Effect of Fibroblast Growth Factor-2 and Retinoic Acid on Lineage Commitment of Bone Marrow Mesenchymal Stem Cells

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In this study, we examined the effect of a combination of fibroblast growth factor-2 (FGF-2) and retinoic acid (RA) on osteoblast and adipocyte lineage commitment and differentiation of human bone marrow mesenchymal stem cells (BMSCs). Pretreatment of human BMSCs with FGF-2 or RA for 5 days followed by osteoblast differentiation induction showed high calcium deposition compared to control. A combination of FGF-2 and RA further induced calcium deposition compared to FGF-2 or RA alone. The enhanced mineral deposition was accompanied with the increased expression of osteoblast differentiation markers, alkaline phosphatase and osteocalcin. On the other hand, FGF-2 pretreatment followed by adipocyte differentiation induction also showed increased formation of lipid droplets in human BMSCs, whereas RA pretreatment suppressed formation of lipid droplets. However, a combination of FGF-2 and RA increased formation of lipid droplets and expression of adipocyte marker genes, including adiponectin, ADIPOQ, FABP4, peroxisome proliferator-activated receptor γ (PPAR γ), and C/EBP α . During pretreatment of BMSCs with FGF-2, RA or in combination, the cells expressed similar levels of MSC surface markers such as CD29, CD44, CD90, and CD105, indicating that they maintain stem cell potential. To determine how RA cooperates with FGF-2 in osteoblast and adipocyte lineage commitment, the expression of RA receptors and intracellular lipid-binding proteins was examined. A combination of FGF-2 and RA strongly induced the expression of RA receptor α , β , γ , PPAR β/δ , CRABP-II, and FABP5. Collectively, these results demonstrate that combined pretreatment of human BMSCs with FGF-2 and RA enhances the commitment into osteoblast and adipocyte lineages through modulation of the expression of RA-related genes.

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Key Words: Human bone marrow mesenchymal stem cells; Lineage commitment; Differentiation; Fibroblast growth factor-2; Retinoic acid

INTRODUCTION

Human bone marrow mesenchymal stem cells (BMSCs) are capable of self-renewal and multiple lineage differentiation [1], and are thus one of the promising adult stem cells for tissue regeneration. With their relatively easy isolation and expansion *in vitro*, human BMSCs have been used in a variety of therapeutic clinical trials and tissue engineering applications [2,3]. However, lineage commitment and differentiation of human BMSCs toward target cells may need to be augmented for efficient tissue regeneration. A variety of factors can control *in vitro* modulation of the commitment and differentiation of hu-

man BMSCs for various applications.

Fibroblast growth factor (FGF) was originally identified as strong inducer of proliferation of fibroblasts [4]. Among 23 FGF family members, FGF-2 regulates multiple biological responses. It is known to be involved in not only *in vivo* embryonic limb development [5] but also *in vitro* cell proliferation and differentiation. FGF-2 is also a strong mitogen that stimulates proliferation of MSCs isolated from various species such as canine, rat, and human [6]. However, FGF-2 shows variable effects on osteochondrogenic differentiation. Iwasaki et al. [7] reported that FGF-2 inhibits osteochondrogenic differentiation of periosteum-derived cells. In contrast, another group showed that FGF-2 increases osteogenic and chondrogenic differentiation potentials of human BMSCs by inactivation of TGF- β signaling [8]. FGF-2 also stimulates osteogenic and chondrogenic differentiation of adipose-derived stromal cells by upregulating differentiation marker genes [9]. Therefore,

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FGF-2 has differential effects on the proliferation and differentiation of MSCs.

Vitamins are also involved in multiple biological processes, including bone formation and proliferation of MSCs [10,11]. Retinoic acid (RA) is an acidic form of vitamin A that has been used to treat dermatological disorders and acute promyelocytic leukemia [12]. In adult human BMSCs and human embryonic stem cells, RA can induce neuronal differentiation [13]. RA also has differential effects on the proliferation and differentiation of MSCs. In mouse adipose tissue-derived stem cells, RA can induce osteogenic differentiation and repair critical-sized calvarial defects [14]. RA functions as a mitogenic and anti-apoptotic factor in placental MSCs [15], whereas it has a negative effect on the proliferation of human BMSCs [16]. Therefore, both FGF-2 and RA produce variable effects on the proliferation and differentiation of MSCs toward osteoblast lineages.

In this study, we demonstrate that the combination of FGF-2 and RA results in the enhanced commitment of human BMSCs into osteoblast and adipocyte lineages without changing MSC characteristics, and the enhanced commitment was accompanied with upregulation of RA receptors (RARs) and intracellular lipid-binding proteins (iLBPs).

MATERIALS AND METHODS

Isolation and culture of human BMSCs

Bone marrow aspirates were obtained from patients during total hip replacement surgery. Informed consent was obtained from patients prior to surgery and experimental procedures were approved by the Institutional Research Board of Kyungpook National University Hospital (KNUHBIO_08-1007). Bone marrow aspirates were diluted with phosphate-buffered saline (PBS) and the cells were layered over a Percoll density gradient (Histopaque, Sigma-Aldrich, St. Louis, MO, USA). After separation in Percoll, bone marrow mononuclear cells were collected and washed twice with PBS and once with minimum essential medium alpha (α -MEM) (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% FBS. The cells were plated in a 100-mm culture dish and incubated at 37°C with 5% CO₂. After 24 h, non-adherent cells were removed. Adherent cells were expanded, analyzed with MSC CD markers, and considered as human BMSCs. Human BMSCs for all experiments were used before the fourth passage. The culture medium was changed every 2 to 3 days.

Cell proliferation assay

Human BMSCs were seeded in a 96-well plate at a density of 700 cells/well. Cells were incubated in α -MEM for 24 h, and

then treated with the indicated concentration of all-trans RA (Sigma-Aldrich, St. Louis, MO, USA), FGF-2 (R&D system, Minneapolis, MN, USA), or a combination of RA and FGF-2, for 3, 5, or 7 days. Proliferation was measured using a CellTiter 96 proliferation assay kit (Promega, Madison, WI, USA), according to the manufacturer's instructions. Briefly, media were removed and 20 μ L of Promega CellTiter 96 Aqueous One Solution Cell Proliferation Assay was added in 100 μ L serum-free Dulbecco's modified essential medium per well. After 2.5 h of incubation at 37°C, optical density was measured at 490 nm using an ELISA plate reader (Biotek, Winooski, VT, USA).

Osteogenic and adipogenic differentiation of human BMSCs

Human BMSCs pretreated with FGF-2, RA, or FGF-2+RA for 5 days were trypsinized and re-plated in 24-well plates at a density of 2.5×10^4 cells/well. On the following day, osteoblastic differentiation was induced with osteogenic medium (α -MEM supplemented with 10% FBS, 50 μ g/mL ascorbic acid, 10 mM β -glycerophosphate, and 10 nM dexamethasone) for 2 weeks. Osteoblastic differentiation was assessed by alizarin red S staining, and the expression of osteoblast marker genes was analyzed with reverse transcription polymerase chain reaction (RT-PCR). Adipogenic differentiation was induced with adipogenic medium (α -MEM supplemented with 10% FBS, 1 μ M dexamethasone, 111 μ g/mL isobutylmethylxanthine, 0.2 mM indomethacin, and 10 μ g/mL insulin) for 21 days. Adipogenic differentiation was assessed by Oil-red O staining. Calcium and oil accumulation was quantified using the Image J program (National Institutes of Health, Bethesda, MD, USA).

RT-PCR

Total RNA was extracted with TRI reagent (MRC, Cincinnati, OH, USA) and 1 μ g of total RNA was reverse transcribed with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. PCR reactions were performed with specific primers, shown in Table 1, under the following conditions: 94°C for 2 min, 94°C for 1 min, 50–60°C for 1 min, 72°C for 1 min, and 72°C for 5 min. PCR cycles for each gene were optimized with human osteoblast cDNAs. Densitometric analysis was performed using Quantity One 1-D analysis software (Bio-Rad, Hercules, CA, USA). The relative expression was determined based on the expression ratio of a target gene versus glyceraldehyde 3-phosphate dehydrogenase.

Surface marker identification by fluorescence-activated cell sorting

Human BMSCs were incubated with FGF-2, RA, or FGF-

Table 1. List of RT-PCR primers

Target		Sequence 5' → 3'
<i>GAPDH</i>	Sense:	5'-CCACTGGCGTGTTCACCAC-3'
	Antisense:	5'-CCTGCTTCACCACCTTCTTG-3'
<i>ALP</i>	Sense:	5'-GCGAACGTATTTCTCCAGACCCAG-3'
	Antisense:	5'-TTCCAAACAGGAGAGTCGCTTCAA-3'
<i>COL1A1</i>	Sense:	5'-GCGAACGTATTTCTCCAGACCCAG-3'
	Antisense:	5'-TTCCAAACAGGAGAGTCGCTTCAA-3'
<i>ON</i>	Sense:	5'-TGCCTGATGAGACAGAGGTG-3'
	Antisense:	5'-AAGTGGCAGGAAGAGTCGAA-3'
<i>OC</i>	Sense:	5'-GGTGCAGCCTTTGTGTCCAAGC-3'
	Antisense:	5'-GGCAAGGGGAAGAGGAAAGAAGG-3'
<i>FABP4</i>	Sense:	5'-GGCCAGGAATTTGACCAAGTC-3'
	Antisense:	5'-ACAGAATGTTGTAGAGTTCAATGCGA-3'
<i>ADIPOQ</i>	Sense:	5'-CATGACCAGGAAACCACGACT-3'
	Antisense:	5'-TGAATGCTGAGCGGTAT-3'
<i>PPARγ</i>	Sense:	5'-CATTCTGGCCACCAACTT-3'
	Antisense:	5'-CCTTGCATCCTTCACAAGCA-3'
<i>C/EBPα</i>	Sense:	5'-CACGAAGCACGATCAGTCCAT-3'
	Antisense:	5'-GGCACAGAGGCCAGATACAAG-3'
<i>RARα</i>	Sense:	5'-GTGTCAACGGGACAAGAACT-3'
	Antisense:	5'-GTCCGAGAAGGTCATGGTGT-3'
<i>RARβ</i>	Sense:	5'-GAAACAGGCCTTCTCAGTGC-3'
	Antisense:	5'-GGTGACTGACTGACCCCACT-3'
<i>RARγ</i>	Sense:	5'-AACAAGGTGACCAGGAATCG-3'
	Antisense:	5'-GTCGGAGAAGGTCATGGTGT-3'
<i>CRABP-II</i>	Sense:	5'-GGTGAATGTGATGCTGAGGA-3'
	Antisense:	5'-TTCTCTGGTCCACGAGGTCT-3'
<i>FABP5</i>	Sense:	5'-TCAGCAGCTGGAAGGAAGAT-3'
	Antisense:	5'-TGAACCAATGCACCATCTGT-3'
<i>PPARβ/δ</i>	Sense:	5'-ACTGAGTTCGCCAAGAGCAT-3'
	Antisense:	5'-GGAAGAGGTACTGGGCATCA-3'

ALP: alkaline phosphatase, COL1A1: type I collagen, ON: osteonectin, OC: osteocalcin, FABP4: fatty acid binding protein 4, ADIPOQ: adiponectin, PPAR γ : peroxisome proliferator-activated receptor γ , C/EBP: CCAAT/enhancer binding protein, RARs: retinoic acid receptors, CRABP-II: cellular retinoic acid-binding protein 2, FABP5: fatty acid-binding protein 5, PPAR β/δ : peroxisome proliferator-activated receptor β/δ , GAPDH: glyceraldehyde 3-phosphate dehydrogenase, RT-PCR: reverse transcription polymerase chain reaction

2+RA for 5 days. Then, the cells were harvested with 0.25% trypsin/EDTA and incubated in 100 μ L of 1% BSA containing monoclonal antibodies directed against CD29, CD44, CD105 (PE-conjugated), and CD90 (FITC-conjugated) (BD Pharmin-gen, Palo Alto, CA, USA) for 30 min at 4°C. The cells were centrifuged at 5000 rpm for 3 min and then washed with 1% BSA. The resuspended cells were fixed for 30 min in ice-cold 4% formaldehyde. Following fixation, the cells were washed twice with 1% BSA and were analyzed using a FACSaria Flow Cy-tometer (BD Diagnostics, Franklin Lakes, NJ, USA).

Statistical analysis

Statistical analysis was performed with Student t-test and *p*

value of less than 0.05 was considered significant.

RESULTS

Effect of FGF-2, RA, and FGF-2+RA on proliferation of human BMSCs

To examine the effect of FGF-2 and RA alone or in combina-tion on proliferation of human BMSCs, cells were treated with the indicated concentration for 3, 5, and 7 days (Fig. 1). Consis-tent with a previous report [16], RA inhibited proliferation of human BMSCs in a dose-dependent manner (Fig. 1). On the other hand, FGF-2 at a concentration of 1 ng/mL significantly stimulated proliferation of BMSCs (Fig. 1). However, FGF-

2-mediated enhanced proliferation was suppressed by RA, but the suppressed level of proliferation was similar to that of control (Fig. 1). These results demonstrate that FGF-2 and RA can modulate proliferation of BMSCs

Expression of MSC surface markers in human BMSCs pretreated with FGF-2 and RA

To examine whether human BMSCs expanded with FGF-2, RA, or FGF-2+RA retain their MSC characteristics, we analyzed the expression of MSC surface markers. After human BMSCs were cultured in the presence of FGF-2, RA, or FGF-2+RA for 5 days, the expression levels of MSC markers including CD29, CD44, CD90, and CD105 were analyzed by flow cytometry. Over 98% of the cells were positive for CD29, CD44, CD90, and CD105, indicating that they retained the expression of MSC surface markers and thus characteristics of BMSCs (Fig. 2).

Effect of FGF-2 and RA on osteoblast differentiation of BMSCs

To determine whether FGF-2 and RA are involved in regulating osteoblast differentiation/commitment, human BMSCs were pretreated with or without FGF-2, RA, or FGF-2+RA for 5 days, and then re-plated and induced with either osteoblast or adipocyte induction media. As shown in Figure 3A, human BMSCs expanded in the presence of FGF-2 and RA, and displayed enhanced calcium deposition compared to control. Interestingly, the combination of FGF-2 and RA followed by osteoblast induction showed the highest level of mineral deposition compared to the other conditions (Fig. 3A). We further confirmed osteoblast differentiation by examining the expression of osteoblast marker genes (Fig. 3B). RT-PCR showed that the expression of osteoblast differentiation marker genes encoding osteocalcin (OC), alkaline phosphatase (ALP), and type I collagen (COL1A1) were increased by FGF-2, RA, or FGF-2+RA. These results suggest that pretreatment with FGF-2, RA, or a combination of both may enhance the commitment of human BMSCs toward the osteoblast lineage.

Effect of FGF-2 and RA on adipocyte differentiation of BMSCs

Next, we examined the effect of FGF-2, RA, or FGF-2+RA on adipocyte differentiation of human BMSCs. The cells were pretreated with FGF-2, RA, or FGF-2+RA for 5 days, and then the pretreated cells were induced toward the adipocyte lineage for more than 21 days. The adipocyte differentiation was assessed by Oil-red O staining (Fig. 4A). Cells cultured with FGF-2 alone showed slightly enhanced oil formation compared to control, while oil formation was decreased by RA alone (Fig. 4A). These findings are in agreement with previous results, which suggest

that RA inhibits growth and adipogenic differentiation of human BMSCs [17]. In contrast, pretreatment of human BMSCs with a combination of FGF-2 and RA followed by adipocyte induction strongly stimulated formation of lipid droplets (Fig. 4A), suggesting that the combination of FGF-2 and RA induced commitment of human BMSCs to the adipocyte lineage. To confirm the enhanced adipocyte differentiation, the expression of adipocyte differentiation marker genes was analyzed. FGF-2 pretreatment marginally increased the expression of *ADIPOQ*, *FABP4*, *PPAR γ* , and *C/EBP α* , while RA pretreatment downregulated all of the adipocyte marker genes. However, most adipocyte marker genes were strongly induced by the combination of FGF-2 and RA compared to FGF-2 or RA alone (Fig. 4B). These results suggest that human BMSCs in response to a combination of FGF-2 and RA undergo commitment to the adipocyte lineage.

Expression of RARs, PPAR β/δ , and iLBPs in human BMSCs in response to FGF-2 and RA

According to Schug et al. [18], when RA binds to FABP5, the RA-FABP5 complex is delivered to the PPAR β/δ receptor in the nucleus, activating the cell proliferation program. When RA binds to CRABP-II, on the other hand, the RA-CRABP-II complex is delivered to the RARs, inhibiting cell proliferation. To understand the molecular mechanisms underlying the cooperation between RA and FGF-2 in the enhanced commitment and differentiation of human BMSCs, we examined the mRNA expression levels of the RARs and iLBPs using RT-PCR (Fig. 5). FGF-2 and RA induced the expression of CRABP-II and RARs, which are involved in growth inhibition. Interestingly, the expression of CRABP-II and RARs was further induced by FGF-2 plus RA. Moreover, expression of FABP5 and PPAR β/δ , which are involved in cell proliferation, was also weakly induced by FGF-2 plus RA. These results suggest that combined treatment of FGF-2 and RA in human BMSCs causes increased expression of RARs and iLBPs. Thus, human BMSCs may become more sensitive to RA stimulation.

DISCUSSION

Human BMSCs have been shown to be a valuable resource for cell therapies and tissue regeneration [2,3]. Most cell therapeutic and tissue engineering approaches simply transplant BMSCs rather than functionally modified cells. To improve regeneration by BMSCs, it is necessary to properly modulate the functionality of naïve BMSCs. For instance, lineage-specific commitment of naïve BMSCs may greatly stimulate regeneration of damaged tissue after implantation.

FGF-2 has been shown to play multiple roles in human BM-

Figure 1. The effect of FGF-2 and RA on proliferation of BMSCs. (A) BMSCs were seeded in 96-well plates at a density of 700 cells/well. The cells were cultured in the presence of FGF-2 (1 ng/mL), various concentrations of all-trans RA (50 to 1250 nM), or a combination of RA and FGF-2 for 3, 5, and 7 days. At the indicated time, proliferation was measured by a cell proliferation assay kit. FGF-2: fibroblast growth factor-2, RA: retinoic acid, BMSCs: bone marrow mesenchymal stem cells.

Figure 2. The expression level of MSC surface markers on BMSCs expanded with RA and FGF-2. BMSCs were cultured in the presence of FGF-2 (1 ng/mL), RA (50 nM), or a combination of the two. After 5 days, cells were harvested and labeled with anti-CD29, CD44, CD90, and CD105 antibodies conjugated with PE or FITC, and then the expression levels were determined by flow cytometry. FGF-2: fibroblast growth factor-2, RA: retinoic acid, BMSCs: bone marrow mesenchymal stem cells, PE: phycoerythrin, FITC: fluorescein isothiocyanate.

Figure 3. Osteoblast differentiation of BMSCs expanded with FGF-2 and RA. (A) Mineralization was assessed by alizarin red S staining. Cells pretreated with FGF-2, RA, or FGF-2 plus RA for 5 days, then replated at a density of 2.5×10^4 cells/well in 24-well plates. On the next day, BMSCs were induced osteoblast differentiation for 2 weeks and stained with alizarin red S (left). Quantification of calcium accumulation was performed using the ImageJ program (right). (B) Expression of osteoblast differentiation marker genes was analyzed by semi-quantitative RT-PCR. Densitometric analysis of RT-PCR was performed using Quantity One 1-D analysis software (below). FGF-2: fibroblast growth factor-2, RA: retinoic acid, BMSCs: bone marrow mesenchymal stem cells, RT-PCR: reverse transcription polymerase chain reaction.

SCs. We have previously reported that FGF-2 in combination with dexamethasone promotes the proliferation and osteoblastic differentiation of adipose tissue-derived MSCs [19]. These results suggest that FGF-2 synergistically interacts with other factors in terms of cellular proliferation and lineage commitment/differentiation of MSCs. Since RA inhibits proliferation

and adipocyte differentiation of MSCs, and osteoblast differentiation occurs at the expense of adipocyte differentiation of BMSCs, we asked whether RA can interact with FGF-2 in promoting the proliferation and differentiation of human BMSCs. As reported previously [16], RA inhibited proliferation of human BMSCs in a dose-dependent manner (Fig. 1). However,

Figure 5. The expression levels of RARs, PPARβ/δ, and iLBPs. BMSCs were plated and treated with FGF-2 (1 ng/mL), RA (50 nM), or a combination of the two for 5 days. Then, cells were harvested and RNA was isolated. The expression level of genes was analyzed by RT-PCR. A representative image of gene expression is shown (upper panel), and the expression level of genes was quantified by using Quantity One 1-D analysis software (bottom). FGF-2: fibroblast growth factor-2, RA: retinoic acid, BMSCs: bone marrow mesenchymal stem cells, RT-PCR: reverse transcription polymerase chain reaction.

FGF-2 partially rescued the RA-mediated suppression of proliferation of human BMSCs (Fig. 1). These results suggest that FGF-2 can interact with RA in promoting the growth of human BMSCs.

We also asked whether interaction between FGF-2 and RA occurs in the multiple lineage commitment or differentiation of BMSCs. Alizarin red S staining showed that the combination of FGF-2 and RA induced the highest levels of calcium deposition compared to FGF-2 or RA alone (Fig. 3A). These results are consistent with the enhanced mRNA expression of osteoblast differentiation marker genes including *ALP*, *COL1A1*, and *OC* (Fig. 3B). Therefore, FGF-2 and RA in combination seem to increase the osteoblast lineage differentiation potential of human BMSCs. We also asked whether human BMSCs that expanded in the presence of FGF-2 and RA were capable of differentiating into adipocytes. Previously it was shown that RA substantially suppresses adipocyte differentiation in mouse MSCs [17]. RA-mediated suppression of adipocyte differentiation was also observed in human BMSCs (Fig. 4A), suggesting that RA may inhibit adipocyte differentiation with a common mechanism in both human and mouse MSCs. We also found that RA-mediated suppression of adipocyte differentiation was partially rescued by FGF-2 (Fig. 4A). Since FGF-2 has been shown to stimulate adipocyte differentiation, signaling pathways induced by RA and FGF-2 may interact to increase the adipocyte differentiation potential. Therefore, our data strongly suggest that priming human BMSCs with FGF-2 and RA increases their potential for differentiation into osteoblasts and adipocytes. It is also interesting to determine in which differentiation stage is BMSCs pretreated with FGF-2 and RA. Immunotyping analysis of cell surface markers of BMSCs pretreated with FGF-2 and RA showed no significant alteration of the MSC markers (Fig. 2), indicating that human BMSCs can be primed or committed to the osteoblast or adipocyte lineage by FGF-2 and RA without loss of stem cell potential.

During lineage commitment of human BMSCs, RA in combination with FGF-2 appears to further enhance the commitment of BMSCs to differentiate to osteoblasts and adipocytes (Figs. 3 and 4). One possible explanation for this observation is that BMSCs became more sensitive to RA and thus obtained a commitment phenotype. Schug et al. [18] reported that when RA binds to the lipid-binding protein CRABP-II, the complex is delivered to the RAR and cell growth is inhibited. However, when RA binds to FABP5, the complex is transported to PPAR β/δ and the signal induces cell proliferation. In addition, when cells express a high ratio of FABP5:CRABP-II in the cytosol, FABP5 binds PPAR β/δ and promotes survival. Therefore, expression of RAR γ , β , and α , PPAR β/δ , and lipid-binding pro-

tein was analyzed to examine the cooperative action of RA and FGF-2 on the commitment of human BMSCs. RA induced the expression of CRABP-II and RARs, which are involved in growth inhibition, thus accounting for the inhibition of BMSC proliferation by RA (Fig. 1). Interestingly, the expression of CRABP-II and RARs was further induced by FGF-2 plus RA. Consistent with these results, cell proliferation enhanced by FGF-2 was decreased by RA (Fig. 1). Moreover, expression of FABP5 and PPAR β/δ , which are involved in cell proliferation, was also weakly induced by FGF-2 plus RA. Although we did not quantitatively demonstrate to what extent the target genes, RARs, CRABP-II, FABP5, and PPAR β/δ were induced, it is plausible that certain levels of target genes of RA-responsive genes may affect the commitment of BMSCs to form osteoblasts or adipocytes. Further study may be necessary to demonstrate whether target genes of RARs and iLBPs are involved in the lineage commitment of human BMSCs.

In summary, we have shown that when FGF-2 and RA are simultaneously applied to human BMSCs, they can effectively promote the commitment of human BMSCs to osteoblast and adipocyte lineages, without changing their MSC characteristics. The enhanced differentiation potential of human BMSCs induced by FGF-2 and RA was accompanied with increased expression of RARs and iLBPs. Therefore, human BMSCs that have been expanded in the presence of both FGF-2 and RA *in vitro* may be useful for application in bone and adipose tissue engineering.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

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