Brief Report

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Upregulation of Bcl-2 enhances secretion of growth factors by adipose-derived stem cells deprived of oxygen and glucose

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Summary **There is an increasing recognition that beneficial effects of adipose-derived stem cell (ADSC) therapy may depend largely on the secretion of multiple growth factors. This study modified ADSCs with the *Bcl-2* gene in order to increase the secretion of growth factors during oxygen-glucose deprivation (OGD). The phenotypes of human ADSCs that were passaged 4 times were analyzed using flow cytometry. Then, ADSCs were genetically modified with *Bcl-2* and *Bcl-2* gene transduction was verified with Western blotting. Proliferative capacity and multipotent differentiation properties were evaluated in Bcl-2-modified ADSCs. Secretion of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF) was evaluated using an enzyme-linked immunosorbent assay (ELISA) during OGD. Human ADSCs that were passaged 4 times expressed stem cell-associated markers but not a fibroblast marker or a hematopoietic stem cell marker. The *Bcl-2* gene was efficiently transfected into ADSCs; Bcl-2 modification did not affect the proliferative and multilineage differentiation capacity of ADSCs. In addition, Bcl-2 overexpression enhanced the secretion of VEGF, bFGF, and HGF by 14.47%, 16.9%, and 91%, respectively, compared to ADSCs alone that were deprived of oxygen and glucose. These data suggest that Bcl-2 overexpression enhances secretion of angiogenic growth factors by ADSCs deprived of oxygen and glucose.**

Keywords: Adipose-derived stem cells (ADSCs), Bcl-2, gene transduction, growth factor

1. Introduction

Human adipose-derived stem cells (ADSCs) are adult pluripotent stem cells. Their use in stem cell therapy has attracted increasing attention in recent years (*1-3*). ADSCs have the potential to differentiate into multiple lineages, such as bone, cartilage, tendons, nerves, and fat when cultivated under lineage-specific conditions (*4-5*). Compared to bone marrow mesenchymal stem cells, ADSCs can be easily obtained in large amounts with less donor site morbidity and discomfort (*6-8*). Because of their convenient isolation, high level of auto-reproducibility, pluripotentiality, and immunosuppressive properties, ADSCs are increasingly recognized as a promising source of human stem cells for cell therapy. ADSCs have been used in basic and clinical studies for the treatment of many diseases (*9-13*). The therapeutic mechanism of ADSCs in cell therapy is based on their ability to directly differentiate into tissue specific cells as well as the paracrine actions of factors released from ADSCs (*14-16*). However, the harsh microenvironment results in poor viability for transplanted cells, and this may significantly limit the paracrine capacity of ADSCs. Therefore, protecting ADSCs from apoptosis and improving their paracrine function is critical to a successful therapy based on ADSCs.

The Bcl-2 antiapoptotic protein is a member of the Bcl-2 family, which plays an important role in regulating the mitochondrial apoptotic pathway to inhibit cell death (*17*). Bcl-2 overexpression can delay the occurrence of cell death (*18*). Bcl-2 deficiency leads to a loss of a death repressor in specific cells (*19*). As indicated by a previous study (*20*), Bcl-2 overexpression significantly reduces ADSC apoptosis. ADSCs are known to enhance

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the secretion of angiogenic and antiapoptotic factors under hypoxic conditions (*16*). The present study sought to investigate the secretion of growth factors by Bcl-2-modified ADSCs under ischemic conditions induced by oxygen-glucose deprivation (OGD) in an *in vitro* model.

2. Materials and Methods

2.1. *Isolation and culturing of human ADSCs*

Samples of human adipose tissue were obtained from three healthy female donors undergoing thigh liposuction. Informed consent was obtained from these donors and the study protocol was approved by an institutional review board. The aspirated fat was washed with PBS and then digested with 0.1% collagenase type I (Gibco, Life Technologies, CA, USA). The digested tissue was centrifuged to separate the stromal cell fraction from adipocytes and connective tissues. The cells were resuspended with complete Dulbecco's modified Eagle medium (DMEM) (Gibco) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Keygentec, Nanjing, China). The cells were passed through a 200-μm mesh and then centrifuged. The isolated cells were resuspended in complete DMEM, placed into 25-cm^2 flasks, and cultured at 37°C in a humidified atmosphere with 5% CO_2. The medium was replaced every 3 days. Cells were passaged at a ratio of 1:2 per week. Only cells that were passaged 4 times were used in this study.

2.2. *Characterization of human ADSCs*

ADSCs were harvested by treatment with 0.25% trypsin/EDTA (Gibco) and then resuspended at a total number of 2×10^6 cells in 500 μL PBS. ADSCs were labeled for 15 min at 4°C with the following monoclonal antibodies: anti-CD29-FITC (eBioscience, San Diego, CA, USA), anti-CD34-FITC (BD Biosciences, San Diego, CA, USA), anti-CD44-PE (BD Biosciences), anti-CD90-FITC (BD Biosciences), anti-CD133 (293C3)-PE (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), or HLA-DR-FITC (BD Biosciences). The cells were thoroughly washed with PBS before analysis. FITC- or PE-labeled mouse IgG (Biolegend, San Diego, CA, USA) was used as the isotype control. The cells were then analyzed with a BDFACSCanto flow cytometer (Becton-Dickinson, San Jose, CA, USA). Data were analyzed with the Cell Quest software package (Becton Dickinson, Bedford, Mass, USA).

2.3. *Gene transduction in ADSCs*

ADSCs that had been passaged 4 times were seeded in a six-well plate at 1×10^5 cells/well. At 80% confluence, the ADSCs were infected with an adenovirus encoding the enhanced green fluorescent protein gene (*Ad/EGFP*) at different multiplicities of infection (MOI = 0, 100, 200, and 500). At 48 h post-transduction, the transduction efficiency of the *EGFP* gene was examined under a fluorescence microscope (ZEISS, Germany) and with flow cytometry. The optimal MOI was used for *Bcl-2* gene delivery into ADSCs. ADSCs without gene transduction and those transfected with adenovirus carrying *Bcl-2* (Ad/Bcl-2) or adenovirus alone were termed "ADSCs", "Bcl-2-ADSCs", and "vector-ADSCs", respectively. Levels of Bcl-2 protein expression were evaluated using Western blotting.

2.4. *Measurement of doubling time*

ADSCs, vector-ADSCs, and Bcl-2-ADSCs were seeded at a density of 1×10^4 cells per cm^2 in six-well plates and incubated until they reached their logarithmic growth phase. The cells were then harvested at intervals of 48 h and counted with a cell counting plate. Doubling time was calculated according to a formula from a previous study (*21*). Doubling time = 48 h/$\log_2$ (N_2/N_1), where N1 is the first cell count and N2 is the cell count 48 h later.

2.5. *Differentiation of genetically modified human ADSCs*

To induce adipogenic differentiation, Bcl-2-ADSCs were seeded onto plates at 1×10^4 cells/cm^2 in complete DMEM. After culturing for 24 h, the medium was changed to adipogenic induction medium using an Adipogenesis Differentiation Kit (Gibco) and the cells were cultured for 14 days. Then, Oil Red O staining was used to observe oil droplets in the cytoplasm of cells. For chondrogenic differentiation, 5-μL droplets of Bcl-2-ADSCs in complete DMEM (1×10^7 cells/mL) were seeded onto plates. After culturing for 48 h, the medium was changed to chondrogenic induction medium using a Chondrogenesis Differentiation Kit (Gibco). Chondrogenic differentiation was induced for 14 days. Chondrogenic differentiation was assessed using Alcian Blue staining. For osteogenic differentiation, Bcl-2-ADSCs (5×10^3/cm^2) were seeded onto plates in complete DMEM. After culturing for 48 h, the medium was changed to osteogenic induction medium using an Osteogenesis Differentiation Kit (Gibco) and cells were cultured for 24 days. The cells were then stained with a 1% Alizarin Red S solution.

2.6. *Western blotting*

Equal amounts of denatured proteins were loaded into each lane of a gel. After electrophoresis, the resolved proteins were transferred onto a nitrocellulose membrane. The membrane was blocked with PBS containing 0.2% Tween 20 and 5% skim milk for 2 h

at room temperature and then incubated with an anti-human Bcl-2 antibody (Keygentec) overnight at 4°C. The membrane was subsequently incubated with a horseradish peroxidase-conjugated secondary antibody, and specific bands were detected with an ECL kit (Keygentec).

2.7. *Oxygen-glucose deprivation*

ADSCs, vector-ADSCs, and Bcl-2-ADSCs were seeded at a density of 2.5×10^5 cells per well on three six-well plates in complete DMEM and then cultured for 24 h. OGD was then performed as described previously (*22*). Briefly, cells were cultured in glucose-free DMEM (Gibco) and exposed to hypoxic conditions in an airtight chamber with 95% N_2 and 5% CO_2 at 37°C.

2.8. *Determination of levels of growth factors*

Cells were cultured and either deprived of oxygen and glucose or not deprived of oxygen and glucose for up to 48 h. The conditioned media with the three groups of cells (ADSCs, vector-ADSCs, and Bcl-2-ADSCs) were collected to evaluate the levels of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF) using a human VEGF enzyme immunoassay kit (Keygentec), human bFGF (Keygentec), or human HGF enzyme immunoassay kit (Keygentec) in accordance with the manufacturer's protocol.

2.9. *Statistical analysis*

Data are expressed as mean ± S.D. Statistical analyses were performed using the statistical software SPSS (version 13.0; IBM, USA). Differences among the 3 groups of cells (ADSCs, vector-ADSCs, and Bcl-2-ADSCs) were compared using one-way ANOVA. Comparisons between 2 groups were performed with an unpaired Student's t test. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. *Characterization of human ADSCs*

Flow cytometric analysis indicated that ADSCs expressed stem cell-associated markers CD29, CD44, and CD90 but lacked expression of CD34, the fibroblast marker HLA-DR, and the hematopoietic stem cell marker CD133 (Figure 1).

3.2. *Bcl-2 overexpression in genetically modified human ADSCs*

At MOIs of 0, 100, 200, and 500, Ad/EGFP transduction efficiency was 0, 49%, 80%, and 80.5%, respectively, according to flow cytometry. Representative GFP expression is shown in Figure 2A. Based on these results, the transduction efficiency reached its maximum at a MOI of 200 (Figure 2B). After transduction, Bcl-2-ADSCs and vector-ADSCs were morphologically indistinguishable from ADSCs. Thus, an optimal MOI of 200 was chosen for subsequent experiments. Detectable protein expression of Bcl-2 was observed in Bcl-2-

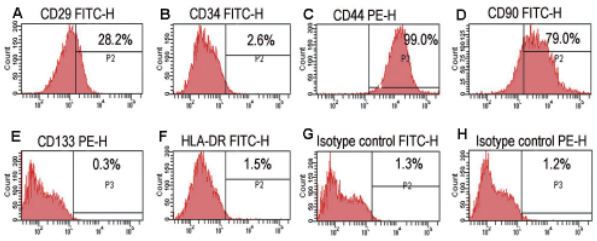

Figure 1. Representative data on the immunophenotypes of human ADSCs that were passaged 4 times. Fluorescence-activated cell sorter analysis indicating the expression of selected surface molecules. **(A)** CD29; **(B)** CD34; **(C)** CD44; **(D)** CD90; **(G** and **H)** Control cells labeled with non-immunoreactive isotype control antibodies. ADSCs expressed the stem cell-associated markers CD29, CD44, and CD90 and did not express CD34, the fibroblast marker HLA-DR, or the hematopoietic stem cell marker CD133.

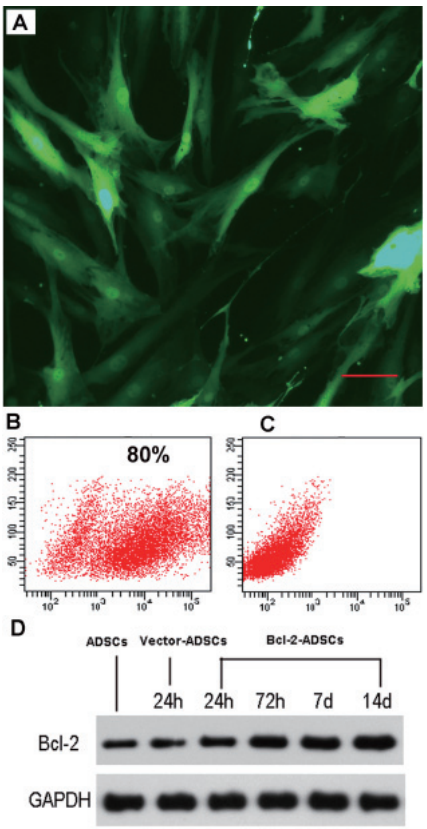

Figure 2. Transduction of ADSCs with an adenoviral vector. **(A)** Representative photomicrograph of ADSCs transfected with Ad/EGFP. Fluorescence-activated cell sorter analysis indicating the transduction efficiency of *EGFP*-transfected ADSCs **(B)** compared to untransfected ADSCs **(C)**. **(D)** Representative western blots showing overexpression of Bcl-2 protein in Bcl-2-ADSCs, which remained at a high level for 14 days post-transduction. GAPDH served as the loading control. Scale bar = 100 μm.

ADSCs as early as 24 h and that level of expression remained high as of day 14 (Figure 2C). In contrast, weak expression of Bcl-2 was detected in vector-ADSCs 24 h post-transduction and in ADSCs, indicating a low level of endogenous Bcl-2 protein in ADSCs.

3.3. *Bcl-2-modified human ADSCs retained their proliferative and multipotent capacity*

ADSCs, vector-ADSCs, and Bcl-2-ADSCs had comparable doubling times (Figure 3A). Bcl-2-ADSCs differentiated into adipocytes, as indicated by Oil Red O staining (Figure 3B, left). Chondrocytes were apparent according to staining with Alcian Blue (Figure 3B, center), and osteoblasts were apparent according to Alizarin Red S staining (Figure 3B, right).

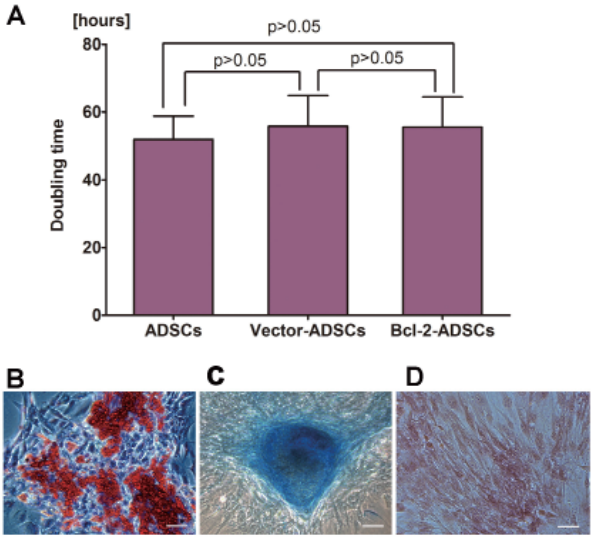

Figure 3. Doubling time and differentiation capacity of Bcl-2-ADSCs. (A) The rate of cell proliferation by ADSCs, vector-ADSCs, and Bcl-2-ADSCs was determined, and their doubling times were determined. There were no significant differences in the doubling time of ADSCs, vector-ADSCs, and Bcl-2-ADSCs. Bcl-2-ADSCs were cultured in adipogenic, chondrogenic, and osteogenic medium. **(B)** Adipogenic differentiation. Staining with Oil Red O. **(C)** Chondrogenic differentiation. Staining with Alcian Blue. **(D)** Osteogenic differentiation. Staining with Alizarin Red S. Values are presented as the mean ± S.D. Scale bar = 50 μm.

3.4. *Bcl-2-modified human ADSCs upregulated growth factors while deprived of oxygen and glucose*

During deprivation of oxygen and glucose, Bcl-2-ADSCs secreted a significantly higher level of VEGF than ADSCs alone (720.83 ± 90.02 versus 629.67 ± 20.45 pg/mL, $p < 0.05$) or than vector-ADSCs (622.50 ± 17.82 pg/mL, $p < 0.05$). Bcl-2-ADSCs secreted a significantly higher level of bFGF than ADSCs alone (383.83 ± 13.86 versus 328.33 ± 4.32 pg/mL, $p < 0.05$) or than vector-ADSCs (332.50 ± 4.85 pg/mL, $p < 0.05$). Bcl-2-ADSCs secreted a significantly higher level of HGF than ADSCs alone (3943.80 ± 260.78 versus 2064.70 ± 387.16 pg/mL, $p < 0.05$) or than vector-ADSCs (1952.00 ± 128.54 pg/mL, $p < 0.05$). Bcl-2-ADSCs secreted a 14.47% higher level of VEGF, a 16.9% higher level of bFGF, and a 91% higher level of HGF compared to ADSCs alone that were cultured while deprived of oxygen and glucose. There were no significant differences in the levels of these three factors in ADSCs and vector-ADSCs deprived of oxygen and glucose ($p > 0.05$). The levels of VEGF and HGF were higher during deprivation of oxygen and glucose than during normal culturing ($p < 0.001$). However, the level of bFGF during deprivation of oxygen and glucose was comparable to that during normal culturing. There were no significant differences in the levels of these three factors in ADSCs, vector-ADSCs, and Bcl-2-ADSCs during normal culturing (Figure 4).

Use of cell-based therapy to treat a number of diseases is a rapidly developing trend in tissue engineering and regenerative medicine. Availability of appropriate cell types remains a crucial issue for therapeutic efficacy and safety. There are several different types of stem cells that have been considered. Although embryonic stem cells have the greatest regenerative potential, their use is limited by ethical reasons, potential tumorigenicity, and immunological rejection (*23*). Use of endothelial progenitor cells from peripheral blood and bone marrow is hampered by expensive procedures of isolation and difficulties in obtaining a sufficient

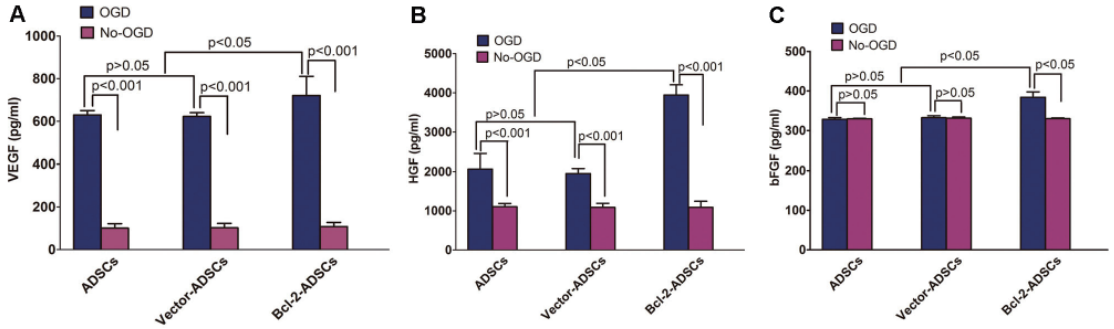

Figure 4. VEGF, HGF, and bFGF secretion by ADSCs, vector-ADSCs, and Bcl-2-ADSCs while deprived of oxygen and glucose or not deprived of oxygen and glucose. (A-C) After incubation for 48 h, the secretion of VEGF, HGF, and bFGF by cells that were not deprived of oxygen and glucose and cells that were deprived of oxygen and glucose ($n = 8$ in each group) in conditioned medium was measured using ELISA. VEGF, HGF, and bFGF concentrations are presented as the mean ± S.D

amount of cells. In addition, *in vitro* cell expansion over multiple passages increases the potential risk of malignancies and chromosomal abnormalities. The harvesting of bone marrow to obtain mesenchymal stromal cells is an invasive and painful procedure. ADSCs have the advantages of easy availability from subcutaneous adipose tissue in large quantities with minimal donor site morbidity and simple collection procedures compared to those for other types of stem cells. Therapy based on ADSCs has emerged as a novel approach to treat a wide range of medical conditions. Thus, ADSCs appear to be favorable candidates for practical stem cell-based therapy.

ADSCs play important roles in cell therapy through multidifferentiation as well as through secretion of growth factors. There is an increasing recognition that the benefits of cell therapy may be mostly attributed to the secretion of multiple complementary growth factors (*24-26*). Numerous studies have examined the paracrine mechanism of ADSCs. *In vitro*, ADSCs can secrete a variety of angiogenic, antiapoptotic, and mitogenic factors, including VEGF, HGF, bFGF, and transforming growth factor-β. Furthermore, the secretion of these growth factors is significantly enhanced when ADSCs are exposed to hypoxic conditions (*16,27*). These growth factors possess angiogenic potential and work collaboratively (*15,28,29*). Angiogenesis involves activation, migration, and proliferation of endothelial cells and is regulated by certain growth factors. These growth factors promote therapeutic angiogenesis, possibly by the combination of direct effects of some growth factors on endothelial cells and indirect effects, including paracrine upregulation of other growth factors (*30*). However, marrow-derived mesenchymal stromal cells have a poor survival once implanted and exposed to ischemic conditions; previous studies revealed that only a small amount of grafted cells had survived *in situ* 4 days after injection (*31-33*). Therefore, ADSC-based therapy may be hindered by low cell survival rates. Previous findings have indicated that Bcl-2 overexpression significantly reduces ADSC apoptosis under harsh conditions (*20*). Using Bcl-2 overexpression to protect ADSCs from apoptosis would establish a good foundation for the paracrine function of ADSCs.

In the present study, flow cytometric analyses revealed that ADSCs that were passaged 4 times expressed the stem cell-associated surface markers CD29, CD44, and CD90 but not the fibroblast marker HLA-DR or the hematopoietic stem cell marker CD133. A point worth mentioning is that ADSCs that were passaged 4 times did not express CD34. The expression profiles coincided with those in previous studies (*21,34,35*). Expression of CD34 and CD34 by ADSCs declined with additional passages. Western blotting indicated that the exogenous *Bcl-2* gene was efficiently transferred into ADSCs and resulted in a high of Bcl-2 expression at the protein level. ADSCs that were genetically modified with Bcl-2 retained their differentiation and proliferative capacity. Bcl-2 overexpression significantly increased the secretion of growth factors when the cells were cultured while deprived of oxygen and glucose. The present study, to the extent known, is the first to find that Bcl-2-ADSCs release higher levels of multiple growth factors, such as VEGF, bFGF, and HGF, than do vector-ADSCs and ADSCs alone while deprived of oxygen and glucose. Previous studies have indicated that the use of single angiogenic growth factors to strengthen neovascularization in patients with atherosclerotic disease results in only modest success (*36,37*). Angiogenesis and collateral growth are likely to require multiple growth factors acting in concert (*38,39*). The present study provides the first direct evidence that Bcl-2 upregulation enhances the potential secretion of growth factors by ADSCs under ischemic conditions. Bcl-2 modification has the advantage of enhancing the levels of a variety of growth factors instead of a single factor when cells are exposed to an ischemic environment early on. Bcl-2-modifid ADSCs can secrete a range of angiogenic growth factors that act in a synergistic manner to promote angiogenesis. This is highly preferable to the increased expression of a single angiogenic factor. The underlying mechanism by which Bcl-2 upregulation enhances the potential secretion of growth factors by ADSCs under ischemic conditions needs to be studied.

In conclusion, the present results indicated that Bcl-2 genetically modified ADSCs retain their proliferative and multilineage differentiation potential. In addition, Bcl-2 upregulation *via* gene transduction significantly enhanced the levels of paracrine factors secreted by ADSCs under ischemic conditions. These findings provide an experimental basis on which to enhance the potential secretion of growth factors by ADSCs under ischemic conditions.

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References

1. Halvorsen YD, Franklin D, Bond AL, Hitt DC, Auchter C, Boskey AL. Paschalis EP, Wilkison WO, Gimble JM. Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived stromal cells. Tissue Eng. 2001; 7:729-741.
2. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem

cells. Mol Biol Cell. 2002; 13:4279-4295.
3. Prunet-Marcassus B, Cousin B, Caton D, André M, Pénicaud L, Casteilla L. From heterogeneity to plasticity in adipose tissues: Site-specific differences. Exp Cell Res. 2006; 312:727-736.
4. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng. 2001; 7:211-228.
5. Mizuno H. Adipose-derived stem and stromal cells for cell-based therapy: Current status of preclinical studies and clinical trials. Curr Opin Mol Ther. 2010; 12:442-449.
6. Karaçal N, Cobanoğlu U, Ambarcioğlu O, Kutlu N. The effect of fibrin glue on fat graft survival. J Plast Reconstr Aesthet Surg. 2007; 60:300-303.
7. Chajchir A, Benzaquen I. Fat-grafting injection for soft-tissue augmentation. Plast Reconstr Surg. 1989; 84:921-934.
8. Ahmed Ali. Contouring of the gluteal region in women: Enhancement and augmentation. Ann Plast Surg. 2011; 67:209-214.
9. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, Aiba-Kojima E, Iizuka F, Inoue K, Suga H, Yoshimura K. Cell-assisted lipotransfer: Supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng. 2006; 12:3375-3382.
10. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: Supportive use of adipose-derived stem/stromal cells. Aesth Plast Surg. 2008; 32:48-55.
11. Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. J Craniomaxillofac Surg. 2004; 32:370-373.
12. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RC, Ma Y. Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant. 2007; 11:814-817.
13. Léobon B, Roncalli J, Joffre C, Mazo M, Boisson M, Barreau C, Calise D, Arnaud E, André M, Pucéat M, Pénicaud L, Prosper F, Planat-Bénard V, Casteilla L. Adipose-derived cardiomyogenic cells: *In vitro* expansion and functional improvement in a mouse model of myocardial infarction. Cardiovasc Res. 2009; 83:757-767.
14. Planat-Bénard V, Menard C, André M, Puceat M, Perez A, Garcia-Verdugo JM, Pénicaud L, Casteilla L. Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. Circ Res. 2004; 94:223-229.
15. Yoshimura K, Eto H, Kato H, Doi K, Aoi N. *In vivo* manipulation of stem cells for adipose tissue repair/reconstruction. Regen Med. 2011; 6(6 Suppl):33-41.
16. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004; 109:1292-1298.
17. Reed JC. Bcl-2 family proteins. Oncogene. 1998; 17:3225-3236.
18. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell. 1993; 75:229-240.
19. Shelat PB, Plant LD, Wang JC, Lee E, Marks JD. The membrane-active tri-block copolymer pluronic F-68 profoundly rescues rat hippocampal neurons from oxygen-glucose deprivation-induced death through early inhibition of apoptosis. Neurosci. 2013; 30:12287-12299.
20. Cui Z, Shen L, Lin Y, Wang S, Zheng D, Tan Q. Inhibition of oxygen-glucose deprivation-induced apoptosis of human adipose-derived stem cells by genetic modification with antiapoptotic protein bcl-2. Aesthetic Plast Surg. 2014; 38:779-787.
21. Gonda K, Shigeura T, Sato T, Matsumoto D, Suga H, Inoue K, Aoi N, Kato H, Sato K, Murase S, Koshima I, Yoshimura K. Preserved proliferative capacity and multipotency of human adipose-derived stem cells after long-term cryopreservation. Plast Reconstr Surg. 2008; 121:401-410.
22. Li J, Qu Y, Chen D, Zhang L, Zhao F, Luo L, Pan L, Hua J, Mu D. The neuroprotective role and mechanisms of TERT in neurons with oxygen-glucose deprivation. Neuroscience. 2013; 252:346-358.
23. Calderon D, Planat-Benard V, Bellamy V, Vanneaux V, Kuhn C, Peyrard S, Larghero J, Desnos M, Casteilla L, Pucéat M, Menasché P, Chatenoud L. Immune response to human embryonic stem cell-derived cardiac progenitors and adipose-derived stromal cells. J Cell Mol Med. 2012; 16:1544-1552.
24. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005; 11:367-368.
25. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch Dermatol Res. 2009; 301:329-336.
26. Rubina K, Kalinina N, Efimenko A, Lopatina T, Melikhova V, Tsokolaeva Z, Sysoeva V, Tkachuk V, Parfyonova Y. Adipose stromal cells stimulate angiogenesis *via* promoting progenitor cell differentiation, secretion of angiogenic factors, and enhancing vessel maturation. Tissue Eng Part A. 2009; 15:2039-2050.
27. Li W, Ma N, Ong LL. Nesselmann C, Klopsch C, Ladilov Y, Furlani D, Piechaczek C, Moebius JM, Lützow K, Lendlein A, Stamm C, Li RK, Steinhoff G. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells. 2007; 25:2118-2127.
28. Hisaka Y, Ieda M, Nakamura T, Kosai K, Ogawa S, Fukuda K. Powerful and controllable angiogenesis by using gene-modified cells expressing human hepatocyte growth factor and thymidine kinase. J Am Coll Cardiol. 2004; 43:1915-1922.
29. Suga H, Eto H, Shigeura T, Inoue K, Aoi N, Kato H, Nishimura S, Manabe I, Gonda K, Yoshimura K. IFATS collection: Fibroblast growth factor-2-induced hepatocyte growth factor secretion by adipose-derived stromal cells inhibits postinjury fibrogenesis through a c-Jun N-terminal kinase-dependent mechanism. Stem Cells. 2009; 27:238-249.
30. Folkman J, Shing Y. Angiogenesis. J Biol Chem. 1992; 267:10931-10934.
31. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and antideath strategies. J Mol Cell Cardiol. 2001; 33:907-921.
32. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart.

Circulation. 2002; 105:93-98.
33. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. Circ Res. 2004; 95:9-20.
34. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, Sato K, Inoue K, Nagase T, Koshima I, Gonda K. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J Cell Physiol. 2006; 208:64-76.
35. Mizuno H, Tobita M, Uysal AC. Concise review: Adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells. 2012; 30:804-810.
36. Simons M, Annex BH, Laham RJ, Kleiman N, Henry T, Dauerman H, Udelson JE, Gervino EV, Pike M, Whitehouse MJ, Moon T, Chronos NA. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: Double-blind, randomized, controlled clinical trial. Circulation. 2002; 105:788-793.
37. Henry TD, Annex BH, McKendall GR, *et al*. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation. 2003; 107:1359-1365.
38. Semenza GL. Angiogenesis in ischemic and neoplastic disorders. Annu Rev Med. 2003; 54:17-28.
39. Jiang M, Wang B, Wang C, He B, Fan H, Shao Q, Gao L, Liu Y, Yan G, Pu J. *In vivo* enhancement of angiogenesis by adenoviral transfer of HIF-1α-modified endothelial progenitor cells (Ad-HIF-1α-modified EPC for angiogenesis). Int J Biochem Cell Biol. 2008; 40:2284-2295.

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