Fermented Oyster Extract-induced Osteoblast Differentiation and Bone Formation by Activating the Wnt/β-catenin Signaling Pathway

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Abstract

The pacific oyster, *Crassostrea gigas*, is well-known as a nutritious food. Recently, we revealed that fermented extract of *C. gigas* (FO) inhibited ovariectomy-induced osteoporosis, resulting from suppression of osteoclastogenesis. However, since the beneficial effect of FO on osteogenesis is poorly understood, in this study it was examined in mouse preosteoblast MC3T3-E1 cells, human osteosarcoma MG-63 osteoblast-like cells, and zebrafish larvae. We found that FO increased mitochondrial activity from days 1 to 7; however, total cell number of MC3T3-E1 cells gradually decreased without any change in cell viability, which suggests that FO stimulates the differentiation of MC3T3-E1 cells. FO also promoted the expression of osteoblast marker genes, including *runt-related transcription factor 2 (mRUNX2), alkaline phosphatase (mALP), collagen type I α1 (mCol1α1), osteocalcin (mOCN), osterix (mOSX), bone morphogenetic protein 2 (mBMP2),* and *mBMP4* in MC3T3-E1 cells accompanied by a significant increase in ALP activity. FO also increased nuclear translocation of RUNX2 and OSX transcription factors, ALP activity, and calcification *in vitro* along with the upregulated expression of osteoblast-specific marker proteins such as RUNX2, ALP, Col1α1, OCN, OSX, and BMP4. Additionally, FO enhanced bone mineralization (calcein intensity) in zebrafish larvae at 9 days post-fertilization comparable to that in the β-glycerophosphate (GP)-treated group. All the tested osteoblast marker genes, including *zRUNX2a*, *zRUNX2b*, *zALP*, *zCol1a1*, *zOCN*, *zBMP2*, and *zBMP4*, were also remarkably upregulated in the zebrafish larvae in response to FO. It also promoted tail fin regeneration in adult zebrafish as same as the GP-treated groups. Furthermore, not only FO positively regulate β-catenin expression and Wnt/β-catenin luciferase activity, but pretreatment with a Wnt/β-catenin inhibitor (FH535) also significantly decreased FO-mediated bone mineralization in zebrafish larvae, which indicates that FO-induced osteogenesis depends on the Wnt/β-catenin pathway. Altogether, the current study suggests that the supplemental intake of FO has a beneficial effect on osteogenesis.

Keywords: *Crassostrea gigas, oyster, bone formation, mineralization, Wnt/β-catenin*

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