Exogenous Polyamines Promote Osteogenic Differentiation In Human Osteoblasts

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Introduction: Polyamines have been implicated in the growth and development of bone and cartilage (Matsui-Yuasa et al., 1985; Vittur et al., 1986). The polyamine spermine has been shown to promote osteogenic differentiation of goat adipose tissue-derived mesenchymal stem cells (ADSCs) (Tjabringa et al., 2006; 2008). In addition, spermine is found to inhibit both nitric oxide (NO) production and COX2 gene expression, both of which are involved in the mechanical adaptation of bone during mechanical loading (Tjabringa et al., 2006). Recently, our published work further suggested that exogenous putrescine, spermidine and spermine reciprocally regulate osteogenic and adipogenic gene expression as well as terminal differentiation in human bone marrow-derived mesenchymal stem cells (hBMSCs) (Lee et al., 2013). In this study, human osteoblasts (hOBs) were treated with exogenous polyamines, as well as α-difluoromethylornithine (DFMO), the irreversible inhibitor of the polyamine biosynthetic enzyme, ornithine decarboxylase (ODC), to investigate the crosstalk between polyamine metabolism and osteogenic differentiation pathways.

Methods: Human osteoblasts (hOBs) were provided by Cell Applications, Inc. (San Diego, CA, USA). Cells were seeded at 10⁴ cells/well in 24-well plates for alizarin red S staining and alkaline phosphatase (ALP) activity assay, and at 5x10⁴ cells/well in 6-well plates for total RNA extraction. Two days after seeding, hOBs were treated with putrescine (PUT), spermidine (SPD), spermine (SPM) or DFMO prepared in osteogenic induction medium (OIM, 10⁻⁷ M dexamethasone, 10 mM β-glycerolphosphate and 50 mM L-ascorbate 2-phosphate in DMEM) for 7 or 14 days. In the presence of PUT, SPD or SPM, the differentiation medium was supplemented with additional 1 mM aminoguanidine to inhibit bovine serum amine oxidase.

Results: We studied the effect of exogenous polyamines and DFMO on the mRNA level of several osteogenic genes, including Runx2 and ALP, the early-onset genes responsible for osteogenic differentiation, as well as osteopontin and osteocalcin, which are late-onset genes related to extracellular matrix mineralization. The mRNA level of Runx2 and ALP was significantly elevated when hOBs were cultured in the presence of exogenous polyamines or DFMO for 7 days, compared to that of hOBs cultured in OIM alone (Fig. 1A and 1B). The gene expression of osteopontin and osteocalcin was unaffected by exogenous polyamines or DFMO at this stage (Fig. 1C and 1D), as osteopontin and osteocalcin are usually up-regulated in hOBs after treatment with OIM for 14 days. On the other hand, when hOBs were treated with OIM supplemented with various concentrations of polyamines or DFMO for 14 days, ALP activity was significantly enhanced compared to hOBs cultured in OIM alone (Fig. 2), in accordance with the increase in the mRNA expression of ALP (Fig. 1B). Furthermore, matrix mineralization was accelerated in the presence of exogenous polyamines or DFMO (Fig. 3). These results suggest that exogenous polyamines and DFMO may direct hOBs toward the osteoblast lineage by promoting osteogenic gene expression, ALP activity and matrix mineralization.

Discussion: It was suggested in our previous study that osteogenic differentiation of hBMSCs may be correlated with suppression of ODC. This study proves that when ODC was inhibited by DFMO, osteogenic differentiation of hOBs was enhanced. These observations suggest that intracellular polyamine homeostasis may be critical in determining the differentiation fate of hOBs and hBMSCs.

Significance: This study correlates polyamine metabolism with osteogenic differentiation, and provides a new direction for the development of novel bone-stimulating drugs.

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**Fig. 1.** Effect of exogenous polyamines and DFMO on the osteogenic gene expression of hOBs. Human OBs were treated with 1 mM PUT, 1 mM SPD, 1 mM SPM or 10 mM DFMO in OIM for 7 days. Relative gene expression of (A) Runx2, (B) ALP, (C) osteopontin and (D) osteocalcin was determined by real-time PCR. Error bars represent standard deviations (n≥3). *p<0.05 and **p<0.01, compared to the control (DMEM). #p<0.05 and ##p<0.01, compared to OIM.
Fig. 2. Effect of exogenous polyamines and DFMO on the ALP activity of hOBs. Human OBs were treated with various concentrations of exogenous polyamines or DFMO in OIM for 14 days, followed by reaction with Pierce 1-Step NBT/BCIP Solution (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

Fig. 3. Effect of exogenous polyamines and DFMO on matrix mineralization of hOBs. Human OBs were treated with various concentrations of exogenous polyamines or DFMO in OIM for 14 days, and were stained with alizarin red S to determine the level of matrix mineralization.