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# The role of growth factors in maintenance of stemness in bone marrow-derived mesenchymal stem cells

Young Woo Eom [a], Ji-Eun Oh [a], Jong In Lee [b], Soon Koo Baik [a,c], Ki-Jong Rhee [d], Ha Cheol Shin [e], Yong Man Kim [e], Chan Mug Ahn [f], Jee Hyun Kong [b], Hyun Soo Kim [e,*], Kwang Yong Shim [b,*]

[a] Cell Therapy and Tissue Engineering Center, Wonju College of Medicine, Yonsei Univ., Wonju, Republic of Korea
[b] Department of Hematology-Oncology, Wonju College of Medicine, Yonsei Univ., Wonju, Republic of Korea
[c] Department of Internal Medicine, Wonju College of Medicine, Yonsei Univ., Wonju, Republic of Korea
[d] Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei Univ., Wonju, Republic of Korea
[e] Pharmicell Co., Ltd., Sungnam, Republic of Korea
[f] Department of Basic Science, Wonju College of Medicine, Yonsei Univ., Wonju, Republic of Korea

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ABSTRACT

Mesenchymal stem cells (MSCs) are an active topic of research in regenerative medicine due to their ability to secrete a variety of growth factors and cytokines that promote healing of damaged tissues and organs. In addition, these secreted growth factors and cytokines have been shown to exert an autocrine effect by regulating MSC proliferation and differentiation. We found that expression of EGF, FGF-4 and HGF were down-regulated during serial passage of bone marrow-derived mesenchymal stem cells (BMSCs). Proliferation and differentiation potentials of BMSCs treated with these growth factors for 2 months were evaluated and compared to BMSCs treated with FGF-2, which increased proliferation of BMSCs. FGF-2 and -4 increased proliferation potentials at high levels, about 76- and 26-fold, respectively, for 2 months, while EGF and HGF increased proliferation of BMSCs by less than 2.8-fold. Interestingly, differentiation potential, especially adipogenesis, was maintained only by HGF treatment. Treatment with FGF-2 rapidly induced activation of AKT and later induced ERK activation. The basal level of phosphorylated ERK increased during serial passage of BMSCs treated with FGF-2. The expression of LC3-II, an autophagy marker, was gradually increased and the population of senescent cells was increased dramatically at passage 7 in non-treated controls. But FGF-2 and FGF-4 suppressed LC3-II expression and down-regulated senescent cells during long-term (i.e. 2 month) cultures. Taken together, depletion of growth factors during serial passage could induce autophagy, senescence and down-regulation of stemness (proliferation via FGF-2/-4 and differentiation via HGF) through suppression of AKT and ERK signaling.


## 1. Introduction

MSCs are a promising source for cell-based tissue engineering and regenerative medicine. MSC transplantation is considered safe and has been widely tested in clinical trials of cardiovascular, neurological, and immunological diseases with encouraging results [1–6]. MSCs have been used in both preclinical and clinical studies for cell therapy because MSCs have several positive properties including migration to sites of injury [7,8], differentiation into target cells in microenvironments [9–12], secretion of paracrine factors that play key roles in regeneration of damaged tissue (reviewed in [13]), modulation of inflammatory and/or immune reactions to reduce the risks of rejection after xeno- or allo-transplantation [14]. The ability of MSCs to treat a wide spectrum of diseases is believed to be due to their potentials to trans-differentiate into damaged cell types. However, trans-differentiation of MSCs into target cells is not readily observable in animal models and thus results from these studies are insufficient to demonstrate the therapeutic properties of MSCs. Recent reports have suggested that paracrine factors secreted by MSCs play important roles in the therapeutic benefits of these cells by increasing angiogenesis, inhibiting apoptosis, protecting damaged cells and inducing proliferation of progenitor or stem cells [13,15,16].

* Corresponding authors. Address: Department of Hematology-Oncology, Wonju College of Medicine, Yonsei University, 162 Ilsan-dong, Wonju, Gangwon-do, 220-701, Republic of Korea. Fax: +82 33 745 3066 (K.Y. Shim). Pharmicell Co., Ltd., Sicox Tower, Sangdaewon 1-dong, Jungwon-gu, Seongnam-si, Gyeonggi-do, 462-737, Republic of Korea. Fax: +82 31 777 9326 (H.S. Kim).
*E-mail addresses:* khsmd@pharmicell.com (H.S. Kim), kyshim@yonsei.ac.kr (K.Y. Shim).

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During ex vivo expansion, various factors secreted by MSCs were also important in the regulation of proliferation and differentiation potentials in an autocrine manner. Although MSCs can be expanded ex vivo in a relatively short period of time [17,18], the "stemness", which can be defined by their potential to proliferate and differentiate, gradually decreases during serial passage. Therefore, regulation of stemness in MSCs is one of the important issues for achieving a maximum effect in stem cell therapy. MSCs express multiple paracrine factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, FGF-4, FGF-6, FGF-7, FGF-9, FGF-17, transforming growth factor (TGF)-β1, TGF-β2, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF)-AA and insulin-like growth factor (IGF)-1. These factors affect a plethora of host responses such as angiogenesis, cellular migration, apoptosis, proliferation and differentiation [16,19–21]. The proliferation of mesenchymal stem cells is regulated by paracrine factors such as FGF-2, FGF-4, interleukin (IL)-6 and stromal-derived factor (SDF)-1, whereas FGF-2, EGF, TGF-β, and other are involved in differentiation [22,23].

Autophagy is a catabolic process of self-eating induced by nutrient limitation and cellular stress, which governs recycling of unnecessary proteins and organelles for survival [24,25]. The functions of autophagy include regulation of cell growth, cell survival, cell death and development. The lack of a specific survival factor also induces autophagy. In neural precursor cells, FGF-2 deprivation induced double-membrane vacuoles in the cytoplasm [26]. Young et al. suggested that autophagy and its consequent protein turnover mediate the acquisition of senescence [27].

To use MSCs in cell-based tissue engineering and regenerative medicine, cellular expansion through serial passage of the isolated MSCs is crucial since large numbers of cells are required. However, serial passaging could alter the phenotype and genotype of the culture-expanded cells due to the adaptation of the cells to the cell culture environment. Therefore, it is critical to actively maintain "stemness" for cell-based tissue engineering and regenerative medicine. Hence, to understand the importance of the growth factors that regulate stemness in BMSCs, we examined the changes in expression of growth factors during serial passage of BMSCs which can induce changes in cell culture environments and regulate the stemness of BMSCs. We also evaluated the extent of AKT and ERK activation by growth factors during serial passage of BMSCs. Finally, we examined changes in the status of autophagy, senescence and differentiation potentials in MSCs treated with growth factors during serial passage.

## 2. Materials and methods

### 2.1. Cell culture

Bone marrow (BM) samples from three healthy donors (aged 21–40 years) were obtained with informed consent from Pharmicell Co., Ltd. (Sungnam, Korea). This study was approved by the Institutional Review Board of Yonsei University Wonju College of Medicine. BMSCs were isolated and cultured as previously described [28]. Briefly, mononuclear cells from BM aspirates were isolated by density-gradient centrifugation and then plated in 75 $cm^2$ flasks ($2 \times 10^5$ cells/$cm^2$) with low-glucose Dulbecco's modified Eagle's medium (LG-DMEM) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin. The cells were cultured at 37 °C in 5% $CO_2$ and after 5 days the medium was changed to remove non-adherent cells. Thereafter, the cell culture medium was changed twice weekly. When the cells reached 90% confluence (passage 0) the BMSCs were trypsinized and passaged at a density of $1 \times 10^3$ cells/$cm^2$. At passage 1, expanded cells were stored in liquid nitrogen. For experiments, cryopreserved cells were thawed, passaged once and used for this study. Population doubling time was determined by dividing the total number of hours in culture by the number of doublings. To evaluate stemness regulation by growth factors, FGF-2 (1 ng/ml), FGF-4 (10 ng/ml), EGF (10 ng/ml), and HGF (10 ng/ml) (all from R&D Systems) were added to the cells during serial passage for 2 months.

### 2.2. RT-PCR

Total RNA was extracted from $1 \times 10^5$ cells using TRIzol Reagent. Total RNA (2 μg) was reverse-transcribed with AMV reverse transcriptase XL for 1 h at 42 °C in the presence of oligo-dT primer. PCR was performed using *Taq* DNA polymerase. Amplified products were electrophoresed on a 2% agarose gel and photographed using a FluorChem FC2 system. The sequences of oligonucleotide primers used for RT-PCR and the expected sizes of the PCR products are listed in Table 1.

### 2.3. Immunoblotting

When BMSCs reached 90% confluence, a total of $1 \times 10^5$ 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 min, subjected to SDS–PAGE and transferred to an Immobilon membrane. 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 LC3 (1:1000, Medical & Biological Laboratories, Nagoya, Japan), α-tubulin, ERK, phospho-ERK, AKT and phospho-AKT (1:1000, purchased from Cell Signaling Technology, Danvers, MA, USA). Bound primary antibodies were detected with HRP-conjugated secondary antibodies (1:2000, Santa Cruz Biotech, Santa Cruz, CA, USA), treated with EZ-Western Lumi Pico (DOGEN, Seoul, Korea) and visualized using FluorChem FC2 system (Alpha Innotech, Santa Clara, CA, USA).

### 2.4. Cell Differentiation

Adipogenic differentiation was determined by plating the BMSCs ($2 \times 10^4$ cells/$cm^2$) in 6-well plates and culturing for 1 week. The medium was then changed to an adipogenic medium

**Table 1**
RT-PCR primers for validation of gene expression.

| | Primer sequence (5′→3′) | Length (bp) | Annealing temperature (°C) |
|---|---|---|---|
| EGF | GGTCAATGCAACCAACTTCA<br>GGCATTGAGTAGGTGATTAG | 383 | 55 |
| FGF-2 | CTGTACTGCAAAAACGGG<br>AAAGTATAGCTTTCTGCC | 349 | 53 |
| FGF-4 | CGGGGCGTGGTGAGCATCTT<br>CGGTTCCCCTTCTTGGTCTTCCC | 209 | 64 |
| GAPDH | CAAGGCTGAGAACGGGAAGC<br>AGGGGGCAGAGATGATGACC | 194 | 60 |
| HGF | ATGCATCCAAGGTCAAGGAG<br>TTCCATGTTCTTGTCCCACA | 349 | 56 |
| IGF-2 | GACCGCGGCTTCTACTTCAG<br>AAGAACTTGCCCACGGGGTAT | 203 | 62 |
| IL-6 | GTAGCCGCCCCACACAGACAGCC<br>GCCATCTTTGGAAGGTTC | 173 | 60 |
| TGF-β1 | AAGTGGACATCAACGGGTTC<br>GTCCAGGCTCCAAATGTAGG | 302 | 60 |
| VEGF | CCGAGGCACAGAGAGACAG<br>TACGGATAAACAGTAGCACCAA | 612 | 58 |

[10% FBS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 10 μg/mL insulin, and 100 μM indomethacin in high glucose (HG)-DMEM] for an additional 3 weeks. Cells were fixed in 4% paraformaldehyde for 10 min, stained with fresh Oil Red-O solution to stain lipid droplets and photographed. Oil Red O was then eluted with isopropanol and the extracted Oil Red O quantitated by measuring the optimal absorbance at 540 nm.

For osteogenic differentiation, cells ($2 \times 10^4$ cells/cm$^2$) were plated in 6-well plates and cultured in osteogenic medium (LG-DMEM medium supplemented with 10% FBS, 10 mM β-glycerophosphate, $10^{-7}$ M dexamethasone, and 0.2 mM ascorbic acid) for 2 weeks. To determine osteogenic differentiation, the release of *p*-nitrophenol from *p*-nitrophenyl phosphate by the ALP enzyme was observed.

### 2.5. Senescence-associated β-galactosidase (SA-β-gal) staining

BMSCs were stained for β-gal activity as described by Dimri et al. [29]. Briefly, $4 \times 10^4$ cells were seeded in 12-well plates and cultured for 2 days. β-Gal activity was assessed with a senescence β-gal staining kit (Cell Signaling Technology) according to the manufacturer's instructions. The percentage of senescent cells was represented by the number of stained cells in the total population.

### 2.6. Statistical analysis

Data are expressed as mean ± standard deviation. Statistical significance was estimated by the Student's t-test and a paired t-test. Significance was defined as *p*-value of $\leqslant 0.05$.

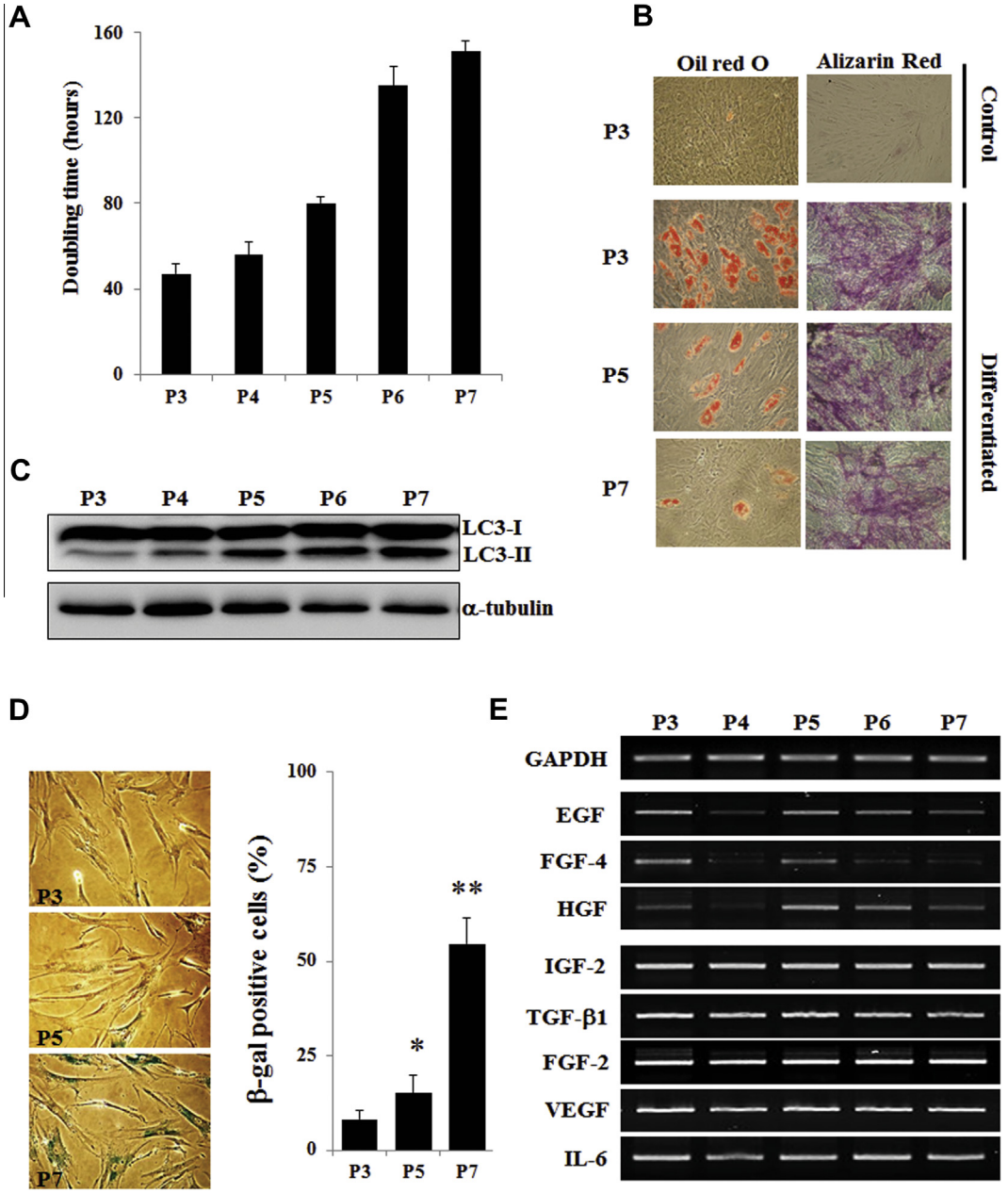


**Fig. 1.** Stemness of BMSCs during serial passage. (A) Population doubling (PD) times of MSCs during serial passage for 2 months. (B) Differentiation potential of cells during serial passage. Adipogenic or osteogenic differentiated MSCs from P3, P5 and P7 were stained with Oil-red O or alizarin red. (C) Western blot analysis of LC3-II during serial passage. (D) SA-β-gal activity during serial passage. SA-β-gal-positive cells were photographed (100× magnification) with a phase contrast microscope and enumerated. At least 200 cells were counted from six different fields, and the percentage of positive cells is shown. Results are expressed as mean ± SD ($n = 6$); $^{*}P < 0.05$, $^{**}P < 0.01$ versus passage 3. (E) RT-PCR analysis of mRNA expression for paracrine factors during serial passage.

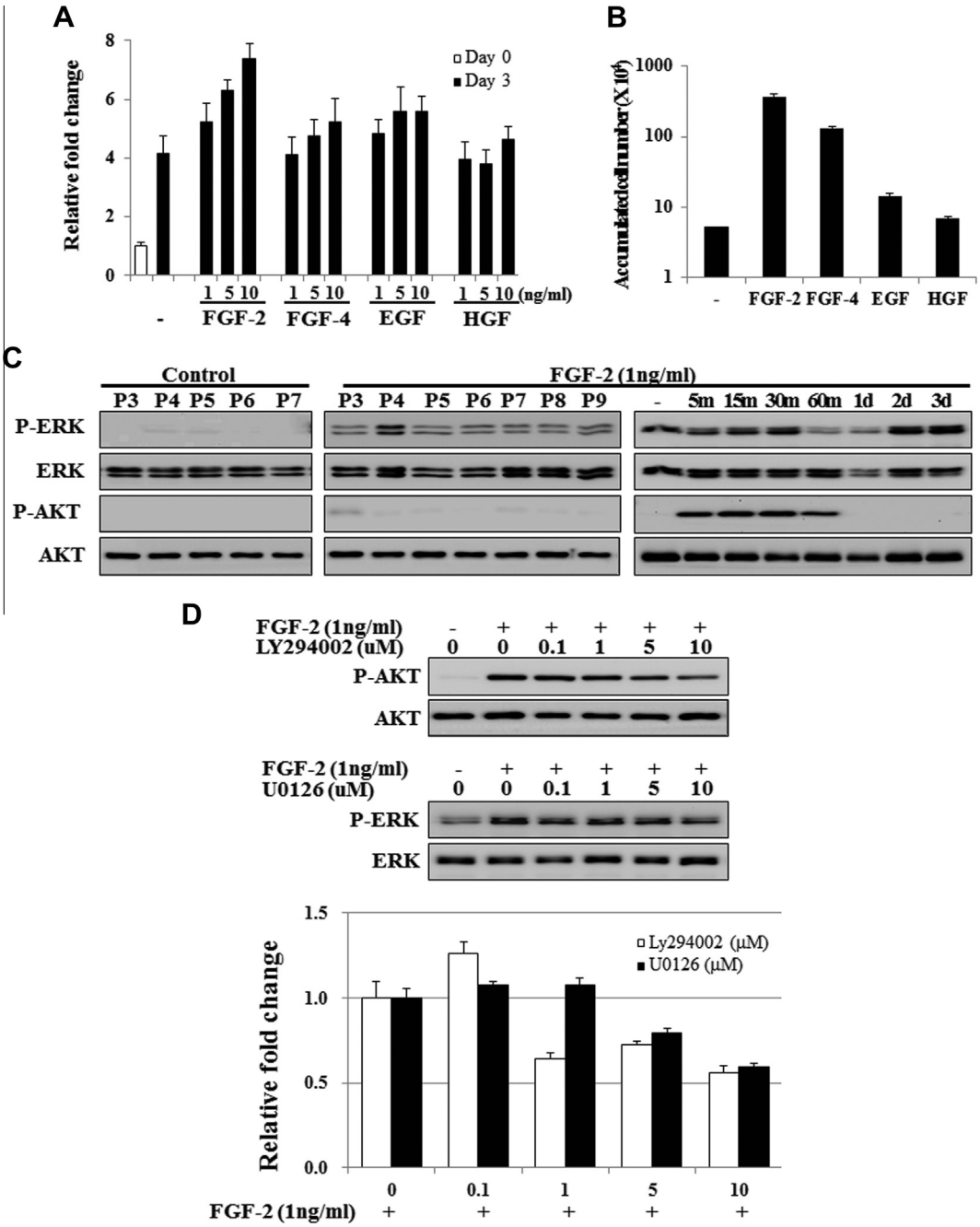


**Fig. 2.** Proliferation potential of BMSCs treated with growth factors. (A) Increase of growth rates by treatment with FGF-2, FGF-4, EGF, or HGF for 3 days. (B) Accumulated cell number by exogenously added growth factors during serial passage for 2 months. (C) Activation of AKT and ERK by FGF-2. (D) Inhibitory effects of LY294002 and U0126 on growth of BMSCs. Increases in growth rates caused by FGF-2 treatment were reversed by inhibitors of AKT and ERK in a dose-dependent manner.

## 3. Results

### 3.1. Stemness of BMSCs during long-term culture

Stemness of MSCs gradually decreases during long-term culture for large-scale expansion. To determine changes in stemness, BMSCs were passaged at $1 \times 10^3$ cells/cm$^2$ for 2 months. BMSCs grown till passage 7 (P7) and the population doubling time examined. We found that the doubling time gradually increased from 47 ± 5 h at P3 to approximately 150 h at P7 (Fig. 1A). We next examined the differentiation potential of the passaged cells. We found that adipogenesis and osteogenesis potential was decreased proportionately to the number of passages. In particular, adipogenic differentiation potential was dramatically decreased in cells from P7 (Fig. 1B). Interestingly, conversion of LC3-I to LC3-II, an indication of autophagy, gradually increased gradually as the passage number increased as well as an increase in the senescent cell population (Fig. 1C and D). To identify paracrine factors that may be affecting the stemness of BMSCs, we examined the expression levels of several paracrine factors by RT-PCR. While the expression levels of EGF, FGF-4, and HGF tended to decrease gradually during long-term culture, mRNA expression levels of IGF-2, TGF-β1, FGF-2, VEGF and IL-6 remained unchanged (Fig. 1E).

### 3.2. Effect of exogenously added growth factors on stemness of BMSCs

To determine whether growth factors that decreased gradually during long-term culture can regulate the stemness of BMSCs, BMSCs were cultured for 2 months with FGF-2, FGF-4, EGF or HGF. EGF, FGF-4, and HGF were the main growth factors that decreased gradually during long-term culture of BMSCs in our system (Fig. 1C). Although we did not observe a decrease in mRNA levels of FGF-2, we included FGF-2 in the experiment because FGF-2 has been shown to decrease in other studies [22]. BMSCs treated with FGF-2, FGF-4, and EGF enhanced cellular proliferation in a dose-dependent manner for 3 days, with the greatest proliferation potential being observed in FGF-2-treated cells (Fig. 2A). In contrast, HGF treatment did not enhance cellular proliferation. During 2 months of culture, addition of FGF-2, FGF-4 and EGF increased the accumulated cell number of BMSCs (FGF-2, 74-fold; FGF-4, 26-fold; EGF, 2.8-fold) whereas HGF had no effect. The number of passages was 9 or 7 in cells treated with FGF-2 and FGF-4 or EGF and HGF for 2 months, respectively (Fig. 2C).

Next, to assess the activation of AKT and ERK, which are generally activated by proliferative stimuli, we treated BMSCs with FGF-2 and examined for phosphorylated AKT and ERK by Western blot analysis. Levels of phosphorylated AKT and phosphorylated ERK were undetected in non-treated cells whereas phosphorylated ERK was detected throughout the long-term culture of FGF-2 treated cells (Fig. 3A). FGF-2 treated cells exhibited a transient increase in AKT activation up to 60 min (Fig. 3B). We found that cells treated with AKT an ERK inhibitors suppressed BMSC proliferation suggesting that MSC proliferation induced by FGF-2 acts through both AKT and ERK pathway (Fig. 3C).

We next determined if addition of exogenous growth factors could maintain differentiation potential. After BMSCs were cultured with or without growth factors (FGF-2, FGF-4, EGF, and HGF) for 2 months, cells from each culture condition were differentiated into adipocytes and osteocytes. Although osteogenic differentiation potential of cells treated with growth factors was similar to that of controls, adipogenic differentiation potential was maintained in cells treated with HGF (Fig. 4A-C). In particular, adipogenic differentiation was dramatically inhibited by the c-Met inhibitor, SU11274 (Fig. 4D).

### 3.3. Effects of exogenously added growth factors on autophagy and senescence of BMSCs

To determine whether decreases in expression of growth factors during long-term culture can induce autophagy and senescence, the expression levels of LC3-II, a widely used autophagy marker, and SA-β-Gal activities were analyzed. In control cells, LC3-II was gradually increased during passaging. Addition of FGF-2 and FGF-4 did not induce any increase in LC3-II expression, while EGF and HGF induced expression of LC3-II gradually during passaging (Fig. 4A). Senescence phenotype was observed to be correlated with autophagy. β-Gal-positive cells were highly observed in control cells and those treated with EGF and HGF, but senescent cells

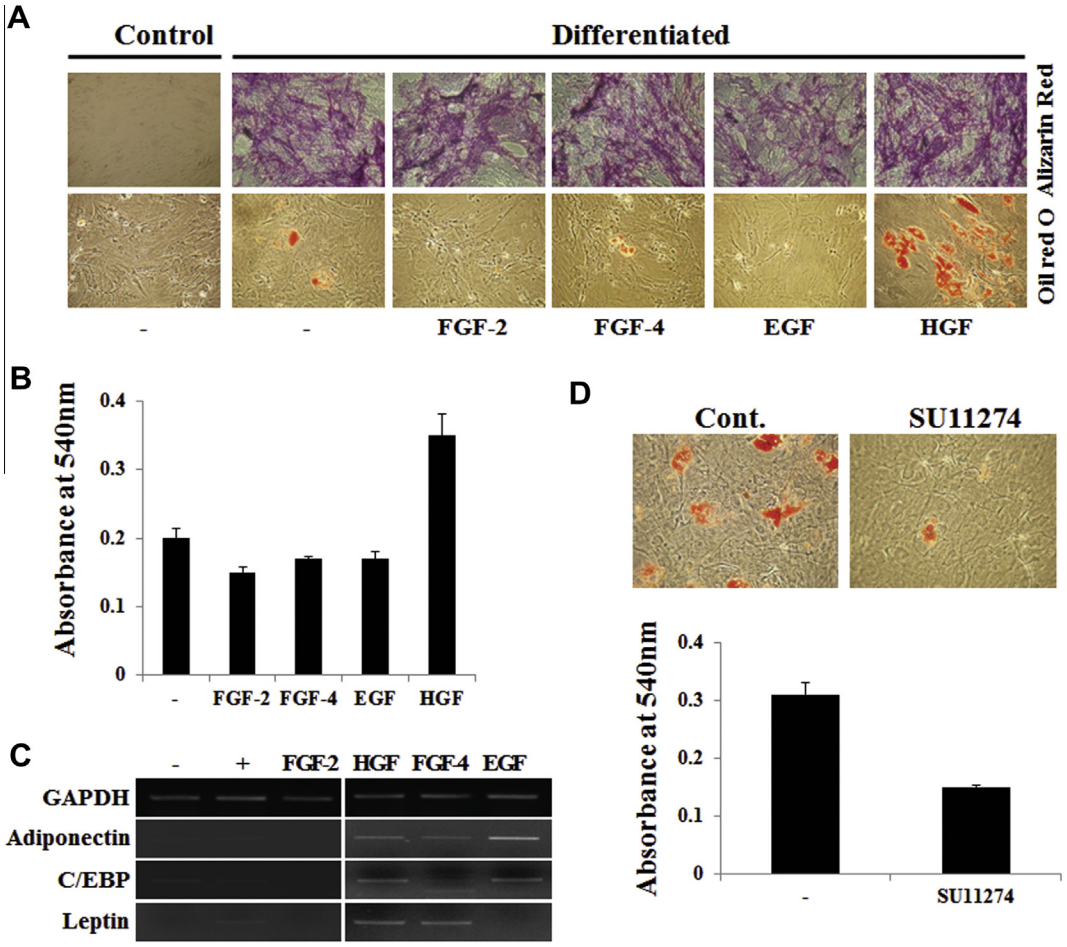


**Fig. 3.** Differentiation potential of BMSCs treated with growth factors. (A) Adipogenesis or osteogenesis after serial passage for 2 months and treatment with FGF-2, FGF-4, EGF, or HGF. Adipogenic differentiation potential was evaluated by elution of Oil-Red O-stained lipid droplets with isopropanol (B) or marker gene expression for adipogenesis detected by RT-PCR (C). (D) Inhibition of adipogenesis by c-met inhibitor (SU11274) in BMSCs (P5) cultured normally.

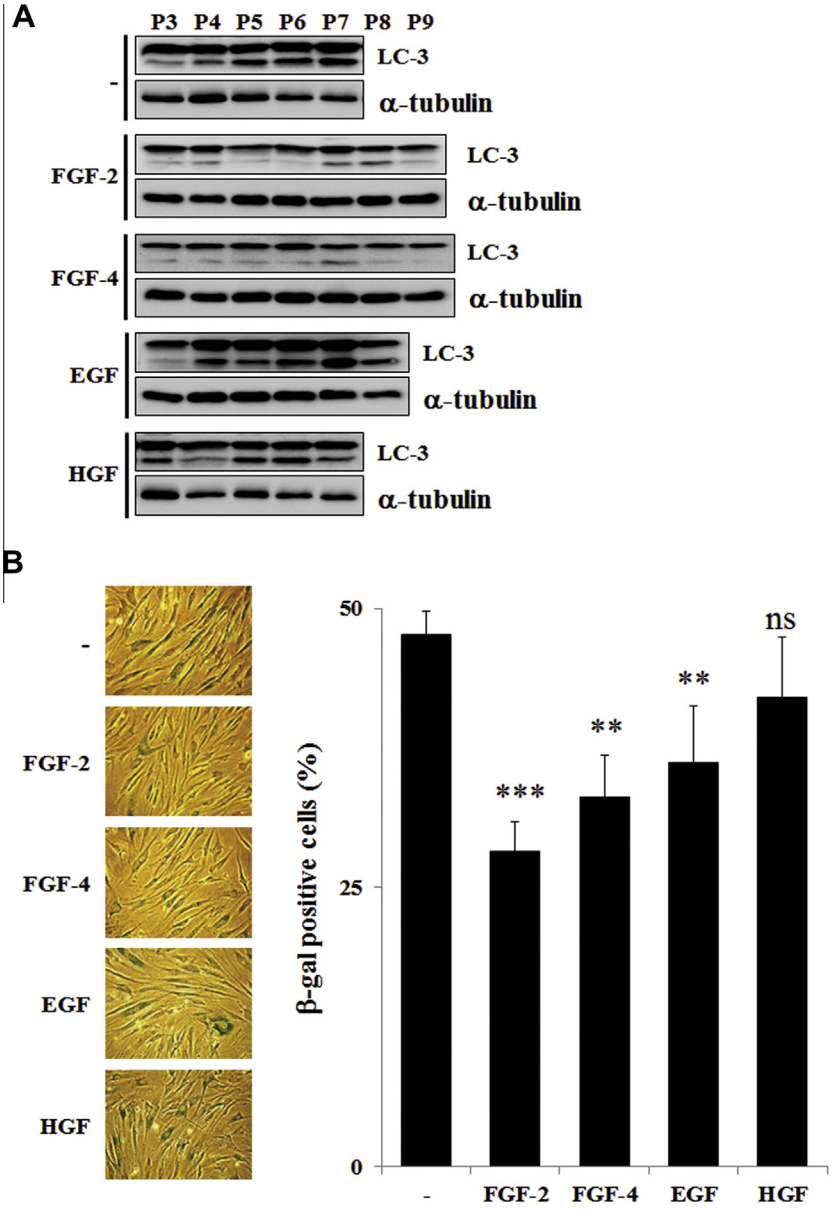


**Fig. 4.** Effect of growth factors on autophagy and senescence. (A) Expression of LC3-II during serial passage with or without growth factors. (B) Effect of growth factors on SA-β-gal activity. SA-β-gal-positive cells were photographed (100×) with a phase contrast microscope and enumberated. At least 200 cells were counted from six different fields and the percentages of positive cells were determined. Results are expressed as mean ± SD ($n$ = 6∼7); $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}p<0.001$ versus control.

were detected only rarely in FGF-2 and FGF-4-treated groups after 2 months of culture.

## 4. Discussion

We report that growth factors expressed by MSCs regulate the maintenance of stemness. FGF-2 and FGF-4 played a key role in the maintenance of proliferation potential, while HGF was important to the maintenance of differentiation potential during long-term culture of about 2 months. Proliferation of BMSCs was regulated by AKT and ERK activation, while FGF-2 induced acute AKT phosphorylation and later (2–3 days) ERK activation. The basal level of phosphorylated ERK in BMSCs treated with FGF-2 was sustained up to the end of passaging for 2 months. After long-term culture, differentiation potential, especially adipogenic activity, was maintained at a high level by HGF, whereas FGF-2, FGF-4 and EGF did not help maintain the adipogenic differentiation potential. Interestingly, c-MET inhibitor SU11274 suppressed adipogenic differentiation of BMSCs. These results suggested that growth factors expressed by BMSCs can be divided into two groups, one group that functions in proliferation and includes factors such as FGF-2 and FGF-4, and another group of factors, such as HGF, that plays a role in differentiation. Growth factors that played roles in increasing proliferation (such as FGF-2 and FGF-4) could suppress the autophagy and senescence phenotype, while HGF played a key role in maintaining differentiation, but could not induce suppression of autophagy and senescence.

MSCs express various growth factors that regulate proliferation and differentiation in an autocrine manner. In our results, growth factors were divided into two groups, which played roles in proliferation (FGF-2 and FGF-4) or differentiation (HGF) of BMSCs. Taken together, during long-term culture, the expression of growth factors was decreased gradually and, subsequently, decreasing growth factors induced the retardation of proliferation or differentiation according to the kinds of growth factors. We hypothesized

that combination treatment of growth factors may be very important to maintain stemness of BMSCs for stem cell therapy. So, we determined whether combination treatments like FGF-2 and HGF or FGF-4 and HGF could increase both proliferation and differentiation potentials. We found that combined treatment of growth factors increased proliferation, but not differentiation (data not shown) of BMSCs. Furthermore, a high dose of FGF-2 induced growth suppression, autophagy and senescence (data not shown). These data suggest that the kinds, doses, and combination of growth factors must be considered to obtain a high quality of BMSCs maintaining the stemness needed for stem cell therapy. In conclusion, growth factor deficiencies could induce autophagy, senescence, and growth retardation of BMSCs. Therefore, to obtain BMSCs possessing stemness after long-term culture, a combined treatment of growth factors will be needed to suppress autophagy and senescence and increase stemness.

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## References

[1] O.Y. Bang, J.S. Lee, P.H. Lee, et al., Autologous mesenchymal stem cell transplantation in stroke patients, Ann. Neurol. 57 (2005) 874–882.
[2] S.L. Chen, W.W. Fang, F. Ye, et al., Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction, Am. J. Cardiol. 94 (2004) 92–95.
[3] H.M. Lazarus, O.N. Koc, S.M. Devine, et al., Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients, Biol. Blood Marrow Transplant. 11 (2005) 389–398.
[4] P.H. Lee, J.W. Kim, O.Y. Bang, et al., Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy, Clin. Pharmacol. Ther. 83 (2008) 723–730.
[5] O. Ringden, M. Uzunel, I. Rasmusson, et al., Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease, Transplantation 81 (2006) 1390–1397.
[6] R.S. Ripa, M. Haack-Sorensen, Y. Wang, et al., Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: results from the Stem Cells in Myocardial Infarction (STEMMI) trial, Circulation 116 (2007) I24–30.
[7] A.I. Caplan, Mesenchymal stem cells, J. Orthop. Res. 9 (1991) 641–650.
[8] D.J. Prockop, Marrow stromal cells as stem cells for nonhematopoietic tissues, Science 276 (1997) 71–74.
[9] R. Barzilay, E. Melamed, D. Offen, Introducing transcription factors to multipotent mesenchymal stem cells: making transdifferentiation possible, Stem cells 27 (2009) 2509–2515.
[10] Y. Sato, H. Araki, J. Kato, et al., Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion, Blood 106 (2005) 756–763.
[11] D.Q. Tang, L.Z. Cao, B.R. Burkhardt, et al., In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow, Diabetes 53 (2004) 1721–1732.
[12] C. Toma, M.F. Pittenger, K.S. Cahill, et al., Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart, Circulation 105 (2002) 93–98.
[13] J. Doorn, G. Moll, K. Le Blanc, et al., Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements, Tissue Eng. Part B Rev. 18 (2012) 101–115.
[14] D.J. Prockop, S.D. Olson, Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions, Blood 109 (2007) 3147–3151.
[15] A.I. Caplan, J.E. Dennis, Mesenchymal stem cells as trophic mediators, J. Cell. Biochem. 98 (2006) 1076–1084.
[16] S.K. Sze, D.P. de Kleijn, R.C. Lai, et al., Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells, Mol. Cell. Proteomics 6 (2007) 1680–1689.
[17] D.C. Colter, R. Class, C.M. DiGirolamo, et al., Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow, Proc. Natl. Acad. Sci. USA 97 (2000) 3213–3218.
[18] I. Sekiya, B.L. Larson, J.R. Smith, et al., Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality, Stem cells 20 (2002) 530–541.
[19] B.S. Park, K.A. Jang, J.H. Sung, et al., Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging, Dermatol. Surg. 34 (2008) 1323–1326.
[20] A.I. Caplan, D. Correa, The MSC: an injury drugstore, Cell Stem Cell 9 (2011) 11–15.
[21] A. Shabbir, D. Zisa, H. Lin, et al., 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. 299 (2010) H1428–1438.
[22] S.S. Fatimah, G.C. Tan, K. Chua, et al., Stemness and angiogenic gene expression changes of serial-passage human amnion mesenchymal cells, Microvasc. Res. 86 (2013) 21–29.
[23] K.L. Pricola, N.Z. Kuhn, H. Haleem-Smith, et al., Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism, J. Cell. Biochem. 108 (2009) 577–588.
[24] D.J. Klionsky, Autophagy: from phenomenology to molecular understanding in less than a decade, Nat. Rev. Mol. Cell Biol. 8 (2007) 931–937.
[25] N. Mizushima, B. Levine, A.M. Cuervo, et al., Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075.
[26] C. Cardenas-Aguayo Mdel, J. Santa-Olalla, J.M. Baizabal, et al., Growth factor deprivation induces an alternative non-apoptotic death mechanism that is inhibited by Bcl2 in cells derived from neural precursor cells, J. Hematother. Stem Cell Res. 12 (2003) 735–748.
[27] A.R. Young, M. Narita, M. Ferreira, et al., Autophagy mediates the mitotic senescence transition, Genes Dev. 23 (2009) 798–803.
[28] Y.O. Jang, Y.J. Kim, S.K. Baik, et al., Histological improvement following administration of autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: a pilot study, Liver Int. 34 (2014) 33–41.
[29] G.P. Dimri, X. Lee, G. Basile, et al., A biomarker that identifies senescent human cells in culture and in aging skin in vivo, Proc. Natl. Acad. Sci. USA 92 (1995) 9363–9367.