International Journal of Oral Biology, Vol. 37, No. 1, March 31 2012, p. 31~36
Copyright ⓒ 2012, *The Korean Academy of Oral Biology*

International Journal of Oral Biology

Dlx3 and Dlx5 Inhibit Adipogenic Differentiation of Human Dental Pulp Stem Cells

Hye-Lim Lee, Hyun Nam, Gene Lee, and Jeong-Hwa Baek*

Department of Molecular Genetics, School of Dentistry and Dental Research Institute, Seoul National University, Seoul 110-749, Korea

(received Feb 6, 2012 ; revised Feb 23, 2012 ; accepted Mar 15, 2012)

Dlx3 and Dlx5 are homeobox domain proteins and are well-known regulators of osteoblastic differentiation. Since possible reciprocal relationships between osteogenic and adipogenic differentiation in mesenchymal stem cells exist, we examined the regulatory role of Dlx3 and Dlx5 on adipogenic differentiation using human dental pulp stem cells. Over-expression of Dlx3 and Dlx5 stimulated osteogenic differentiation but inhibited adipogenic differentiation of human dental pulp stem cells. Dlx3 and Dlx5 suppressed the expression of adipogenic marker genes such as C/EBPα, PPARγ, aP2 and lipoprotein lipase. Adipogenic stimuli suppressed the mRNA levels of Dlx3 and Dlx5, whereas osteogenic stimuli enhanced the expression of Dlx3 and Dlx5 in 3T3-L1 preadipocytes. These results suggest that Dlx3 and Dlx5 exert a stimulatory effect on osteogenic differentiation of stem cells through the inhibition of adipogenic differentiation as well as direct stimulation.

Key words: Dlx3, Dlx5, adipocyte differentiation, human dental pulp stem cells

Introduction

The Distal-less (Dlx) protein is a homeobox domain (HD) protein. In vertebrates, there are six Dlx genes arranged in three clusters (Dlx1/Dlx2, Dlx3/Dlx4, Dlx5/Dlx6) [1]. Dlx is expressed in limb bud, brachial arch and epi-mesoderm during development and is required for the development and maintenance of these structures. Among the Dlx genes, Dlx3 and Dlx5 are known to be a positive regulator of osteoblast differentiation [2,3]. Dlx3 is expressed in pre-osteoblast, osteoblast and osteocyte in bone [4]. Bone morphogenetic protein 2 (BMP2), an inducer of osteoblast differentiation, increases Dlx3 expression and Dlx3 expression is induced earlier than that of Runx2 [5]. Dlx3 over-expression promotes the expression of osteoblast marker genes and directly regulates the transcription of Runx2 and osteocalcin by binding to their promoters [2,6]. However, *in vivo* function of the Dlx3 in osteoblast differentiation and bone formation is not clear because the *dlx3*$^{-/-}$ mice are embryonic lethal due to placental deficiency [7]. Dlx5 expression is also induced by BMP2 [8]. Over-expression of Dlx5 induces the expression of Runx2, alkaline phosphatase (ALP) and osteocalcin and increases mineralized nodule formation *in vitro* [2,3]. Dlx5-deficient mice showed reduced calcification and abnormal craniofacial development [9].

Mesenchymal stem cells are multipotent that can give rise not only to osteoblasts, but also to a range of other cell types, including adipocytes, chondrocytes and myoblasts [10]. Among these fates, differentiation to the osteoblast and adipocyte lineages has particular relevance to the maintenance of normal bone homeostasis. Because osteoblasts and adipocytes differentiate from a common progenitor, inducers of differentiation toward one lineage may inhibit cell differentiation into another lineage. Osteoporosis and aging-related osteopenia were shown to be associated with an increased adipocyte accumulation in bone marrow, suggesting that there are the inverse relationships between osteoblast differentiation and adipocyte differentiation in bone marrow [11-13].

Although there were a significant number of reports about the role of Dlx3 and Dlx5 in relation to osteoblast differentiation, we could not find the report about the regulatory role of Dlx3 and Dlx5 in adipocyte differentiation. Therefore, in this study, we examined the effect of Dlx3 and Dlx5 on

*Corresponding author: Jeong-Hwa Baek, Department of Molecular Genetics, School of Dentistry, Seoul National University, 28 Yeongun-dong, Jongno-gu, Seoul 110-749, Korea. Tel: +82-2-740-8688; Fax: +82-2-741-3193; E-mail: baekjh@snu.ac.kr

adipocyte differentiation in mesenchymal stem cells derived from human dental pulp tissues.

Materials and methods

Reagents

Alpha modified Eagle's medium (αMEM) and fetal bovine serum (FBS) was purchased from BioWhittaker (Walkersville, MD, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Hyclone (Logan, UT, USA). BMP2 was purchased from R&D Systems (Minneapolis, MN, USA). The easy-BLUE^{TM} for total RNA extraction and *Star*Taq^{TM} polymerase for polymerase chain reaction (PCR) amplification were obtained from iNtRON Biotechnology (Sungnam, Korea). AccuPower RT-PreMix for the first-strand cDNA synthesis was from Bioneer (Daejeon, Korea). PCR primers were synthesized by TaKaRa Korea (Seoul, Korea). Ascorbic acid, β-glycerophosphate, dexamethasone, indomethacin, isobutylmethylxanthin and insulin were purchased from Sigma (St. Louis, MO, USA).

Cell culture and transient transfection

Human dental pulp stem cells were prepared from exfoliated deciduous pulp tissues as previously described [14]. Single-cell suspensions were cultured in α-MEM supplemented with 10% FBS and 1% antibiotics-antimycotics. Medium was changed every 3 days and cells were sub-cultured at 70% confluency. Murine preadipocytic 3T3-L1 cells (Korean cell line bank; Seoul, Korea) were cultured in DMEM supplemented with 10% FBS and 1% antibiotics-antimycotics.

For the transient transfection of Dlx3 and Dlx5 expression plasmids, electroporation was performed using a Gene Pulser X Cell (Bio-Rad laboratories, Richmond, CA, USA) according to the manufacturer's instructions. The construction of Dlx3 and Dlx5 expression vectors has been described previously [15,16].

To induce osteogenic differentiation, human dental pulp stem cells and 3T3-L1 cells were cultured in osteogenic medium (growth medium supplemented with 0.1 μM dexamethasone, 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid) for the indicated period. When indicated, BMP2 (300 ng/ml) was added to osteogenic medium. To induce adipogenic differentiation, cells were incubated in adipogenic medium (growth medium supplemented with 0.1 μM dexamethasone, 50 μM indomethacin, 0.5 mM isobutylmethylxanthine and 10 μg/ml insulin) for the indicated period. At the end of the culture period, osteogenic and adipogenic differentiation was confirmed by Alizarin red S and Oil red O staining, respectively.

Fluorescense-assisted cell sorter (FACS) analysis

Expression of stem cell surface markers in human dental pulp stem cells was determined by FACS analysis as previously described [14]. The cells were detached and washed with phosphate-buffered saline supplemented with 2% FBS. Quantities of 100,000-500,000 cells were incubated with the primary antibody for 30 min on ice. The following antibodies were used; FITC-conjugated mouse anti-human CD14, CD31, CD44 and CD45 antibodies; PE-conjugated mouse anti-human CD29, CD73 and CD117 antibodies; PE.Cy5-conjugated mouse anti-human CD90 antibody; APC-conjugated mouse anti-human CD34 and HLA-DR antibodies (all from BD PharMingen, San Jose, CA, USA); APC-conjugated mouse anti-human CD105 antibody (eBioscience, San Diego, CA, USA) and biotin-conjugated mouse anti-human HLA class I antibody (BD PharMingen). After washing, cells were fixed with 4% paraformaldehyde at 4°C before analysis. Fluorescence intensity was measured on a FACS Calibur (BD PharMingen), and data were analyzed using FLOWJO software (Tree Star, Inc., Ashland, OR, USA).

Reverse Transcription-PCR (RT-PCR)

To evaluate mRNA expression, semi-quantitative RT-PCR was performed in the range of linear amplification. Total RNA was isolated using easy-BLUE^{TM} RNA Extraction Reagents. cDNA was synthesized from total RNA using the AccuPower^{TM} RT-PreMix and was subsequently subjected to PCR amplification using *Star*Taq^{TM} polymerase. The PCR products were electrophoresed in a 1.2% agarose gel and visualized under UV light by ethidium bromide staining. Human genes and their primer sequences for PCR were as follows (in parentheses, the annealing temperature used for the PCR reaction and the product size are described): cAMP response element binding protein (CREB) forward (f) 5'-ACCAGCAGAGTGGAGATGCT-3', CREB reverse (r) 5'-CTGCCCACTGCTAGTTTGGT-3' (58°C, 476 bp); peroxisome proliferator-activated receptor γ (PPARγ)-f 5'-GAC CACTCCCACTCCTTTGA-3', PPARγ-r 5'-CGACATTC AATTGCCATGAG-3' (58°C, 257 bp); adipocyte protein 2 (aP2)-f 5'-CATCAGTGTGAATGGGGATG-3', aP2-r 5'-GTGGAAGTGACGCCTTTCAT-3' (58°C, 541 bp); lipoprotein lipase (LPL)-f 5'-ACAGGGCGGCCACAAGTTTT-3', LPL-r 5'-ATGGAGAGCAAAGCCCTGCTC-3' (60°C, 717 bp); CCAAT/enhancer-binding proteins α (C/EBPα)-f 5'-CACGAAGCACGATCAGTCCAT-3', C/EBPα-r 5'-GGC ACAGAGGCCAGATACAAG-3' (58°C, 110 bp); Dlx3-f 5'-AAGGTCCGAAAGCCGCGTA-3', Dlx3-r 5'-CTGCTGC TGTAAGTGGGGT-3' (55°C, 414 bp) GAPDH-f 5'-CCAT CTTCCAGGAGCGAGATC-3' and GAPDH-r 5'-GCCTT CTCCATGGTGGTGAA-3' (56°C, 321 bp). Mouse genes and their primer sequences for PCR are as follows Runx2-f 5'-CCGCACGACAACCGCACCAT-3', Runx2-r 5'-CGC TCCGGCCCACAAATCTC-3' (56°C, 289 bps); bone sialoprotein (BSP)-f 5'-GTCAACGGCACCAGCACCAA-3', BSP-r 5'-GTAGCTGTATTCGTCCTCAT-3' (58°C, 375 bps); ALP-f 5'-AGGCAGGATTGACCACGG-3', ALP-r 5'-TG TAGTTCTGCTCATGGA-3' (56°C, 439 bps); Dlx3-f 5'-ATGAGTGGCTCCTTCGATCGCAAGCT-3', Dlx3-r 5'-T CAGTACACAGCCCCAGGGTTGGGC-3' (60°C, 421 bp)

Dlx5-f 5'-TCTCTAGGACTGACGCAAACA-3', Dlx5-r 5'-GTTACACGCCATAGGGTCGC-3' (56°C, 120 bp) GAPDH-f 5'-TCACCATCTTCCAGGAGCG-3' and GAPDH-r 5'-CTGCTTACCACCTTCTTGA-3' (56°C, 571 bps).

Results and discussion

Dental pulp tissues have been shown to be an excellent source for stem cells because it can be obtained from the deciduous dentition requiring extraction as part of a planned serial extraction for management of occlusion [17]. Previous studies have shown that *in vitro* culture conditions can induce dental pulp stem cells to differentiate into osteogenic, chondrogenic, adipogenic and myogenic cells [17,18]. Therefore, in this study, we used dental pulp stem cells derived from human exfoliated deciduous pulp tissues. The cells showed fibroblast-like morphology (Fig. 1A). Immune-phenotyping of the cells of passage number 3 showed that they were negative for hematopoietic markers (CD14, CD34, CD45, CD117 and HLA-DR) and endothelial marker (CD31), whereas more than 90% of cells were positive for mesenchymal stem cell markers (CD29, CD44, CD73, CD90, CD105, CD73 and HLA-I) (Fig. 1B). These results are consistent with the previous report about stem cells from human exfoliated deciduous teeth, and indicate that these cells are mesenchymal stem cells [19].

We then examined the effect of Dlx3 and Dlx5 over-expression on adipogenic differentiation. Dental pulp stem cells were transiently transfected with the expression vector for Dlx3 or Dlx5 and incubated under the adipogenic conditions for 21 days. Oil red O staining showed that the accumulation of lipid droplets was diminished in Dlx-transfected cells compared with pcDNA-transfected cells (Fig. 2). As a control, these cells were also incubated in the osteogenic medium for 21 days, and osteogenic induction was verified by Alizarin red staining (Fig. 2). Consistent with the previous reports, over-expression of Dlx3 or Dlx5 enhanced osteogenic differentiation and matrix mineralization [2,5].

To further verify the inhibitory effect of Dlx3 and Dlx5 on adipogenic differentiation, we examined the expression levels of adipogenic marker genes. After the incubation for 21

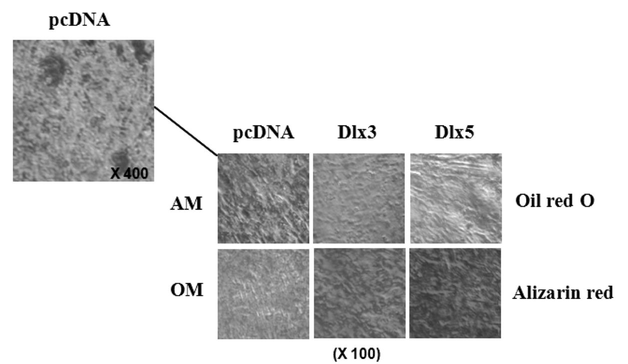

Fig. 2. Over-expression of Dlx3 or Dlx5 inhibited adipogenic differentiation of human dental pulp stem cells while stimulating osteogenic differentiation. The cells were transiently transfected with pcDNA or with expression vector for Dlx3 or Dlx5 and incubated in adipogenic medium (AM) or osteogenic medium (OM) for 21 days with medium change every other day. At the end of the culture period, Oil red O and Alizarin red staining was performed to examine adipogenic and osteogenic differentiation, respectively. Microscopic images of Oil red O- or Alizarin red-stained cells were presented (× 100). (*Upper left panel*) Higher magnification image (× 400) of Oil red O-stained pcDNA-transfected cells.

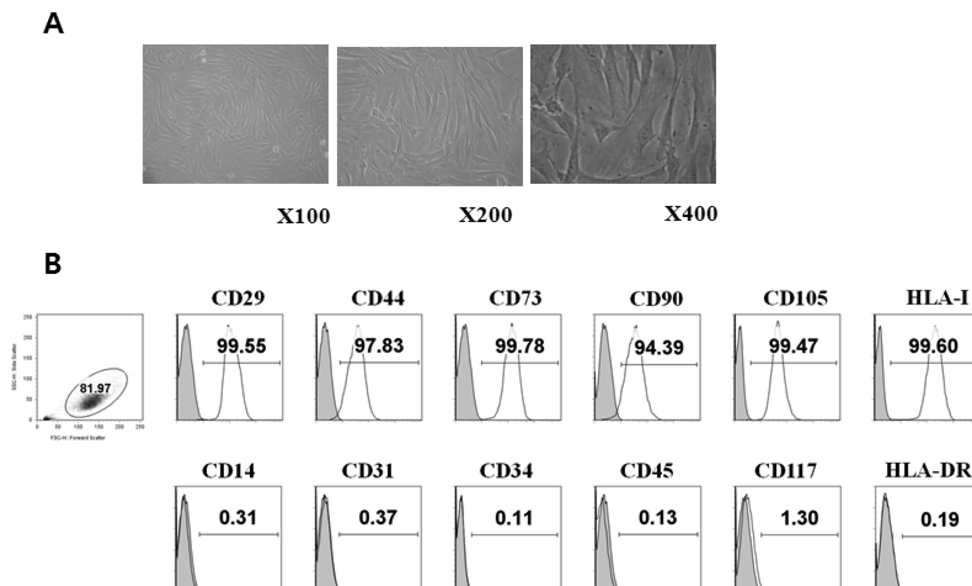

Fig. 1. Human dental pulp stem cells showed fibroblast-like morphology and mesenchymal stem cell surface marker expression. (A) Microscopic images of human dental pulp stem cells of passage number three. (B) Results of immune-phenotyping of human dental pulp stem cells (passage 3) for detection of surface marker expression.

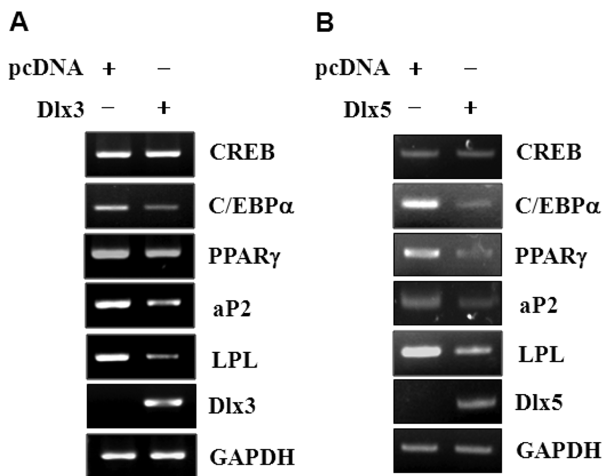

Fig. 3. Over-expression of Dlx3 and Dlx5 suppressed adipogenic marker gene expression of human dental pulp stem cells. The cells were transiently transfected with pcDNA or with expression vector for Dlx3 (A) or Dlx5 (B) and incubated in adipogenic medium for 21 days. At the end of the culture period, total RNA was isolated and semi-quantitative RT-PCR was performed.

days in adipogenic medium, the cells were harvested for RT-PCR. As expected, over-expression of Dlx3 and Dlx5 suppressed the expression of adipogenic marker genes such as C/EBPα, PPARγ, aP2 and LPL but not that of CREB (Fig. 3). These results indicate that Dlx3 and Dlx5 inhibit adipogenic differentiation through the inhibition of adipogenic gene expression.

Compared with pcDNA-transfected cells, the level of Dlx3 or Dlx5 was higher in Dlx-transfected cells (Fig. 3). Because we used transient transfection method to over-express Dlx, it is unlikely that Dlx3 or Dlx5 mRNA in 21 day-cultured cells is mainly derived from exogenously introduced expression vectors. Therefore, we examined whether adipogenic differentiation itself regulates the expression levels of Dlx3 and Dlx5. For this experiment, we used 3T3-L1 preadipocyte cell line. 3T3-L1 cells were incubated in the growth medium, adipogenic medium or osteogenic medium for 48 h and we examined the expression levels of Dlx3 and Dlx5 by RT-PCR. Adipogenic stimuli suppressed the expression of both Dlx3 and Dlx5 whereas osteogenic stimuli increased their expression levels (Fig. 4B). To confirm that osteogenic condition can induce osteogenic differentiation of 3T3-L1 cells, we incubated the cells under osteogenic condition for 6 days and examined the expression levels of osteogenic marker genes. RT-PCR results showed that the levels of Runx2, BSP and ALP mRNA increased by osteogenic stimuli (Fig. 4A). These results suggest that increased expression of Dlx3 and Dlx5 mRNA observed in Fig. 3 is mainly indirect effect of inhibition of adipogenic differentiation of dental pulp stem cells.

Differentiation of mesenchymal stem cells to the specific lineage depends on the phenotype-specific transcription factors. The representative transcription factors involved in

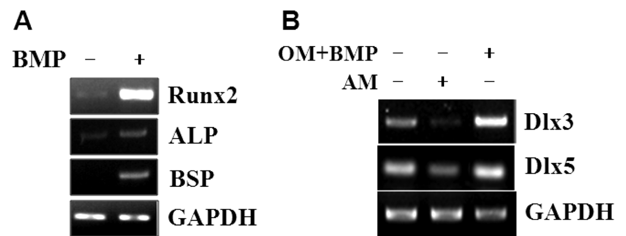

Fig. 4. Adipogenic stimuli suppressed the expression levels of Dlx3 and Dlx5 whereas osteogenic stimuli increased their expression levels in 3T3-L1 cells. (A) The cells were incubated in osteogenic medium in the presence or absence of BMP2 (300 ng/ml) for 6 days. (B) The cells were incubated in growth medium, adipogenic medium (AM) or osteogenic medium (OM) supplemented with BMP2 (300 ng/ml) for 48 h. At the end of the culture period, total RNA was isolated and semi-quantitative RT-PCR was performed. BSP, bone sialoprotein; ALP, alkaline phosphatase

osteoblast differentiation include Runx2, Osterix, ATF4, Dlx3 and Dlx5 [20]. As for adipocyte differentiation, transcription factors including C/EBPα, C/EBPβ, PPARγ and CREB are the main determinants of adipogenic differentiation [21]. Among them, C/EBPα and PPARγ are regarded as the essential transcription factors because the deletion of any of these transcription factors blocked adipogenic differentiation [21,22]. As described above, there are inverse relationships between adipogenesis and osteogenesis in the bone marrow. Several transcription factors have been suggested to be involved in this reciprocal regulation, including Runx2 and PPARγ [23]. Osteogenic stimuli, such as mechanical stress, increase the expression and transcriptional activity of Runx2 while suppressing PPARγ expression, resulting in the shift of mesenchymal stem cell fate toward the osteogenic lineage [24,25]. In contrast, adipogenic stimuli, such as immobilization and an antidiabetic medicine rosiglitazone, show opposite effects; that is, enhancement of PPARγ expression and suppression of Runx2 expression are observed, resulting in enhanced adipogenesis in place of osteogenic differentiation [26,27]. Therefore, the transcription factors that regulate the expression of Runx2 and/or PPARγ will exert an effect on stem cell fate determination toward osteoblast and/or adipocyte lineage. Previous studies have shown that Dlx3 and Dlx5 mediate BMP2-induced Runx2 expression in pre-osteoblastic cells [2,5,28]. In this study, we showed that Dlx3 and Dlx5 suppressed the expression of PPARγ. Furthermore, we also demonstrated that expression levels of Dlx3 and Dlx5 were up-regulated by osteogenic stimuli while being down-regulated by adipogenic stimuli. Taken together with previous reports, these results suggest that Dlx3 and Dlx5 play an important regulatory role in fate determination of mesenchymal stem cells toward osteoblast lineage by inhibition of adipogenesis as well as by stimulation of osteoblastogenesis. Furthermore, it is suggested that down-regulation of Dlx3 and Dlx5 is necessary for proper adipogenic differentiation.

Acknowledgement

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0068779).

References

1. Ghanem N, Jarinova O, Amores A, Long Q, Hatch G, Park BK, Rubenstein JL, Ekker M. Regulatory roles of conserved intergenic domains in vertebrate Dlx bigene clusters. Genome Res. 2003;13:533-43.
2. Hassan MQ, Javed A, Morasso MI, Karlin J, Montecino M, van Wijnen AJ, Stein GS, Stein JL, Lian JB. Dlx3 transcriptional regulation of osteoblast differentiation: temporal recruitment of Msx2, Dlx3, and Dlx5 homeodomain proteins to chromatin of the osteocalcin gene. Mol Cell Biol. 2004; 24:9248-61.
3. Ryoo HM, Hoffmann HM, Beumer T, Frenkel B, Towler DA, Stein GS, Stein JL, van Wijnen AJ, Lian JB. Stage-specific expression of Dlx-5 during osteoblast differentiation: involvement in regulation of osteocalcin gene expression. Mol Endocrinol. 1997;11:1681-94.
4. Li H, Marijanovic I, Kronenberg MS, Erceg I, Stover ML, Velonis D, Mina M, Heinrich JG, Harris SE, Upholt WB, Kalajzic I, Lichtler AC. Expression and function of Dlx genes in the osteoblast lineage. Dev Biol. 2008;316:458-70.
5. Hassan MQ, Tare RS, Lee SH, Mandeville M, Morasso MI, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. BMP2 commitment to the osteogenic lineage involves activation of Runx2 by DLX3 and a homeodomain transcriptional network. J Biol Chem. 2006;281:40515-26.
6. Cha JH, Ryoo HM, Woo KM, Kim GS, Baek JH. Tricho-dento-osseous syndrome mutant Dlx3 shows lower transactivation potential but has longer half-life than wild-type Dlx3. Int J Oral Biol. 2007;32:119-25.
7. Morasso MI, Grinberg A, Robinson G, Sargent TD, Mahon KA. Placental failure in mice lacking the homeobox gene Dlx3. Proc Natl Acad Sci U S A. 1999;96:162-7.
8. Jun JH, Ryoo HM, Woo KM, Kim GS, Baek JH. Bone morphogenetic protein 2-induced MAPKs activation is independent of the Smad1/5 activation. Int J Oral Biol. 2009;34:115-21.
9. Acampora D, Merlo GR, Paleari L, Zerega B, Postiglione MP, Mantero S, Bober E, Barbieri O, Simeone A, Levi G. Craniofacial, vestibular and bone defects in mice lacking the Distal-less-related gene Dlx5. Development (Cambridge, England). 1999;126:3795-809.
10. 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;284:143-7.
11. SaidakZ, Hay E, Marty C, Barbara A, Marie PJ. Strontium ranelate rebalances bone marrow adipogenesis and osteoblastogenesis in senescent osteopenic mice through NFATc/Maf and Wnt signaling. Aging cell. 2012; 10.1111/j.1474-9726.2012.00804.x.
12. Meunier P, Aaron J, Edouard C, Vignon G. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. Clin Orthop Relat Res. 1971;80:147-54.
13. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging cell. 2004;3:379-89.
14. Nam H, Lee G. Identification of novel epithelial stem cell-like cells in human deciduous dental pulp. Biochem Biophys Res Commun. 2009;386:135-9.
15. Cha JH, Ryoo HM, Woo KM, Kim GS, Baek JH. Dlx3 plays a role as a positive regulator of osteoclast differentiation. Int J Oral Biol. 2007;32:85-91.
16. Kim YJ, Lee MH, Wozney JM, Cho JY, Ryoo HM. Bone morphogenetic protein-2-induced alkaline phosphatase expression is stimulated by Dlx5 and repressed by Msx2. J Biol Chem. 2004;279:50773-80.
17. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000;97:13625-30.
18. Zhang W, Walboomers XF, Wolke JG, Bian Z, Fan MW, Jansen JA. Differentiation ability of rat postnatal dental pulp cells in vitro. Tissue Eng. 2005;11:357-68.
19. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003;100:5807-12.
20. Marie PJ. Transcription factors controlling osteoblastogenesis. Arch Biochem Biophys. 2008;473:98-105.
21. Wu Z, Puigserver P, Spiegelman BM. Transcriptional activation of adipogenesis. Curr Opin Cell Biol. 1999;11: 689-94.
22. Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol. 1996;16:4128-36.
23. Muruganandan S, Roman AA, Sinal CJ. Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. Cell Mol Life Sci. 2009;66:236-53.
24. David V, Martin A, Lafage-Proust MH, Malaval L, Peyroche S, Jones DB, Vico L, Guignandon A. Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis. Endocrinology. 2007;148:2553-62.
25. Sen B, Xie Z, Case N, Ma M, Rubin C, Rubin J. Mechanical strain inhibits adipogenesis in mesenchymal stem cells by stimulating a durable beta-catenin signal. Endocrinology. 2008;149:6065-75.
26. Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. Endocrinology. 2005;146:1226-35.

27. Minaire P, Edouard C, Arlot M, Meunier PJ. Marrow changes in paraplegic patients. Calcified Tissue Int. 1984; 36:338-40.
28. Lee MH, Kim YJ, Kim HJ, Park HD, Kang AR, Kyung HM, Sung JH,Wozney JM, Ryoo HM. BMP-2-induced Runx2 expression is mediated by Dlx5, and TGF-beta 1 opposes the BMP-2-induced osteoblast differentiation by suppression of Dlx5 expression. J Biol Chem. 2003;278: 34387-94.