Adiponectin increases BMP-2 expression in osteoblasts via AdipoR receptor signaling pathway

INTRODUCTION:
Bone is a complex tissue composed of several cell types which are continuously undergoing a process of renewal and repair termed “bone remodeling”. The two major cell types responsible for bone remodeling are osteoclasts, which resorb bone, and osteoblasts, which form new bone. Although the mechanisms of osteoporosis are not entirely clear, they are likely relate to decreased availability or effects of bone growth factors, such as bone morphogenetic proteins (BMPs). BMPs, structurally related to the transforming growth factor-β superfamily. Among BMP family members, BMP-2 has been extensively studied and demonstrated to play a crucial role in inducing osteoblast differentiation and bone formation during embryonic skeletal development and postnatal bone remodeling. Adiponectin is a key mediator of the metabolic syndrome that is caused by visceral fat accumulation. However, the effects of adiponectin on osteoblasts are remain unclear. Here we found that adiponectin increased mRNA and protein levels of BMP-2 by using RT-PCR assay and ELISA. In addition, adiponectin increases BMP-2 expression through AdipoR, AMPK, p38 and NF-κB signaling pathway.

METHODS:
Cell culture: The human osteosarcoma cell lines (U2OS and MG-63,) and mouse osteoblastic cell line MC3T3-E1 was purchased from American Type Culture Collection Cells were cultured in DMEM supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). The conditionally immortalized human fetal osteoblastic cell line (hFOB; CRL-11372) was maintained in a 1:1 mixture of DMEM/Ham's F-12 medium containing 10% FBS supplemented with Geneticin (300 µg/ml). Serum and maintained at 37°C in a humidified atmosphere of 5% CO2. The murine primary osteoblastic cells (pOB cells) were prepared from fetal mouse. The cells were grown on the plastic cell culture dishes in 95% air, 5% CO2 with α-MEM that was supplemented with 20 mM HEPES and 10% heat-inactivated fetal calf serum, 2 mM-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). The characteristics of osteoblasts were confirmed by morphology and the expression of alkaline phosphatase.

Western blot analysis; Transfection assay; Quantitative real-time PCR; ELISA assay; Kinase assay; Electrophoretic Mobility Shift Assays.

RESULTS
Bone morphogenetic protein (BMP) plays important roles in osteoblastic differentiation and bone formation. To examine the effects of adiponectin on BMP-2 expression in osteoblastic cells. Cells were treated with various concentrations of adiponectin and the BMP-2 expression were determined by ELISA and real-time PCR. Stimulation of cells with adiponectin increased protein and mRNA expression of BMP-2 (Fig 1). Adiponectin-induced BMP-2 expression. Stimulation of hFOB cells with adiponectin increased AdipoR1 expression (Fig. 2A). Transient transfection of siRNA against AdipoR1 but not AdipoR2 expressed in liver. Next, we examine whether AdipoR receptor is involved in adiponectin-mediated BMP-2 expression. Stimulation of hFOB cells with adiponectin increased AdipoR1 and AdipoR2 expression (Fig. 2A). Transient transfection of siRNA against AdipoR1 but not AdipoR2 effectively inhibited the expression of BMP-2 in osteoblasts (Fig. 2B). These data suggest that adiponectin/AdipoR1 receptor interaction plays a key role in BMP-2 expression in osteoblasts. Next, we performed western blot analysis to elucidate the signal transduction pathways involved in the adiponectin-induced up-regulation of BMP-2. Stimulation of cells with adiponectin induced AMPK, p38 and IKKα/β phosphorylation in a time-dependent manner (Fig 3A). Adiponectin-induced BMP-2 expression was greatly reduced by treatment with AMPK inhibitor (Ara A ; Compound C) and p38 inhibitor (SB 203580) (Fig. 3B). Transfection of cells with AMPKα1, but not AMPKα2 siRNA reduced adiponectin-mediated BMP-2 expression. Taken together, these data suggest that the activation of the AMPK and p38 pathways are required for the adiponectin-induced expression of BMP-2 in osteoblasts. Activations of NF-κB pathway after adiponectin treatment was demonstrated, and adiponectin-mediated expression of BMP-2 was inhibited by the specific inhibitor and mutant of NF-κB cascades. Therefore, NF-κB activation also involved in adiponectin-induced BMP-2 expression.

DISCUSSION:
Our results provide evidence that adiponectin enhances BMP-2 expression in osteoblastic cells, and AdipoR1 receptor, AMPK, p38 and NF-κB signaling pathways may be involved in increasing BMP-2 expression by adiponectin.