# Functional analysis of zinc finger and BTB domain containing 16 (ZBTB16) during osteoblastic differentiation of periodontal ligament-derived human multipotent mesenchymal stromal cells

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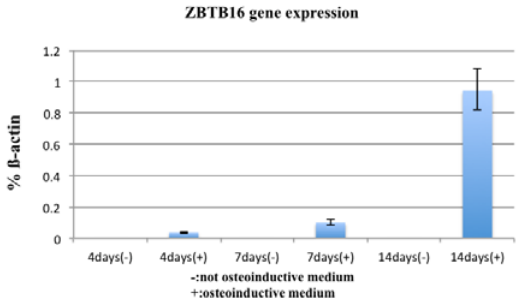
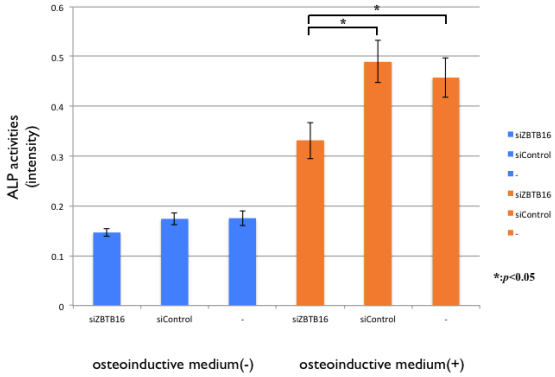
**Statement of Purpose:** Human multipotent mesenchymal stromal cells (hMSCs) possess the ability to differentiate into osteoblast, however the molecular basis of osteoblast-specific gene expression and differentiation is still unknown. In this study, we analyzed the changes of gene expression during osteoblastic differentiation in periodontal ligament-derived hMSCs using a new-generation sequencer.

**Methods:** At least 3 samples of human periodontal tissue were used as a source of hMSCs in this study. Each tissue was dispersed in alpha-minimum essential medium with GlutaMAX (α-MEM) (Invitrogen, Carlsbad, CA) containing 0.8 PZ-U/mL collagenase type I (Serva Electrophoresis, Heidelberg, Germany) and 1200 PU/mL dispase (Sanko Junyaku, Tokyo, Japan). A single-cell suspension was spread in a T25 Primaria culture flask (Falcon). Then, cells were cultured in complete medium [α-MEM supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin containing 10% fetal bovine serum (Moregate Biotech, Queensland, Australia)] at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$. For osteoblastic differentiation, cells were cultured in complete medium with or without osteoinductive supplements, which contained 50 μg/mL L-ascorbic acid phosphate magnesium salt (Wako Pure Chemical, Tokyo, Japan), 10 mmol/L β-glycerophosphate (βGP) (Sigma-Aldrich, St. Louis, MO), and 10 nmol/L dexamethasone (DEX) (Fuji Pharma, Tokyo, Japan). Total RNA was purified and concentrated using RiboMinus Eukaryote Kit (Invitrogen) and RNeasy MinElute Cleanup Kit (Qiagen). Ribosomal RNA-depleted total RNA was used to construct a library using SOLiD Total RNA-Seq Kit (Life Technologies). The library DNA was subjected to whole-exome enrichment using SureSelect Human All Exon Kit (Agilent Technologies, Santa Clara, CA). The enriched library DNA was then sequenced using SOLiD System (Life Technologies). After the whole transcriptome analysis (WTA), up-regulated genes were selected and confirmed by means of Taqman gene expression assays. Functional experiments were performed using small interfering RNA (siRNA). hMSCs were transiently transfected with siRNA of selected genes, and cultured with or without osteoinductive medium for 5 days. Then, alkaline phosphatase (ALP) activity was measured.

**Results:** Several genes were up or down-regulated during the osteoinduction in hMSCs. One of up-regulated genes was zinc finger and BTB domain containing 16(ZBTB16), also known as promyelocytic leukaemia zinc finger (PLZF). The gene expression of ZBTB16 was not detected without osteoinductive medium. In contrast, the expression of ZBTB16 was detected when cells were cultured with osteoinductive medium and increased day by day (Figure1).




Furthermore ALP activity was decreased by siZBTB16 when hMSCs were cultured with osteoinductive medium (Figure2).




Statistical significance ($p < 0.05$) was evaluated with Student's *t*-test.

**Conclusions:** In this study, the expression of ZBTB16 was enhanced by osteoinductive medium in hMSCs, and the expression level was increased for 14 days. The gene silencing of ZBTB16 during osteoinduction inhibited the osteoblastic differentiation of hMSCs, thus ZBTB16 may positively affect the osteoblastogenesis. In addition, the expression of ZBTB16 can be a marker of osteoblastic phenotype, because it was not observed without osteoinductive medium.

**References:**

Ikeda R, et al. J. Biol. Chem.2005;280:8523-8530.
Inoue I, et al. J Pharmacol Sci.2006;100:205-210.