Maintenance of Proliferation and Adipogenic Differentiation by Fibroblast Growth Factor-2 and Dexamethasone Through Expression of Hepatocyte Growth Factor in Bone Marrow-derived Mesenchymal Stem Cells

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Several studies have investigated the various effects of dexamethasone (Dex) on the proliferation and differentiation of mesenchymal stem cells (MSCs). Previously, we reported that co-treatment with L-ascorbic acid 2-phosphate and fibroblast growth factor (FGF)-2 maintained differentiation potential in MSCs through expression of hepatocyte growth factor (HGF). In this study, we investigated the effects of co-treatment with FGF-2 and Dex on the proliferation and differentiation potential of MSCs during a 2-month culture period. Co-treatment with FGF-2 and Dex increased approximately a 4.7-fold higher accumulation rate of MSC numbers than that by FGF-2 single treatment during a 2-month culture period. Interestingly, co-treatment with FGF-2 and Dex increased expression of HGF and maintained adipogenic differentiation potential during this culture period. These results suggest that co-treatment with FGF-2 and Dex preserves the proliferation and differentiation potential during long-term culture.

Key Words: Mesenchymal stem cell, Hepatocyte growth factor, Fibroblast growth factor-2, Dexamethasone, Adipogenesis

INTRODUCTION

Mesenchymal stem cells (MSCs) have been developed as a drug to treat incurable diseases because MSCs have several positive properties including migration ability into injured sites (Caplan, 1991; Prockop, 1997), trans-differentiation potential (Pittenger et al., 1999; Toma et al., 2002; Tang et al., 2004; Sato et al., 2005; Barzilay et al., 2009), secretion of paracrine factors (Doorn et al., 2012), and immunomodulatory potential (Prockop and Olson, 2007). Currently, five drugs for MSC cell therapy are sold and used in human for patients with acute myocardial infarction (Hearticellgram-

AMI), osteoarthritis (Cartistem), Crohn's disease (Cupistem), amyotrophic lateral sclerosis (Neuronata-R), and graft-versus-host disease (Prochymal). Although some studies reported that MSCs could be expanded ex vivo in a relatively short period of time (Colter et al., 2000; Sekiya et al., 2002), their proliferation and differentiation potential under conventional culture conditions gradually decreased during prolonged serial passage (Mendes et al., 2002; Stenderup et al., 2003; Siddappa et al., 2007; Yang et al., 2015). Therefore, maintenance of stemness, which is defined by their proliferation and differentiation potential, is one of the key points for developing MSC therapeutics to reach maximum clinical benefits.

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Dexamethasone (Dex) is a synthetic glucocorticoid hormone that can play key role in regulation of metabolism and immune reaction. The effects of Dex on the proliferation and differentiation of MSCs have been documented by many research groups. The exact mechanisms of Dex on MSC differentiation are still poorly identified, but it has been used for full differentiation of MSCs into osteoblasts (Cheng et al., 1994; Jaiswal et al., 1997; Aubin, 1998; D'Ippolito et al., 1999; Walsh et al., 2001; Hardy and Cooper, 2011; Mostafa et al., 2012). Moreover, Dex is also used for differentiation of MSCs into chondroblasts, myocytes, and adipocytes in vitro (Pittenger et al., 1999; Gao and Caplan, 2003; Caplan, 2005). Dex mediates increase of transcription of several genes and interference of wnt signaling pathways during differentiation of MSCs (Beresford et al., 1994; Cheng et al., 1996; Fromigue et al., 1997; Kim et al., 1999; Hong et al., 2005; Wang et al., 2005; Hamidouche et al., 2008; Wang et al., 2008). Dex also regulates migration and proliferation of MSCs (Xiao et al., 2010; Yun et al., 2011). At high concentrations (100 nM), Dex suppressed MSC proliferation (Walsh et al., 2001), whereas at low concentrations Dex (10 nM) favored the expansion of MSCs and enhanced their osteogenic potential (Both et al., 2007). Previously, we reported that long-term treatment with fibroblast growth factor (FGF)-2 and FGF-4 increased proliferation potential, but not differentiation potential, whereas hepatocyte growth factor (HGF) maintained just differentiation potential without increasing proliferation potential during serial passage (Eom et al., 2014). Additionally, L-ascorbic acid 2-phosphate and FGF-2 treatment maintained both proliferation and differentiation potential in MSCs through activation of AKT and ERK and expression of HGF (Bae et al., 2015). In this study, we investigated the effects of co-treatment of FGF-2 and low dose of Dex (10 nM) on proliferation and differentiation potential of MSCs during a 2-month culture period.

MATERIALS AND METHODS

Cell culture

MSCs isolated from bone marrow samples from three healthy donors (aged 21~40 years), who agreed to donate bone marrow and signed an informed consent from Phar-

micell (Sungnam, Korea). This study was approved by the Institutional Review Board of Yonsei University Wonju College of Medicine (No. 2010-57). Briefly, mononuclear cells from bone marrow aspirates were isolated by density-gradient centrifugation and then plated in 75-cm² flasks (2×10^5 cells/cm²) with low-glucose Dulbecco's modified Eagle's medium (LG-DMEM) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin. Isolated mononuclear cells were cultured at 37°C in 5% CO₂ for five days and then the medium was changed to remove non-adherent cells. Thereafter, the culture medium was changed twice weekly. When the cells reached 90% confluence the MSCs were trypsinized and passaged at a density of 1×10^3 cells/cm². At passage 1, expanded cells were stored in liquid nitrogen. To analyze the effects of FGF-2 and Dex on stemness maintenance of MSCs, cryopreserved cells were thawed, passaged once, and then used for the experiment. To evaluate proliferation and differentiation potential, FGF-2 (1 ng/ml; R&D Systems, Minneapolis, MN, USA) and/or Dex (10 nM; Sigma-Aldrich, St. Louis, MO, USA) were added to cultures.

Adipogenic differentiation

To determine adipogenic differentiation potential, MSCs (2×10^4 cells/cm²) were plated on 6-well plates and then cultured for one week. The medium was then changed to an adipogenic medium containing 10% FBS, 1 μ M dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 10 μ g/mL insulin, and 100 μ M indomethacin in high glucose (HG)-DMEM. After three weeks, differentiated cells were fixed with 4% paraformaldehyde for 10 minutes, stained for lipid droplets with fresh Oil Red O solution, and then photographed. Oil Red O was then eluted with isopropanol and the extracted Oil Red O were quantitated by measuring the optimal absorbance at 540 nm.

MTT assay

MSCs (1×10^3 cells/cm²) were plated in 96-well plates and cultured for 3-day with LG-DMEM with or without FGF-2 (1 or 10 ng/ml), Dex (0.5, 1, 5, 10, 20 nM), LY294002 (1, 10, or 25 μ g/ml; Calbiochem, Darmstadt, Germany), or U0126 (1, 10, or 25 μ g/ml; Calbiochem).

After a 3-day culture, 0.5 mg of methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma) dissolved in PBS was added to each well (final concentration, 5 mg/ml) and incubated at 37°C for 3 h. MTT formazan was dissolved in 100 µl DMSO, incubated for a further 15 min with stirring and then absorbance was read at 570 nm on a microplate reader.

Immunoblotting

A total of 1×10^5 cells were lysed in sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (62.5 mM Tris (pH 6.8), 1% SDS, 10% glycerol, and 5% β-mercaptoethanol), boiled for 5 minutes, subjected to SDS-PAGE and transferred to an Immobilon-P transfer membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked with 5% skim milk in TBST (Tris-buffered saline containing 0.1% Tween 20) and then incubated with primary antibodies against ERK, phospho-ERK, AKT, and phospho-AKT (1:1,000, Cell Signaling Technologies). Bound primary antibodies were detected with HRP-conjugated secondary antibodies (1:2,000, Santa Cruz Biotechnology, Dallas, TX, USA), treated with EZ-Western Lumi Pico (DOGEN, Seoul, Korea) and visualized using FluorChem FC2 system (Cell Biosciences).

RT-PCR

Total RNA was extracted from 1×10^5 cells using TRIzol reagent according to the manufacturer's instructions (Gibco, Grand Island, NY, USA). RNA (2 µg) was reverse-transcribed with M-MLV reverse transcriptase (Bioneer, Daejeon, Korea) for one hour at 42°C in the presence of oligo-dT primer. PCR was performed using *Taq* DNA polymerase (Bioneer). Specific primers used for RT-PCR assays were 5'-ATGC-ATCCAAGGTCAAGGAG-3' (sense), 5'-TTCCATGTTC-TTGTCCCACA-3' (antisense) for HGF, and 5'-CAAGGC-TGAGAACGGAAGC-3' (sense), 5'-AGGGGGCAGA-GATGATGACC-3' (antisense) for GAPDH. cDNA was amplified for 30 cycles for HGF or 27 cycles for GAPDH. Amplified products were electrophoresed on a 2% agarose gel and photographed using the FluorChem FC2 system (Cell Biosciences, Santa Clara, CA, USA).

Statistical analyses

Data are expressed as mean ± standard deviation from three independent experiments. Statistical significance was estimated by the paired Student's *t*-test and one-way ANOVA. Significance was defined as *P*-value of ≤ 0.05 .

RESULTS

Proliferation and differentiation potential by FGF-2 and/or Dex in MSCs

Previously, we reported that co-treatment with L-ascorbic acid 2-phosphate and FGF-2 maintained both proliferation and differentiation potential in MSCs through activation of AKT and ERK and expression of HGF, respectively (Bae et al., 2015). To analyze the effects of low Dex concentration on proliferation and differentiation of MSCs, MSCs were treated with FGF-2 and/or Dex for three days or two months. Although it has been demonstrated that low dose of Dex (10 nM) can increase proliferation of MSCs, in our system, MSC proliferation only slightly increased by low dose of Dex. However, co-treatment with FGF-2 (1 ng/ml) and Dex (10 nM) for three days increased proliferation of MSCs as much as increase of proliferation by 10 ng/ml of FGF-2 (Fig. 1A). Furthermore, after two months of culture, co-treatment with FGF-2 (1 ng/ml) and Dex (10 nM) increased the MSC accumulation rate approximately 4.73-fold compared to cells treated with FGF-2 alone (Fig. 1B). Next, we tested whether addition of FGF-2 and/or Dex could maintain differentiation potential of MSCs. MSCs were cultured in the presence of FGF-2 (1 ng/ml) and/or Dex (1 nM) for two months and then the cells were differentiated into adipocytes. Adipogenic differentiation potential was maintained only in cells treated with both FGF-2 and Dex, but not in cells treated with FGF-2 or Dex alone (Fig. 1C and D). These results suggest that co-treatment with FGF-2 and Dex increased the proliferation potential and maintained the adipogenic differentiation potential of MSCs.

Activation of AKT and ERK by FGF-2 and Dex in MSCs

Since the proliferation potential of MSCs co-treated with FGF-2 and Dex increased approximately 4.73-fold com-

Fig. 1. Proliferation and differentiation potential of MSCs treated with FGF-2 and/or Dex. (A) Increase of growth rates by treatment with FGF-2 or Dex after three days. (B) Accumulation of cell numbers by exogenously added FGF-2 and/or Dex during serial passage over two months. (C-D) Adipogenesis after serial passage of MSCs over two months. Adipogenic differentiation potential was evaluated by elution of Oil Red O-stained lipid droplets with isopropanol (D). Error bars represent mean $\pm$ SD of triplicate wells. Data are shown from one of three independent experiments.

Fig. 2. Activation of AKT and ERK in MSCs treated with FGF-2 and Dex. (A) Regulation of AKT and ERK activation by FGF-2 and Dex, LY294002, or U0126. (B) Inhibitory effects of LY294002 and U0126 on growth of MSCs. * P <0.05. Error bars represent mean $\pm$ SD of triplicate wells. Data are shown from one of three independent experiments.

pared to MSCs treated with FGF-2 alone after two months of culture, we analyzed the activation of AKT and ERK, which are key signaling molecules to induce cell proliferation.

Co-treatment with FGF-2 and L-ascorbic acid 2-phosphate induced phosphorylation of both AKT and ERK. The activation of AKT and ERK were inhibited by LY294002

Fig. 3. HGF expression in MSCs treated with FGF-2 and/or Dex. (A) RT-PCR analysis of HGF mRNA expression after treatment with FGF-2 and/or Dex for three days. (B) The expression level of HGF in MSCs treated with FGF-2 and/or Dex were analyzed by densitometry. GAPDH was used as the housekeeping gene. Error bars represent mean $\pm$ SD of triplicate wells. Data are shown from one of three independent experiments.

(PI3K inhibitor) and U0126 (MEK inhibitor), respectively (Fig. 2A). Moreover, proliferation of MSCs induced by co-treatment with FGF-2 and Dex was also suppressed by both LY294002 and U0126 treatment after a 3-day culture period (Fig. 2B). These data suggest that co-treatment with FGF-2 and Dex increase proliferation potential of MSCs via activation of both AKT and ERK.

HGF expression by FGF-2 and Dex in MSCs

Previously, we reported that HGF maintained differentiation potential of MSCs, while proliferation potential was not increased by HGF treatment during serial passage (Eom et al., 2014). Additionally, co-treatment with L-ascorbic acid 2-phosphate and FGF-2 maintained both proliferation and differentiation potential in MSCs through activation of AKT

and ERK and expression of HGF (Bae et al., 2015). To determine whether HGF expression is responsible for maintenance of adipogenic differentiation potential, we examined HGF expression by RT-PCR. Interestingly, co-treatment with FGF-2 and Dex increased HGF expression approximately 68% (Fig. 3), but FGF-2 or Dex treatment alone reduced HGF expression. These results suggest that co-treatment with FGF-2 and Dex maintain differentiation potential of MSCs via up-regulation of HGF.

DISCUSSION

To use MSCs in treatment of incurable diseases, *ex vivo* expansion through long-term culture of MSCs is needed since MSCs are present in the bone marrow in very low numbers. However, the potential of MSC to proliferate and differentiate during long-term serial passage is known to decrease gradually. Therefore, maintenance of proliferation and differentiation potential during long-term culture is one of the critical issues for therapeutic use of MSCs. Numerous studies have been performed to improve the expansion efficiency of MSCs and maintain their differentiation potential. MSCs are affected by various cytokines and growth factors including vascular endothelial growth factor (VEGF), FGF-2, FGF-4, FGF-6, FGF-7, FGF-9, FGF-17, transforming growth factor (TGF)- β 1, TGF- β 2, HGF, keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF)-AA, interleukin (IL)-6, stromal-derived factor (SDF)-1 and insulin-like growth factor (IGF)-1. These factors can regulate proliferation and differentiation potentials of MSCs in an auto-crine manner and affect a plethora of host responses such as angiogenesis, cellular migration, apoptosis, proliferation and differentiation (Sze et al., 2007; Park et al., 2008; Shabbir et al., 2010; Caplan and Correa, 2011). Of these, FGF-2, FGF-4, EGF, IL-6 and SDF-1 is known to regulate proliferation in MSCs and FGF-2, EGF, TGF- β , and HGF are involved in differentiation in MSCs (Pricola et al., 2009; Fatimah et al., 2013; Eom et al., 2014). Therefore, to maintain proliferation and differentiation potential of MSCs during long-term culture, combination treatment of growth factors may be an effective strategy to maintain proliferation and differentiation potential of MSCs. However, we found com-

bination treatment with FGF-2 and HGF, which can regulate proliferation and differentiation potential, respectively, did not maintain differentiation potential of MSCs during long-term culture in spite of increasing proliferation (data not shown). Moreover, a high dose of FGF-2 reduced proliferation and induced autophagy and senescence in short period compared to low dose of FGF-2 (data not shown). These findings suggest that combination treatment of growth factors must be considered to obtain a high quality of MSCs maintaining the proliferation and differentiation potential needed for achieving a maximum effect in stem cell therapy.

In this study, we found that co-treatment with FGF-2 and Dex maintains the proliferation and differentiation potential of MSCs during long-term culture (up to two months). Activation of PI3K/AKT and MEK/ERK signaling pathways, which promoted MSC proliferation, was observed by co-treatment with FGF-2 and Dex. Moreover, FGF-2 and Dex maintained differentiation potential of MSCs via HGF expression. Therefore, our data suggest that co-treatment with FGF-2 and Dex would be beneficial in obtaining MSCs that possess proliferation and differentiation potential during long-term culture than that of combination treatments of growth factors.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

- Aubin JE. Advances in the osteoblast lineage. *Biochem Cell Biol.* 1998. 76: 899-910.
- Bae SH, Ryu H, Rhee KJ, Oh JE, Baik SK, Shim KY, Kong JH, Hyun SY, Pack HS, Im C, Shin HC, Kim YM, Kim HS, Eom YW, Lee JI. L-ascorbic acid 2-phosphate and fibroblast growth factor-2 treatment maintains differentiation potential in bone marrow-derived mesenchymal stem cells through expression of hepatocyte growth factor. *Growth Factors.* 2015. 33: 71-78.
- Barzilay R, Melamed E, Offen D. Introducing transcription factors to multipotent mesenchymal stem cells: making transdifferentiation possible. *Stem Cells.* 2009. 27: 2509-2515.
- Beresford JN, Joyner CJ, Devlin C, Triffitt JT. The effects of dexamethasone and 1,25-dihydroxyvitamin D3 on osteogenic differentiation of human marrow stromal cells *in vitro*. *Arch Oral Biol.* 1994. 39: 941-947.
- Both SK, van der Muijsenber AJ, van Blitterswijk CA, de Boer J, de Bruijn JD. A rapid and efficient method for expansion of human mesenchymal stem cells. *Tissue Eng.* 2007. 13: 3-9.
- Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991. 9: 641-650.
- Caplan AI. Mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. *Tissue Eng.* 2005. 11: 1198-1211.
- Caplan AI, Correa D. 2011. The MSC: an injury drugstore. *Cell Stem Cell.* 2011. 9: 11-15.
- Cheng SL, Yang JW, Rifas L, Zhang SF, Avioli LV. Differentiation of human bone marrow osteogenic stromal cells *in vitro*: induction of the osteoblast phenotype by dexamethasone. *Endocrinology.* 1994. 134: 277-286.
- Cheng SL, Zhang SF, Avioli LV. Expression of bone matrix proteins during dexamethasone-induced mineralization of human bone marrow stromal cells. *J Cell Biochem.* 1996. 61: 182-193.
- Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proc Natl Acad Sci U S A.* 2000. 97: 3213-3218.
- D'Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA. Agerelated osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *J Bone Miner Res.* 1999. 14: 1115-1122.
- Doom J, Moll G, Le Blanc K, van Blitterswijk C, de Boer J. Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements. *Tissue Eng Part B Rev.* 2012. 18: 101-115.
- Eom YW, Oh JE, Lee JI, Baik SK, Rhee KJ, Shin HC, Kim YM, Ahn CM, Kong JH, Kim HS, Shim KY. The role of growth factors in maintenance of stemness in bone marrow-derived mesenchymal stem cells. *Biochem Biophys Res Commun.* 2014. 445: 16-22.
- Fatimah SS, Tan GC, Chua K, Fariha MM, Tan AE, Hayati AR. Stemness and angiogenic gene expression changes of serial-passage human amnion mesenchymal cells. *Microvasc Res.* 2013. 86: 21-29.
- Fromigue O, Marie PJ, Lomri A. Differential effects of transforming growth factor beta2, dexamethasone and 1,25-dihydroxyvitamin D on human bone marrow stromal cells. *Cytokine.* 1997. 9: 613-623.
- Gao J, Caplan AI. Mesenchymal stem cells and tissue engineering for orthopaedic surgery. *Chir Organi Mov.* 2003. 88: 305-316.

- Hamidouche Z, Hay E, Vaudin P, Charbord P, Schule R, Marie PJ, Fromiguet O. FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/beta-catenin signaling-dependent Runx2 expression. *FASEB J*. 2008. 22: 3813-3822.
- Hardy R, Cooper MS. Glucocorticoid-induced osteoporosis - a disorder of mesenchymal stromal cells? *Front Endocrinol*. 2011. 2: 24.
- Hong JH, Hwang ES, McManus MT, Amsterdam A, Tian Y, Kalmukova R, Mueller E, Benjamin T, Spiegelman BM, Sharp PA, Hopkins N, Yaffe MB. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science*. 2005. 309: 1074-1078.
- Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. *J Cell Biochem*. 1997. 64: 295-312.
- Kim CH, Cheng SL, Kim GS. Effects of dexamethasone on proliferation, activity, and cytokine secretion of normal human bone marrow stromal cells: possible mechanisms of glucocorticoid-induced bone loss. *J Endocrinol*. 1999. 162: 371-379.
- Mendes SC, Tibbe JM, Veenhof M, Bakker K, Both S, Platenburg PP, Oner FC, de Bruijn JD, van Blitterswijk CA. Bone tissue-engineered implants using human bone marrow stromal cells: effect of culture conditions and donor age. *Tissue Eng*. 2002. 8: 911-920.
- Mostafa NZ, Fitzsimmons R, Major PW, Adesida A, Jomha N, Jiang H, Uludag H. Osteogenic differentiation of human mesenchymal stem cells cultured with dexamethasone, vitamin D3, basic fibroblast growth factor, and bone morphogenetic protein-2. *Connect Tissue Res*. 2012. 53: 117-131.
- Park BS, Jang KA, Sung JH, Park JS, Kwon YH, Kim KJ, Kim WS. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. *Dermatol Surg*. 2008. 34: 1323-1326.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999. 284: 143-147.
- Pricola KL, Kuhn NZ, Haleem-Smith H, Song Y, Tuan RS. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J Cell Biochem*. 2009. 108: 577-588.
- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997. 276: 71-74.
- Prockop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. *Blood*. 2007. 109: 3147-3151.
- Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, Sato T, Miyanishi K, Takayama T, Takahashi M, Takimoto R, Iyama S, Matsunaga T, Ohtani S, Matsuura A, Hamada H, Niitsu Y. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood*. 2005. 106: 756-763.
- Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*. 2002. 20: 530-541.
- Shabbir A, Zisa D, Lin H, Mastri M, Roloff G, Suzuki G, Lee T. Activation of host tissue trophic factors through JAK-STAT3 signaling: a mechanism of mesenchymal stem cell-mediated cardiac repair. *Am J Physiol Heart Circ Physiol*. 2010. 299: H1428-1438.
- Siddappa R, Licht R, van Blitterswijk C, de Boer J. Donor variation and loss of multipotency during *in vitro* expansion of human mesenchymal stem cells for bone tissue engineering. *J Orthop Res*. 2007. 25: 1029-1041.
- Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone*. 2003. 33: 919-926.
- Sze SK, de Kleijn DP, Lai RC, Khia Way Tan E, Zhao H, Yeo KS, Low TY, Lian Q, Lee CN, Mitchell W, El Oakley RM, Lim SK. Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells. *Mol Cell Proteomics*. 2007. 6: 1680-1689.
- Tang DQ, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Atkinson MA, Yang LJ. *In vivo* and *in vitro* characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes*. 2004. 53: 1721-1732.
- 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.
- Walsh S, Jordan GR, Jefferiss C, Stewart K, Beresford JN. High concentrations of dexamethasone suppress the proliferation but not the differentiation or further maturation of human osteoblast precursors *in vitro*: relevance to glucocorticoid-induced osteoporosis. *Rheumatology*. 2001. 40: 74-83.
- Wang FS, Ko JY, Yeh DW, Ke HC, Wu HL. Modulation of Dickkopf-1 attenuates glucocorticoid induction of osteoblast

- apoptosis, adipocytic differentiation, and bone mass loss. *Endocrinology*. 2008. 149: 1793-1801.
- Wang FS, Lin CL, Chen YJ, Wang CJ, Yang KD, Huang YT, Sun YC, Huang HC. Secreted frizzled-related protein 1 modulates glucocorticoid attenuation of osteogenic activities and bone mass. *Endocrinology*. 2005. 146: 2415-2423.
- Xiao Y, Peperzak V, van Rijn L, Borst J, de Bruijn JD. Dexamethasone treatment during the expansion phase maintains stemness of bone marrow mesenchymal stem cells. *J Tissue Eng Regen Med*. 2010. 4: 374-386.
- Yang J, Kwon J, Kim M, Bae Y, Jin H, Park H, Eom YW, Rhee K-J. *In vitro* expansion of umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) under hypoxic conditions. *Biomed Sci Lett*. 2015. 21: 40-49.
- Yun SP, Ryu JM, Han HJ. Involvement of beta1-integrin via PIP complex and FAK/paxillin in dexamethasone-induced human mesenchymal stem cells migration. *J Cell Physiol*. 2011. 226: 683-692.
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