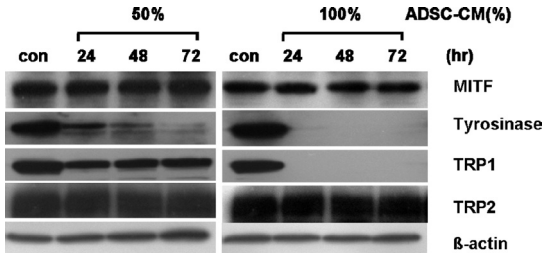

Fig. 3. Effect of ADSC-CM on the Expression of Melanogenic Proteins

Expression of MITF remained unchanged, but expression of tyrosinase and TRP1 were down-regulated by ADSC-CM treatment.

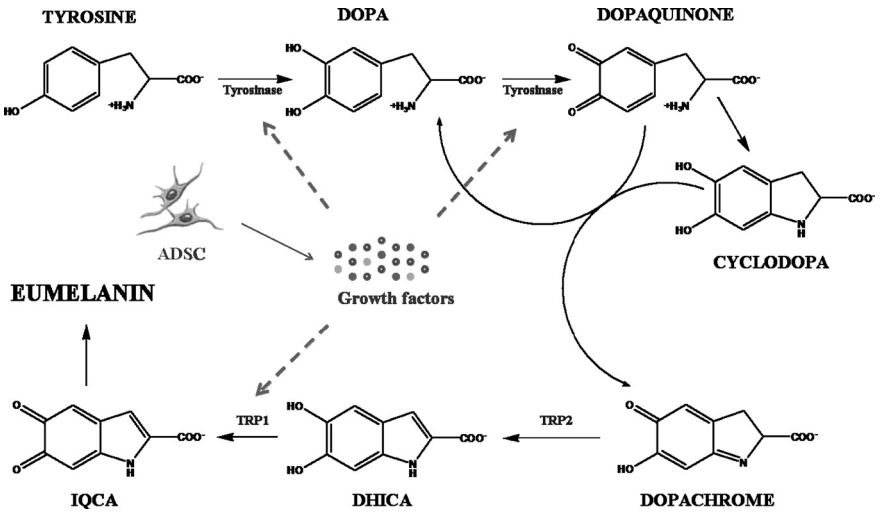

Fig. 4. Scheme Represents the Inhibitory Effect of ADSC on Melanin Synthesis

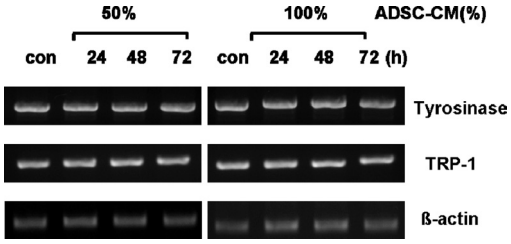

Fig. 5. mRNA Expression of Tyrosinase and TRP1

mRNA expression was not modulated by ADSC-CM treatment.

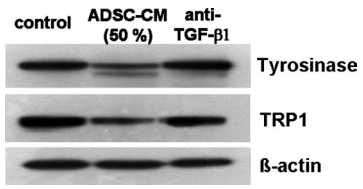

Fig. 6. Controversial Effect of Blocking TGF-β1 on the Expression of Melanogenic Proteins after ADSC-CM Treatment for 24 h

Neutralizing TGF-β1 reversed the down-regulated tyrosinase and TRP1 expression by ADSC-CM.

It is reasonable to conclude that ADSC-CM does not suppress the mRNA expression of tyrosinase and TRP1, but increases the rate of degradation of tyrosinase and TRP1. An increased rate of proteolytic degradation of tyrosinase and TRP1 was demonstrated previously.[2)]

Critical Role of TGF-β1 in the Inhibition of Melanin Synthesis It has been previously demonstrated that TGF-β1 inhibited melanin synthesis and this effect was mediated by high affinity receptors with IC_{50} values of 10^{-11} M.[2)] In an ELISA assay of TGF-β1, a similar level of this growth factor was detected in ADSC-CM,[19)] which might be responsible for the inhibition of melanin synthesis by ADSC-CM. To test this hypothesis, neutralizing antibody of TGF-β1 was added with 50% ADSC-CM for 24 h. As expected, down-regulated expression of tyrosinase and TRP1 by ADSC-CM was almost reversed by blocking TGF-β1 (Fig. 6), which suggests a critical role for this growth factor in the inhibition of melanin synthesis.

DISCUSSION

Control of melanin production is an important process treating abnormal skin pigmentation. Modulators of melanin synthesis generally act on the melanogenic proteins or the transfer of melanosomes from melanocytes to keratinocytes. In this study, ADSC-CM inhibited melanin synthesis in B16 melanoma cells. Tyrosinase activity, which plays a pivotal role in melanin synthesis, was also inhibited by ADSC-CM in a dose-dependent manner. To clarify the underlying mechanisms of the whitening action of ADSCs, expression level of melanogenic proteins were measured by Western blot. As a result, expressions of tyrosinase and TRP1 were down-regulated. TGF-β1, known to inhibit melanin synthesis, was neutralized by the addition of blocking antibody to ADSC-CM, and down-regulated expression of tyrosinase and TRP1 was sufficiently reversed. This is the first indication that ADSC has whitening effect that inhibits melanin synthesis by down-regulating tyrosinase and TRP1, which is mainly mediated by TGF-β1 secretion.

It has been reported that UV radiation-induced proliferation and melanogenesis of melanocytes was reduced by a topical application of an antioxidant such as vitamin C and E, to the skin of hairless mice.[1,20)] Reactive oxygen species are considered to play an important role in regulating the proliferation of melanocytes and keratinocytes, and in the melanogenesis of melanocytes. ADSC-CM is a free radical scavenger and has potent antioxidant activity,[15)] which may result in an inhibition of peroxidation in the skin, and in the inhibition of melanin synthesis. Besides tyrosinase activity and expression, antioxidants inhibit the chemical reaction leading to melanin formation, and either to a change in the type of melanin formed or to interfere with the distribution of pigment and melanosome transfer. However, not only antioxidant action but also secretion of specific cytokines by ADSC may have contributed to the whitening effect of ADSC-CM in this experiment. TGF-β plays an inhibitory role in pigment formation. It has been observed that low concentration of TGF-β1 is enough to interfere with tyrosinase activity and possibly with the intracellular stability of the protein itself.[2,17)] In an ELISA assay of TGF-β1, high level of this growth factor was detected in ADSC-CM,[15,19)] which might be responsible for the inhibition of melanin synthesis by ADSC-CM. To test this hypothesis, neutralizing antibody of TGF-β1 was added with the ADSC-CM in this experiment, and down-regulated expression of tyrosinase and TRP1 were reversed by blocking TGF-β1. Other cytokines such as ILs and TNF-α were detected in ADSC-CM, but concentrations were much lower than the IC_{50} value for tyrosinase activity. For example, 50% inhibition was obtained at 1 nM TNF-α, but its concentration in the ADSC-CM was lower than 10 pM.[21)] Therefore, it is reasonable to conclude that TGF-β1 secreted in the ADSC-CM played a pivotal role in the whitening action in this study.

MITF is a transcription factor having an essential basic helix–lop–helix–leucine zipper structure, and is believed to regulate melanocyte pigmentation, proliferation and survival.[22)] Mutations of the MITF gene in humans are known to cause abnormal pigmentation of skin and hair.[23,24)] In addition, it has been reported that MITF is a major transcriptional regulator of the melanogenic enzymes such as tyrosinase and TRPs.[25,26)] A plethora of growth factor signaling pathways is implicated in melanocyte biology, and some of them have been shown to be relevant to MITF.[17,25,27)] Therefore, the expression level of MITF was examined with ADSC-CM incubation in this study, but the expression level was not changed. TGF-β1 reportedly down-regulates MITF and induces a significant delay in extracellular signal related kinase (ERK) activation that also contributes to depigmentation.[2)] Although whitening action of ADSC-CM was mainly mediated by TGF-β1, we could not clarify why the expression of MITF was not changed in this study. Our suggestion is that other secretory factors of ADSC compensated for the down-regulated expression of MITF by TGF-β1.

In summary, ADSC has a whitening effect *via* a paracrine mechanism. ADSC-CM inhibited melanin synthesis and down-regulated melanogenic enzymes such as tyrosinase and TRP1. Neutralizing TGF-β1 in ADSC-CM sufficiently reversed the down-regulation of melanogenic enzyme expression, which implies that secretion of TGF-β1 plays a pivotal

role in inhibiting melanin synthesis by ADSC. Although further clinical studies are needed, administration of ADSC and their secretary factors have great promise for application in hyperpigmented skin disorders.

REFERENCES

1) Solano F., Briganti S., Picardo M., Ghanem G., *Pigment Cell Res.*, **19**, 550—571 (2006).
2) Martinez-Esparza M., Jimenez-Cervantes C., Beermann F., Aparicio P., Lozano J. A., Garcia-Borron J. C., *J. Biol. Chem.*, **272**, 3967—3972 (1997).
3) Briganti S., Camera E., Picardo M., *Pigment Cell Res.*, **16**, 101—110 (2003).
4) Martinez-Esparza M., Jimenez-Cervantes C., Solano F., Lozano J. A., Garcia-Borron J. C., *Eur. J. Biochem.*, **255**, 139—146 (1998).
5) Parvez S., Kang M., Chung H. S., Cho C., Hong M. C., Shin M. K., Bae H., *Phytother. Res.*, **20**, 921—934 (2006).
6) Haynesworth S. E., Baber M. A., Caplan A. I., *Bone*, **13**, 69—80 (1992).
7) Kim C. G., Lee J. J., Jung D. Y., Jeon J., Heo H. S., Kang H. C., Shin J. H., Cho Y. S., Cha K. J., Kim C. G., Do B. R., Kim K. S., Kim H. S., *Mol. Cells*, **21**, 343—355 (2006).
8) Zuk P. A., Zhu M., Ashjian P., De Ugarte D. A., Huang J. I., Mizuno H., Alfonso Z. C., Fraser J. K., Benhaim P., Hedrick M. H., *Mol. Biol. Cell.*, **13**, 4279—4295 (2002).
9) Zuk P. A., Zhu M., Mizuno H., Huang J., Futrell J. W., Katz A. J., Benhaim P., Lorenz H. P., Hedrick M. H., *Tissue Eng.*, **7**, 211—228 (2001).
10) Kinnaird T., Stabile E., Burnett M. S., Lee C. W., Barr S., Fuchs S., Epstein S. E., *Circ. Res.*, **94**, 678—685 (2004).
11) Kratchmarova I., Kalume D. E., Blagoev B., Scherer P. E., Podtelejnikov A. V., Molina H., Bickel P. E., Andersen J. S., Fernandez M. M., Bunkenborg J., Roepstorff P., Kristiansen K., Lodish H. F., Mann M., Pandey A., *Mol. Cell Proteomics*, **1**, 213—222 (2002).
12) Rehman J., Traktuev D., Li J., Merfeld-Clauss S., Temm-Grove C. J., Bovenkerk J. E., Pell C. L., Johnstone B. H., Considine R. V., March K. L., *Circulation*, **109**, 1292—1298 (2004).
13) Maeda K., Okubo K., Shimomura I., Funahashi T., Matsuzawa Y., Matsubara K., *Biochem. Biophys. Res. Commun.*, **221**, 286—289 (1996).
14) Maeda K., Okubo K., Shimomura I., Mizuno K., Matsuzawa Y., Matsubara K., *Gene*, **190**, 227—235 (1997).
15) Kim W. S., Park B. S., Kim H. K., Park J. S., Kim K. J., Choi J. S., Chung S. J., Kim D. D., Sung J. H., *J. Dermatol. Sci.*, **49**, 133—142 (2008).
16) Martinez-Esparza M., Solano F., Garcia-Borron J. C., *Cell. Mol. Biol.* (Noisy-le-grand), **45**, 991—1000 (1999).
17) Kim D. S., Park S. H., Park K. C., *Int. J. Biochem. Cell Biol.*, **36**, 1482—1891 (2004).
18) Shin N. H., Ryu S. Y., Choi E. J., Kang S. H., Chang I. M., Min K. R., Kim Y., *Biochem. Biophys. Res. Commun.*, **243**, 801—803 (1998).
19) Kim W. S., Park B. S., Sung J. H., Yang J. M., Park S. B., Kwak S. J., Park J. S., *J. Dermatol. Sci.*, **48**, 15—24 (2007).
20) Quevedo W. C., Holstein T. J., Jr., Dyckman J., McDonald C. J., Isaacson E. L., *Pigment Cell Res.*, **13**, 89—98 (2000).
21) Kilroy G. E., Foster S. J., Wu X., Sherwood J., Ruiz S., Heifetz A., Ludlow J. W., Stricker D. M., Potiny S., Green P., Halvorsen Y. D., Cheatham B., Storms R. W., Gimble J. M., *J. Cell. Physiol.*, **212**, 702—709 (2007).
22) Steingrimsson E., Moore K. J., Lamoreux M. L., Ferre-D'Amare A. R., Burley S. K., Zimring D. C., Skow L. C., Hodgkinson C. A., Arnheiter H., Copeland N. G., Jenkins N. A., *Nat. Genet.*, **8**, 256—263 (1994).
23) Tachibana M., *Pigment Cell Res.*, **10**, 25—33 (1997).
24) Tassabehji M., Newton V. E., Read A. P., *Nat. Genet.*, **8**, 251—255 (1994).
25) Bertolotto C., Abbe P., Hemesath T. J., Bille K., Fisher D. E., Ortonne J. P., Ballotti R., *J. Cell Biol.*, **142**, 827—835 (1998).
26) Bertolotto C., Busca R., Abbe P., Bille K., Aberdam E., Ortonne J. P., Ballotti R., *Mol. Cell Biol.*, **18**, 694—702 (1998).
27) Choi H., Ahn S., Lee B. G., Chang I., Hwang J. S., *Pigment Cell Res.*, **18**, 439—446 (2005).