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Adiponectin Stimulates Osteoblast Differentiation Through Induction of COX2 in Mesenchymal Progenitor Cells

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ABSTRACT

In bone marrow, osteoblasts and adipocytes are differentiated from mesenchymal progenitor cells and their differentiation is reciprocally regulated by largely unknown mechanisms. In this study, we investigated downstream signaling cascades of adiponectin, a member of the adipocytokine family, in the regulation of osteoblast differentiation. Adiponectin augmented expression of several osteogenic marker genes and increased osteoblast differentiation in mesenchymal progenitor cells. The expression of cyclooxygenase-2 (COX2) was potently increased by adiponectin, whereas inhibition of COX2 activity abolished the effect of adiponectin on osteogenesis. In addition, adiponectin rapidly stimulated p38 mitogen-activated protein kinase via the adiponectin receptor, AdipoR1, which resulted in c-Jun activation for COX2 expression. Adiponectin also stimulated BMP2 expression in a COX2-dependent manner. Moreover, Runx2, a key osteogenic transcription factor, contributed to the acceleration of osteogenesis in the presence of adiponectin. Collectively, the finding that adiponectin could promote osteogenesis through an intracellular signaling cascade in mesenchymal progenitor cells suggests that adiponectin would be a potential therapeutic target for bone-related diseases. *STEM CELLS 2009;27:2254–2262*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

In bone marrow, common marrow mesenchymal progenitor cells are able to differentiate into several cell types such as adipocytes and osteoblasts [1]. Recent evidence suggests that there is an inverse correlation between adipocyte differentiation and osteoblast differentiation from common progenitor cells [2, 3]. An increase in adiposity due to elevated adipogenesis in bone marrow, which is caused by aging, obesity, and diabetes, is one of the major risk factors of bone-related disorders, including osteoporosis and bone fractures [4–7]. Although the precise mechanism is not thoroughly understood, adipocytokines released from adipocytes, including leptin, resistin, and adiponectin, are associated with the regulation of bone mineral density [8]. For example, leptin-deficient mice exhibit increased bone mass, whereas intracerebroventricular infusion of leptin causes bone loss [9]. However, the roles of other adipocytokines in bone metabolism are largely unknown.

Adiponectin (also known as Acrp30, AdipoQ, apM1, and GBP28) is an adipocytokine exclusively expressed from adipocytes [10]. Adiponectin dysregulation has been implicated in insulin resistance, diabetes, and cardiovascular diseases [11]. One of the most interesting features of adiponectin is that its expression level in adipose tissue and plasma is decreased in obese and diabetic subjects, who have larger fat cells, showing dysregulated energy homeostasis [12]. Several adiponectin receptors or binding molecules, such as AdipoR1, AdipoR2, T-cadherin, and calreticulin, have been reported [10, 13]. Among these, AdipoR1 is ubiquitously expressed—and highly expressed in skeletal muscle—whereas AdipoR2 is abundantly expressed in the liver [10]. AdipoR1- and AdipoR2-mediated adiponectin stimulate glucose uptake and fatty acid oxidation through the activation of AMPK (AMP-activated protein kinase) and p38 mitogen-activated protein kinase (MAPK), which results in increased insulin sensitivity [14, 15]. Although adiponectin stimulates osteoblast proliferation and differentiation via AdipoR1 [16], the downstream signaling cascades of adiponectin during osteogenesis are not thoroughly understood.

Prostaglandin E_2 (PGE_2), which is produced by cyclooxygenase-2 (COX2) in osteoblasts, plays critical roles in bone anabolism [17]. The elevation of PGE_2 by systemic or local infection stimulates bone formation, and increased lamellar bone formation in response to mechanical stress is mediated by COX2 [18–20]. In addition, COX2 knockout mice exhibit decreased mesenchymal progenitor cell differentiation into

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osteoblast lineage when compared with wild-type littermates [21]. Thus, it is likely that regulatory molecules for COX2 expression in osteogenic cells would result in changes in bone metabolism.

Recently, it has been reported that adiponectin is expressed in bone marrow cells and is present in bone marrow regions [1, 22]. In addition, adiponectin represses adipogenesis in bone marrow progenitor cells [23]. These findings led us to test whether adiponectin can affect osteogenesis in mesenchymal progenitor cells. In this study, we demonstrate that adiponectin stimulates osteoblast differentiation in a COX2-dependent manner. During adiponectin-mediated osteogenesis in mesenchymal progenitor cells, AdipoR1 and p38 MAPK are activated to promote the phosphorylation of c-Jun, which is an essential step in turning on osteoblast differentiation via COX2 expression. Collectively, these data suggest that adiponectin could be a positive inducer of osteogenesis in mesenchymal progenitor cells.

Materials and Methods

Cell Culture and Differentiation of Osteoblasts

C3H10T1/2 murine mesenchymal progenitor cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Gaithersburg, MD, http://www.gibcobrl.com) supplemented with 10% fetal bovine serum (FBS). Osteoblast differentiation was induced with DMEM containing 10% FBS, 50 μM ascorbic acid, and 10 mM β-glycerophosphate. Primary bone marrow cells (PBMCs) were isolated from the femur and tibia of 4-week-old C57BL/6J mice and maintained in α-modified essential medium (α-MEM; Gibco BRL) with 10% FBS and 100 nM hydrocortisone. For osteoblast differentiation, PBMCs were cultured in α-MEM with 10% FBS, 50 μM ascorbic acid, and 10 mM β-glycerophosphate.

Purification of Adiponectin from HEK293 Cell Cultures

Recombinant adiponectin protein was purified using a previously described protocol [15]. The purity and oligomerization of adiponectin were examined before use.

siRNA Transfection

For transient transfection, C3H10T1/2 cells were grown to 80% confluence and subsequently transfected with several siRNA (small interference-RNA) using a MicroPorator according to the manufacturer's instructions (pipette-type electroporator; INCYTO Co., Ltd., Cheonan-city, Koreahttp://www.incyto.com). To minimize the possibility of different transfection efficiencies, siRNA-transfected C3H10T1/2 cells were split and treated with or without adiponectin. The siRNA duplexes for AdipoR1, c-Jun, and Runx2 that contained the most efficient capacity recommended by Bioneer were purchased from them (Bioneer, Daejeon, Korea, http://www.bioneer.com). The siRNA sets used were as follows: AdipoR1 forward, 5′-GAC UUG GCU UGA GUG GUG U (dTdT)-3′; c-Jun forward, 5′-GAG AAG AGG AAC CUA UAC U (dTdT)-3′; Runx2 forward, 5′-CGA UCU GAG AUU UGU GGG C (dTdT)-3′.

Transient Transfection, DNA Constructs, and Reporter Assay

For luciferase assays, we transiently transfected pCS4-3 Myc-Runx2 and pII1.3-Luc (1.3 kilobases of osteocalcin promoter) in HEK293 cells using the calcium phosphate method. C3H10T1/2 cells were transiently transfected with pXP-2-COX2-Luc using a MicroPorator. At 12 hours after transfection, adiponectin (10 μg/ml) treated for 12 hours and cell lysates were analyzed for luciferase activity. The pCMV-β-galactosidase plasmid and green fluorescent protein expression plasmid were used as internal controls for transfection efficiency.

Western Blotting

Western blotting was performed as previously described [24]. Antibodies against COX2 (NeoMarkers, USA), p38 MAPK, FLAG, phosphorylated c-Jun (serine 63 residue), and c-Jun (Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.com), β-actin (Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com), GAPDH (AbFrontier, Seoul, Korea, http://www.abfrontier.com), and phosphorylated p38 MAPK (BD Biosciences, San Diego, http://www.bdbiosciences.com) were used. Western blot analyses were visualized with horseradish peroxidase-conjugated secondary antibodies (Sigma-Aldrich) and enhanced chemiluminescence.

Real-Time Quantitative Polymerase Chain Reaction

Total RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, http://www.invitrogen.com) according to the manufacturer's protocol. After isolation of total RNA, the complementary DNA was generated by M-MuLV reverse transcriptase (Fermentas Canada Inc., Burlington, Ontario, Canada, http://www.fermentas.com) and was analyzed by quantitative real-time polymerase chain reaction (qPCR; My-IQ thermocycle; Bio-Rad, Hercules, CA, http://www.bio-rad.com) with SYBR Green (BioWhittaker Molecular Application, Rockland, ME, http://www.biowhittaker.com). All reaction products were normalized to the expression level of mRNA. PCR primer sets used were as follows: AdipoR1 forward, 5′-ACG TTG GAG AGT CAT CCC GTA T-3′; reverse, 5′-CTC TGT GTG GAT GCG GAA GAT-3′; AdipoR2 forward, 5′-TAG CCT CTA TAT CAC CGG AGC T-3′; reverse, 5′-AGA AGG CCT GCA GGA CCT GCA-3′; ALP forward, 5′-GAC TGG TAC TCG GAT AAC GA-3′; reverse, 5′-TGC GGT TCC AGA CAT AGT GG-3′; BMP2 forward, 5′-TGG AAG TGG CCC ATT TAG AG-3′; reverse, 5′-TGA CGC TTT TCT CGT TTG TG-3′; BMP7 forward, 5′-GAA AAC AGC AGC AGT GAC CA-3′; reverse, 5′-GGT GGC GTT CAT GTA GGA GT-3′; c-Jun forward, 5′-TCC CCT ATC GAC ATG GAG TC-3′; reverse, 5′-TGA GTT GGC ACC CAC TGT TA-3′; COX2 forward, 5′-AGA AGG AAA TGG CTG CAG AA-3′; reverse, 5′-GCT CGG CTT CCA GTA TTG AG-3′; GAPDH forward, 5′-TGC ACC ACC AAC TGC TTA G-3′; reverse, 5′-GGA TGC AGG GAT GAT GTT C-3′; osteocalcin forward, 5′-CGC TCT CAG GGG CAG ACA CT-3′; reverse, 5′-GCA CCC TCC AGC ATC CAG TA-3′; osteopontin forward, 5′-TGC CTG ACC CAT CTC AGA AGC A-3′; reverse, 5′-TGA GAG GTG AGG TCC TCA TC-3′; Runx2 forward, 5′-GAA GGA AAG GGA GGA GGG GT-3′; reverse, 5′-TCT GTC TCT CCT TCC CTT CC-3′; 18S forward, 5′-ACC GCA GCT AGG AAT AAT GGA-3′; reverse, 5′-GCC TCA GTT CCG AAA ACC A-3′.

p38 Mitogen-Activated Protein Kinase In Vitro Kinase Assay

HEK293 cells transfected with FLAG-p38 MAPK were lysed on ice using TGN buffer. The total cell extract (500 μg) was immunoprecipitated with anti-FLAG antibodies and washed with 20 mM HEPES (pH 7.4). Kinase assays were performed for 30 minutes at 30°C using 2 μg of glutathione *S*-transferase-c-Jun (GST-c-Jun) as the substrate in the reaction buffer (20 mM HEPES [pH 7.4], 10 mM $MgCl_2$, 12 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 1 μCi [γ-^{32}P] ATP). The reactions were terminated using 5× SDS sample buffer. The products were then resolved by SDS-PAGE, and the level of incorporated [^{32}P] was detected by autoradiography. Following this, the membranes were immunoblotted using anti-FLAG antibodies.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) assays were performed as described previously [24]. The PCR primer sets used in this study were as follows: COX2 promoter forward, 5′-AGC TGT GTG CGT GCT CTG A-3′; reverse, 5′-TCG CAG TTT GAC AAC

TGG C-3′; osteocalcin promoter forward, 5′-CGC TCT CAG GGG CAG ACA CT-3′; reverse, 5′-GCA CCC TCC AGC ATC CAG TA-3′; osteopontin promoter forward, 5′-GGC CAA CCT AAG CTA CCG AA-3′; reverse, 5′-CCA CCA ATC AGG AGG TGG AG-3′.

Enzyme Immunoassay for PGE_2

C3H10T1/2 cells in 12-well plates were incubated in 1 ml of medium with or without adiponectin. Supernatants from these cultures were examined for the presence of PGE_2 using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, http://www.caymanchem.com).

Alkaline Phosphatase Staining

Differentiated osteoblast cells were stained for alkaline phosphatase (ALP) activity using the 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) color development substrate (Promega, Madison, WI, http://www.promega.com).

Statistics

Data were analyzed using Student's t test. $p < .05$ and $p < .01$ were considered significant.

Results

Adiponectin Stimulates Osteogenesis in Mesenchymal Progenitor Cells

To investigate the effect of adiponectin on osteogenesis of mesenchymal progenitor cells, the mesenchymal progenitor cell line C3H10T1/2 and mouse PBMCs were treated with or without adiponectin during osteogenic differentiation. As shown in Figure 1A, adiponectin increased ALP-positive cells in both cell types. Consistently, the expression of osteogenic marker genes, including type I collagen, osteopontin, osteocalcin, and ALP, were stimulated by adiponectin (Fig. 1B). Furthermore, adiponectin overexpression via adenovirus also increased the expression of osteogenic genes such as osterix and osteopontin during osteogenesis (supporting information Fig. S1). In C3H10T1/2 cells, the expression level of osteopontin and osteocalcin was significantly increased at the early stage of osteogenic differentiation (day 2) with adiponectin (Fig. 1C). Similarly, the levels of ALP-positive cells and calcium deposition were greatly increased by adiponectin in PBMCs (supporting information Fig. S2). These results imply that adiponectin might promote osteogenesis at the early stage of osteoblast differentiation in mesenchymal progenitor cells.

Adiponectin Promotes COX2 Expression in Mesenchymal Progenitor Cells

To investigate the effect of adiponectin on the expression of other osteogenic genes, we analyzed the mRNA levels of Runx2 and COX2 together with osteocalcin and osteopontin. In accordance with the above results, the expression of most osteogenic genes, but not Runx2, was elevated by adiponectin (Fig. 2A). Of these, adiponectin rapidly increased both mRNA and protein levels of COX2 within 3 hours of treatment in C3H10T1/2 cells (Fig. 2A, 2B). Increases in COX2 expression by adiponectin were also monitored in PBMCs, preosteoblastic MC3T3-E1 cells (supporting information Fig. S3A, S3B). Previously, it was shown that COX2 plays a role in producing osteogenic stimulator PGE_2 in osteogenesis [17, 21]. At the same time, the level of PGE_2, the product of COX2, was increased by adiponectin in C3H10T1/2 cells (Fig. 2C) and PBMCs (supporting information Fig. S3C). However, COX2 expression was not altered in other adiponectin-sensitive cells, such as FAO hepatocytes and differentiated C2C12 myocytes, whereas the expression of Acyl-CoA oxidase, a well-known adiponectin target gene, was induced by adiponectin in those cells (supporting information Fig. S4). This indicated that adiponectin would increase COX2 expression specifically in mesenchymal progenitor cells and preosteoblastic cells.

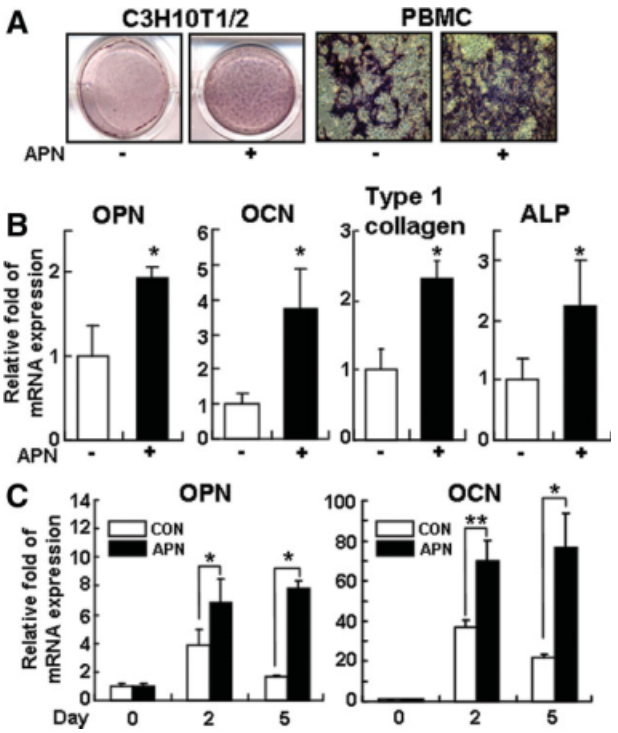

Figure 1. Adiponectin stimulates osteogenesis in mesenchymal progenitor cells. **(A):** C3H10T1/2 cells and primary bone marrow cells were differentiated into osteoblasts in the presence or absence of adiponectin (10 μg/ml) for 6 and 9 days, respectively. Differentiated osteoblasts were monitored by ALP staining to reveal ALP-positive cells. **(B):** C3H10T1/2 cells were differentiated into osteoblasts with or without adiponectin (10 μg/ml) for 6 days. Relative levels of osteopontin, osteocalcin, type 1 collagen, and ALP mRNA were determined using real-time quantitative polymerase chain reaction (qPCR). Values are normalized to the levels of GAPDH mRNA. **(C):** C3H10T1/2 cells were differentiated into osteoblasts with or without adiponectin (10 μg/ml). Cells were harvested at the indicated times. Relative levels of osteopontin and osteocalcin mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. Values are expressed as the mean ± SD. *, $p < .05$; **, $p < .01$. Abbreviations: ALP, alkaline phosphatase; APN, adiponectin; OCN, osteocalcin; OPN, osteopontin; PBMCs, primary bone marrow cells.

To determine the role of COX2 induction by adiponectin for osteogenesis, we examined the effects of COX2 inhibitors on adiponectin-induced osteogenesis. In C3H10T1/2 cells, increased expression of osteopontin, osteocalcin, and COX2 by adiponectin was significantly reduced by the COX2-specific inhibitor, NS398, during osteoblast differentiation (Fig. 2D). Additionally, in PBMCs, ALP-positive cells elevated by adiponectin were decreased by another COX2 inhibitor, salicylate, whereas PGE_2 reversed the effect of salicylate on osteogenesis, as determined by ALP staining assay (supporting information Fig. S5). Taken together, these results suggest that increased COX2 expression is crucial for adiponectin-mediated stimulation of osteogenesis in mesenchymal progenitor cells.

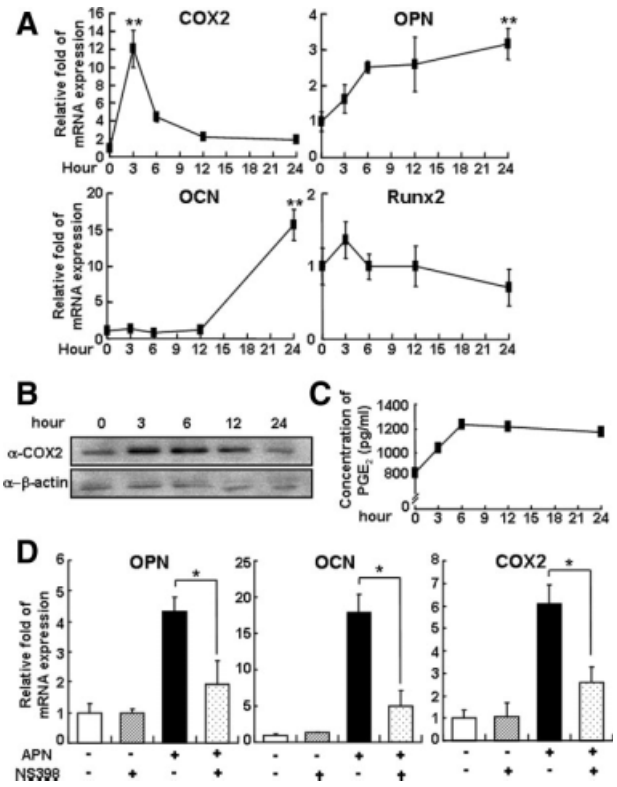

Figure 2. Adiponectin promotes osteogenesis via COX2 induction. **(A–C):** Adiponectin (10 μg/ml) was treated for the indicated time periods in C3H10T1/2 cells. **(A):** Relative amounts of COX2, osteopontin, osteocalcin, and Runx2 mRNA were determined using real-time quantitative polymerase chain reaction (qPCR). Values are normalized to the levels of GAPDH mRNA. **(B):** Western blot analyses were conducted using antibodies against COX2 (α-COX2) and β-actin (α-β-actin). Antibodies against β-actin (α-β-actin) were used as the loading control. **(C):** The concentration of PGE_2 was determined using the PGE_2 EIA kit (Cayman Chemical). **(D):** Adiponectin (10 μg/ml) and NS398 (10 μM), which is a COX2-specific inhibitor, were added to C3H10T1/2 cells until differentiation, day 5. Relative levels of each mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. Values are expressed as the mean ± SD. *, $p < .05$; **, $p < .01$. Abbreviations: APN, adiponectin; COX2, cyclooxygenase-2; OCN, osteocalcin; OPN, osteopontin; PGE_2, prostaglandin E_2; Runx2, Runt-related transcription factor 2.

Induction of COX2 Expression by Adiponectin Is Dependent on c-Jun

Next, we investigated the molecular mechanism by which adiponectin could induce COX2 expression in mesenchymal progenitor cells. As illustrated in Figure 3A, the transcriptional activity of the COX2 promoter was increased by adiponectin in reporter assays with C3H10T1/2 cells. Because the COX2 promoter is tightly regulated by AP-1 factors [25], especially c-Jun, we investigated the mRNA levels of c-Jun following adiponectin treatment. Adiponectin rapidly (approximately 3 hours) promoted c-Jun mRNA production (Fig. 3B). Next, we performed ChIP assays to decipher whether c-Jun is indeed involved in the augmentation of COX2 expression by adiponectin. Adiponectin significantly increased c-Jun recruitment onto the promoter of the COX2 gene (Fig. 3C). Moreover, recruitment of p300, a well-known coactivator for c-Jun [26], onto the COX2 promoter was also increased by adiponectin treatment (Fig. 3C). To investigate the role of c-Jun in COX2 expression with adiponectin, we repressed endogenous

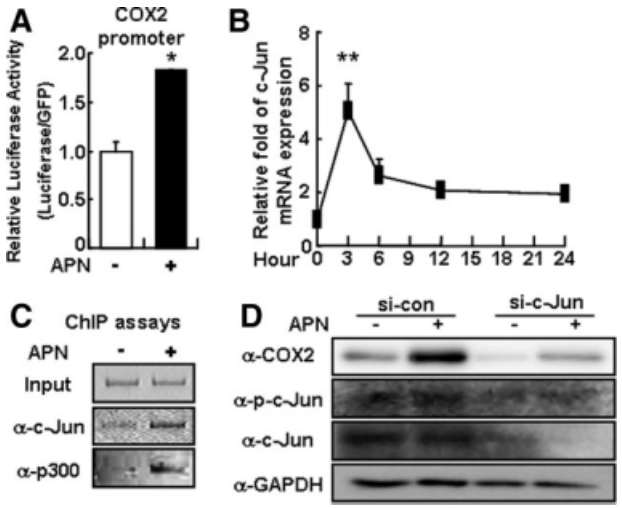

Figure 3. c-Jun mediates COX2 expression by adiponectin. **(A):** A reporter construct containing the COX2 promoter was transfected in C3H10T1/2 cells with and without adiponectin (10 μg/ml). Luciferase activities were determined 12 hours after adiponectin treatment. Transfection efficiency was normalized with green fluorescent protein. **(B):** Adiponectin (10 μg/ml) was treated for the indicated time periods in C3H10T1/2 cells. Relative amounts of c-Jun mRNA were determined using real-time quantitative polymerase chain reaction. Values are normalized to the levels of GAPDH mRNA. **(C):** C3H10T1/2 cells were treated with adiponectin (10 μg/ml) for 3 hours. Chromatin immunoprecipitation assays were performed to monitor the recruitment of c-Jun and its coactivator, p300, onto the COX2 promoter. The input represents 10% of the total input chromatin. **(D):** C3H10T1/2 cells were transfected with scrambled or c-Jun siRNA and treated with and without adiponectin (10 μg/ml) for 3 hours. Western blot analyses were conducted using antibodies against COX2 (α-COX2), phosphorylated c-Jun (α-p-c-Jun), c-Jun (α-c-Jun), and GAPDH (α-GAPDH). Antibodies against GAPDH (α-GAPDH) were used as the loading control. Values are expressed as the mean ± SD. *, $p < .05$; **, $p < .01$. Abbreviations: APN, adiponectin; ChIP, chromatin immunoprecipitation; COX2, cyclooxygenase-2.

c-Jun expression via siRNA. In C3H10T1/2 cells, increased COX2 protein by adiponectin was markedly reduced by c-Jun knockdown (Fig. 3D). Thus, these results strongly suggest that c-Jun plays an important role in the increase of COX2 expression upon adiponectin in mesenchymal progenitor cells.

p38 MAPK Phosphorylates c-Jun by Adiponectin

Because the phosphorylation of c-Jun is important to its protein stability and transcriptional activity [27], we investigated the phosphorylation status of c-Jun with or without adiponectin. In C3H10T1/2 cells, c-Jun phosphorylation was significantly increased by adiponectin within short (<15 minutes) periods (Fig. 4A). Furthermore, phosphorylation of p38 MAPK, a c-Jun upstream kinase [28], was also increased by adiponectin (Fig. 4A). Although the meaning of the biphasic increase in p38 MAPK phosphorylation is still unclear, biphasic p38 MAPK phosphorylation by adiponectin was repeatedly detected. Similarly, phosphorylation of p38 MAPK by adiponectin was increased in PBMCs (supporting information Fig. S6A). To examine whether p38 MAPK directly phosphorylates c-Jun upon exposure to adiponectin, we performed in vitro kinase assays using GST-c-Jun proteins. As shown in Figure 4B, phosphorylation of GST-c-Jun protein by p38 MAPK was augmented by adiponectin. In addition, a specific inhibitor for p38 MAPK, SB203580, greatly inhibited c-Jun phosphorylation (Fig. 4C) and significantly repressed the

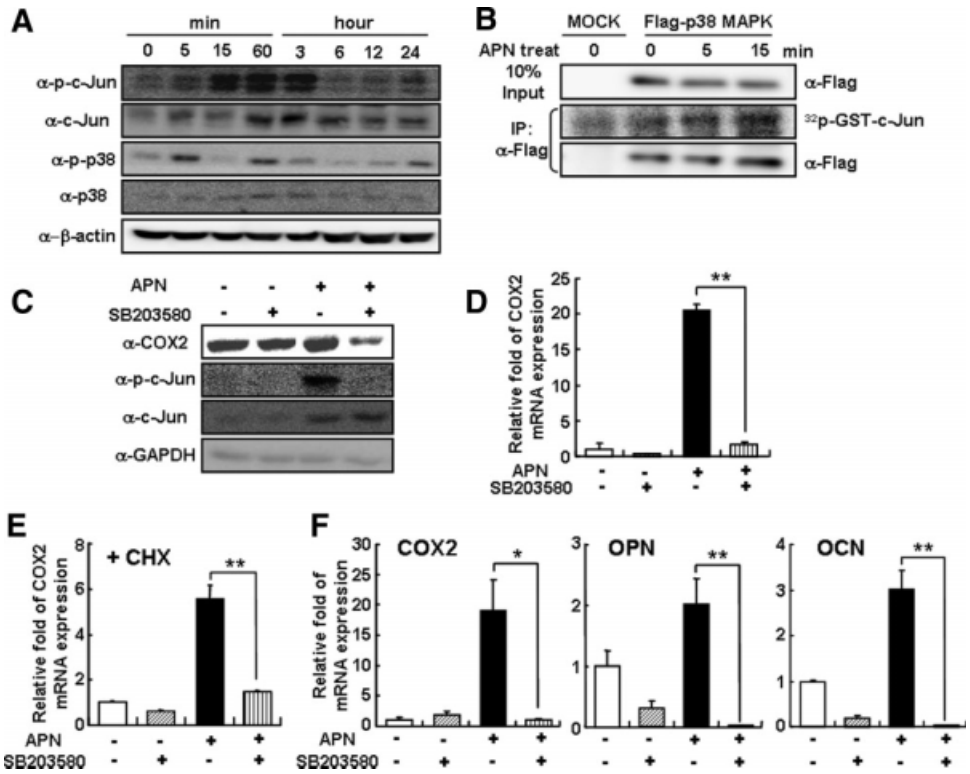

Figure 4. Adiponectin increases c-Jun phosphorylation and COX2 expression via p38 MAPK. **(A):** Adiponectin (10 μg/ml) was treated for the indicated time periods in C3H101/2 cells. The phosphorylation and protein levels of c-Jun and p38 MAPK were monitored by Western blot analyses. Antibodies against β-actin (α-β-actin) were used as the loading control. **(B):** p38 MAPK kinase activity was determined in the absence or presence of adiponectin. HEK293 cells were transfected with FLAG-p38 MAPK and treated with adiponectin for the indicated time periods. Whole cell lysates were immunoprecipitated with anti-FLAG antibodies (α-FLAG), and in vitro kinase assays were carried out using GST-c-Jun protein as a substrate. Western blot analyses were performed using antibodies against FLAG (α-FLAG). The level of FLAG-p38 MAPK was determined as the loading control. The input represents 10% of the total input immunoprecipitation. **(C, D):** SB203580 (10 μM), a p38 MAPK-specific inhibitor, was pretreated for 1 hour before being treated with adiponectin (10 μg/ml) for 3 hours in C3H10T1/2 cells. **(C):** Western blot analyses were conducted and antibodies against GAPDH (α-GAPDH) were used as the loading control. **(D):** Relative amounts of COX2 mRNA were determined using real-time quantitative polymerase chain reaction (qPCR). Values are normalized to the levels of GAPDH mRNA. **(E):** Cycloheximide (10 μM) and SB203580 (10 μM) were pretreated for 1 h. Adiponectin (10 μg/ml) was treated for 3 hours in C3H10T1/2 cells. Relative amounts of COX2 mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. **(F):** C3H10T1/2 cells were differentiated into osteoblasts for 5 days with or without adiponectin (10 μg/ml) and SB203580 (10 μM). Relative amounts of COX2, osteopontin, and osteocalcin mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. Values are expressed as the mean $\pm$ SD. *, $p < .05$; **, $p < .01$. Abbreviations: APN, adiponectin; COX2, cyclooxygenase-2; CHX, cycloheximide; OCN, osteocalcin; OPN, osteopontin.

levels of COX2 protein and mRNA in C3H10T1/2 cells (Fig. 4C, 4D). The p38 MAPK inhibitor consistently suppressed adiponectin-induced PGE_2 levels and COX2 expression in PBMCs (supporting information Fig. S6B, S6C). Because adiponectin promoted both c-Jun mRNA and protein levels, and also c-Jun phosphorylation (Figs. 3B, 4A), we questioned whether adiponectin-dependent COX2 expression is primarily associated with an increase in c-Jun expression or c-Jun protein phosphorylation. To address this, cycloheximide was treated with adiponectin to inhibit de novo synthesis of c-Jun protein. In the presence of cycloheximide, COX2 mRNA expression was greatly increased by adiponectin, although the p38 MAPK inhibitor still repressed the expression of COX2 mRNA (Fig. 4E), implying that an increase in c-Jun phosphorylation by adiponectin via p38 MAPK would be an important prerequisite step to induce COX2 expression. Accordingly, elevated expression of several osteogenic genes, including COX2, osteopontin, and osteocalcin, by adiponectin was greatly prevented by SB203580 during osteogenic differentiation (Fig. 4F). SB203580 also lessened adiponectin-induced ALP-positive staining in PBMCs and MC3T3-E1 cells (supporting information Fig. S6D). Taken together, these results propose that p38 MAPK would increase c-Jun phosphorylation by adiponectin, which would lead to enhanced COX2 expression in mesenchymal progenitor cells.

Adiponectin Activates BMP2 Expression via COX2 in C3H10T1/2 Cells

BMP2 is one of the most important growth factors that stimulates osteogenesis and osteogenic marker genes like osteopontin and osteocalcin [29]. Because endogenous PGE_2 regulates BMP2 expression in human mesenchymal progenitor cells [30], we investigated whether adiponectin is able to control BMP expression through COX2 induction. As illustrated in Figure 5A, adiponectin greatly increased expression of BMP2, but not BMP7, in C3H10T1/2 cells. Then, to study whether elevation of PGE_2 by adiponectin was involved in the regulation of BMP2 expression, C3H10T1/2 cells were co-treated with adiponectin and the COX2 inhibitor NS398. As shown

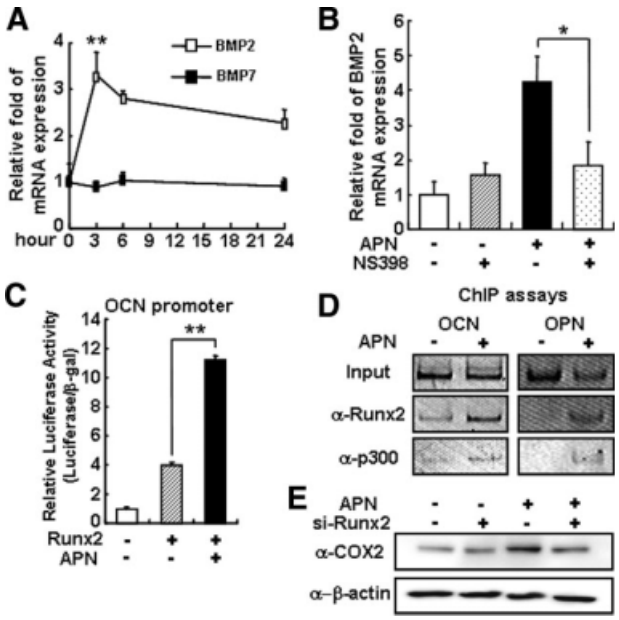

Figure 5. Adiponectin stimulates BMP2 expression and Runx2 transcriptional activity. **(A):** Adiponectin (10 μg/ml) was treated for the indicated time periods in C3H10T1/2 cells. Relative amounts of BMP2 and BMP7 mRNA were determined using real-time quantitative polymerase chain reaction (qPCR). Values are normalized to the levels of GAPDH mRNA. **(B):** Adiponectin (10 μg/ml) and NS398 (20 μM) were added to C3H10T1/2 cells for 3 hours. Relative amounts of mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. **(C):** A reporter construct containing the osteocalcin promoter was co-transfected with a Myc-tagged Runx2 expression vector into HEK293 cells. Runx2 transcriptional activity was determined with and without adiponectin (10 μg/ml). Luciferase activities were determined 24 hours after transfection. Transfection efficiency was normalized using β-gal activity. **(D):** C3H10T1/2 cells were treated with adiponectin (10 μg/ml) for 3 hours. ChIP assays were performed to determine the recruitment of Runx2 and coactivator p300 onto the promoter of osteocalcin and osteopontin. The input represents 10% of the total input chromatin. **(E):** C3H10T1/2 cells were transfected with scrambled or Runx2 siRNA and treated with and without adiponectin (10 μg/ml) for 3 hours. Western blot analyses were conducted using antibodies against COX2 (α-COX2). Antibodies against β-actin (α-β-actin) were used as the loading control. Values are expressed as the mean $\pm$ SD. *, $p < .05$; **, $p < .01$. Abbreviations: APN, adiponectin; COX2, cyclooxygenase-2; CHX, cycloheximide; OCN, osteocalcin; OPN, osteopontin.

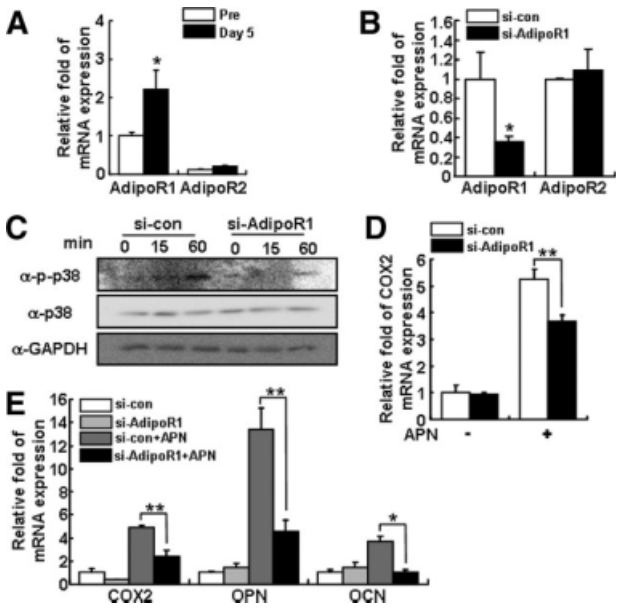

Figure 6. AdipoR1 is the major adiponectin receptor for osteoblast differentiation. **(A):** The levels of AdipoR1 and R2 mRNA were measured by real-time quantitative polymerase chain reaction (qPCR) in the process of osteogenesis with C3H10T1/2 cells. Values are normalized to the levels of 18S rRNA. **(B–E):** C3H10T1/2 cells were transfected with scrambled or AdipoR1 siRNA. **(B):** AdipoR1 and R2 mRNA were measured by real-time qPCR. Values are normalized to the levels of GAPDH mRNA. **(C):** Adiponectin (10 μg/ml) was treated for the indicated time periods. The phosphorylation and protein levels of p38 MAPK were detected by Western blot analyses. Antibodies against GAPDH (α-GAPDH) were used as the loading control. **(D):** Adiponectin (10 μg/ml) was treated for 3 hours. COX2 mRNA was measured by real-time qPCR. Values are normalized to the levels of GAPDH mRNA. **(E):** siRNA transfected C3H10T1/2 cells were differentiated for 5 days with and without adiponectin (10 μg/ml) and relative amounts of COX2, osteopontin, and osteocalcin mRNA were determined using real-time qPCR. Values are normalized to the levels of GAPDH mRNA. Pre-, undifferentiated C3H10T1/2 cells, day 5, C3H10T1/2 cells were osteogenic differentiated for day 5; si-con, scrambled si-RNA; si-AdipoR1, AdipoR1 si-RNA. Values are expressed as the mean $\pm$ SD. *, $p < .05$; **, $p < .01$. Abbreviations: APN, adiponectin; COX2, cyclooxygenase-2; OCN, osteocalcin; OPN, osteopontin.

in Figure 5B, NS398 evidently repressed an adiponectin-dependent increase in BMP2 expression, indicating that increased BMP2 expression by adiponectin is dependent on COX2.

The finding that BMP2 activates Runx2 transcriptional activity through R-Smads [31] and that adiponectin promotes several Runx2 target genes, including osteocalcin and osteopontin (Fig. 1), led us to examine the effect of adiponectin on the transcriptional activity of Runx2. Adiponectin greatly augmented the transcriptional activity of Runx2 on the osteocalcin promoter in the reporter assays (Fig. 5C), although the underlying mechanism by which adiponectin stimulates Runx2 activity is largely unknown. Adiponectin consistently increased the recruitment of Runx2 and the coactivator p300 onto the promoter regions of osteocalcin and osteopontin, which were determined by ChIP assays (Fig. 5D). Furthermore, suppression of BMP2 receptors, including BMPR1a, BMPR1b, and Acvr1, inhibited the recruitment of Runx2 onto the promoter regions of its target genes in the presence of adiponectin (supporting information Fig. S7). This implied that adiponectin could also stimulate the transcriptional activity of Runx2 through BMP2 signaling in mesenchymal progenitor cells.

Since COX2 has been reported as one of the direct targets of Runx2 in osteoblasts [24, 32, 33], we investigated the effect of Runx2 suppression on adiponectin-induced COX2 expression. As shown in Figure 5E, Runx2 knockdown decreased the level of COX2 expression in the presence of adiponectin. Although further studies are required to understand how adiponectin is able to elevate Runx2 activity, these observations indicate that Runx2 activated by adiponectin could promote COX2 expression.

AdipoR1 Is the Major Functional Receptor for Adiponectin Signaling in C3H10T1/2 Cells

Both AdipoR1 and R2 are expressed in mesenchymal progenitor cells and osteoblasts [22, 34], and AdipoR1 appears to be a major receptor for adiponectin in human primary osteoblasts [16]. Although we detected both AdipoR1 and R2 mRNA in mesenchymal progenitor cells and bone marrow regions

(supporting information Fig. S8), the mRNA expression level of AdipoR1 was greater than that of AdipoR2 in C3H10T1/2 cells, PBMCs, and MC3T3-E1 cells (Fig. 6A, supporting information Fig. S9). In addition, AdipoR1 mRNA expression was elevated during differentiation in mesenchymal progenitor cells (Fig. 6A, supporting information Fig. S9). To investigate whether adiponectin signaling is mediated by AdipoR1 during osteogenesis, we specifically repressed AdipoR1 expression (about 38%) using siRNA in C3H10T1/2 cells (Fig. 6B). When the expression of AdipoR1 was suppressed, both p38 MAPK phosphorylation (Fig. 6C) and COX2 expression (Fig. 6D) by adiponectin were greatly decreased. Furthermore, adiponectin-dependent increases of COX2, osteopontin, and osteocalcin expression were reduced by AdipoR1 siRNA during osteoblast differentiation (Fig. 6E). These results suggest that AdipoR1 would play a key role in mediating adiponectin signaling cascade for the regulation of osteoblast differentiation in mesenchymal progenitor cells.

Discussion

Certain cytokines that are secreted from adipocytes and osteoblasts reciprocally influence bone and fat metabolism [9, 35, 36]. However, there are some discrepancies regarding the role of adiponectin in bone metabolism. Several reports suggest that adiponectin enhances bone mineral density in vivo and osteoblast differentiation in vitro [16, 37]. In contrast, serum levels of adiponectin are inversely correlated with bone mineral density [38], and the bone mass of adiponectin-deficient mice is not significantly different from that of wild-type mice [34]. Although adiponectin mRNA was expressed in mesenchymal progenitor cells and osteoblasts, a significantly abundant level of adiponectin was detected in differentiated adipocytes (supporting information Fig. S8). However, several findings suggest that adiponectin has a paracrine role in bone marrow and mesenchymal progenitor cells. For instance, the adiponectin protein level in bone marrow fluid is much higher than that in serum, and 4-week-old mice have more abundant levels of adiponectin in bone marrow fluid than do 14-week-old mice [22]. Regarding adipogenesis, adiponectin inhibits adipocyte differentiation in mesenchymal progenitor cells in a COX2-dependent manner [23]. We also observed that adiponectin lessened adipogenesis in C3H10T1/2 cells (supporting information Fig. S10). Interestingly, osteoblast differentiation in bone marrow progenitor cells from adiponectin-deficient mice is significantly repressed when compared to that of control mice [34]. Consistent with the above reports, we demonstrated that treatment of adiponectin stimulated differentiation of osteoblasts through COX2 induction in several mesenchymal progenitor cells, including C3H10T1/2 cells and PBMCs.

In bone biology, COX2 is an important inducer of osteoblast differentiation [21]. However, we revealed the molecular mechanism by which adiponectin-induced COX2 would stimulate osteoblast differentiation. Previously, COX2 was reported to regulate the expressions of BMPs, including BMP2 and BMP7, in mesenchymal progenitor cells and osteoblasts [30, 39]. Because adiponectin increased BMP2 expression via COX2 induction (Fig. 5), it is likely that the increase in BMP2 expression via COX2 by adiponectin would lead to pleiotrophic increases in osteogenic marker gene expression. We believe that this is the first evidence that adiponectin increases BMP2 expression, where COX2 is an important mediator linking adiponectin and BMP2 expression in the process of osteogenesis of mesenchymal progenitor cells.

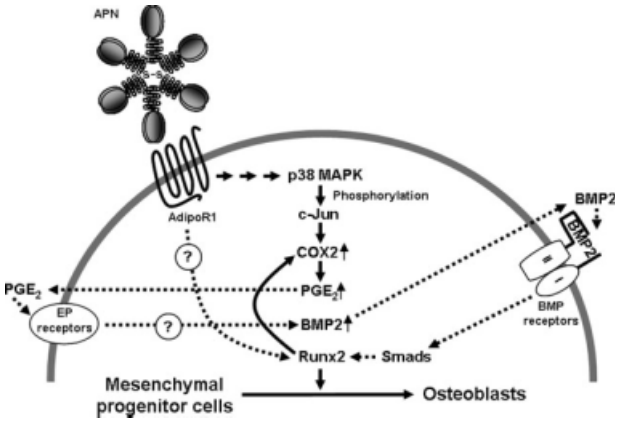

Figure 7. A model for adiponectin signaling to stimulate osteoblast differentiation in mesenchymal progenitor cells. In mesenchymal progenitor cells, adiponectin activates AdipoR1, p38 MAPK, and c-Jun to increase the expression of COX2. The COX2 pathway is essential for increasing BMP2 expression following adiponectin administration. Activated BMP2 signaling might contribute to promoting osteoblast differentiation through activation of Runx2 in mesenchymal progenitor cells. Activated Runx2 by adiponectin would stimulate osteogenic marker genes expression and COX2 expression to enhance osteogenesis. Prostaglandin E (EP) receptors; PGE_2 receptors. Abbreviations: COX2, cyclooxygenase-2; PGE_2, prostaglandin E_2; Runx2, Runt-related transcription factor 2.

Several reports have revealed that adiponectin is able to modulate COX2 expression in PBMCs and cardiac myocytes [23, 40]. Although COX2 induction by adiponectin in cardiac myocytes is dependent on sphingosine kinase-1 [41], we have shown that adiponectin serially activated AdipoR1, p38 MAPK, and the c-Jun signaling pathway to induce COX2 expression in mesenchymal progenitor cells. On the basis of similar data, Luo et al. recently reported that adiponectin activates p38 MAPK via AdipoR1 in human osteoblasts. Unlike p38 MAPK, AMPK was not involved in adiponectin-induced COX2 expression or osteogenesis (supporting information Fig. S11). Furthermore, we first observed that adiponectin stimulated c-Jun phosphorylation. Several lines of evidence suggest that c-Jun is a key transcription factor in COX2 expression upon adiponectin in mesenchymal progenitor cells. First, adiponectin markedly increased c-Jun expression and elevated
c-Jun phosphorylation (Figs. 3B, 4A). Second, recruitment of c-Jun and its cofactor, p300, onto the promoter of COX2 was augmented by adiponectin (Fig. 3C). Third, knockdown of c-Jun suppressed the increase in COX2 expression by adiponectin (Fig. 3D). Together, it is feasible to propose that a sequential signaling cascade (adiponectin → AdipoR1 → p38 MAPK → c-Jun) is involved in inducing COX2 expression upon adiponectin in mesenchymal progenitor cells.

During osteoblast differentiation, the expression and modification of Runx2, a key transcription factor in osteogenesis, play crucial roles [42]. In the present study, we observed that adiponectin promoted the transcriptional activity of Runx2 and Runx2 binding onto the promoter of target genes like osteocalcin and osteopontin (Fig. 5). Moreover, COX2 expression by adiponectin was repressed by Runx2 knockdown (Fig. 5E), suggesting that Runx2 would contribute to stimulating COX2 expression by adiponectin. However, in mesenchymal progenitor cells, adiponectin did not change the levels of Runx2 mRNA (Fig. 2A) and Runx2 phosphorylation, which are associated with increased Runx2 transcriptional activity (data not shown). This implies that adiponectin might indirectly

regulate Runx2 activity through another pathway. Nevertheless, it is of interest to note that the suppression of BMP2 signaling repressed Runx2 recruitment onto its target gene promoters (supporting information Fig. S7). It is possible to explain these observations by the following model in which adiponectin-induced BMP2 signaling might prompt Runx2 transcriptional activity through activation of Smads (Fig. 7). Further studies are required to understand the effect of activated BMP2 expression by adiponectin on transcriptional activity of Runx2 in mesenchymal progenitor cells.

Collectively, it is possible to suggest that adiponectin activates the AdipoR1 → p38 MAPK → c-Jun cascade to promote COX2 expression in mesenchymal progenitor cells and that activated COX2 would increase BMP2 expression and stimulate osteoblast differentiation (Fig. 7). Although further in vivo studies should test whether adiponectin is capable of inducing bone formation in the bone marrow region, the data presented here suggest that adiponectin could be a potent therapeutic agent for diseases with diminished bone regions by activating osteoblast differentiation in mesenchymal progenitor cells.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Caplan AI, Bruder SP. Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. Trends Mol Med 2001;7:259–264.
2 Pei L, Tontonoz P. Fat's loss is bone's gain. J Clin Invest 2004;113:805–806.
3 Gimble JM, Zvonic S, Floyd ZE et al. Playing with bone and fat. J Cell Biochem 2006;98:251–266.
4 Hofbauer LC, Brueck CC, Singh SK et al. Osteoporosis in patients with diabetes mellitus. J Bone Miner Res 2007;22:1317–1328.
5 Rosen CJ, Bouxsein ML. Mechanisms of disease: Is osteoporosis the obesity of bone?Nat Clin Pract Rheumatol 2006;2:35–43.
6 Duque G. Bone and fat connection in aging bone. Curr Opin Rheumatol 2008;20:429–434.
7 Zhao LJ, Jiang H, Papasian CJ et al. Correlation of obesity and osteoporosis: Effect of fat mass on the determination of osteoporosis. J Bone Miner Res 2008;23:17–29.
8 Oh KW, Lee WY, Rhee EJ et al. The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men. Clin Endocrinol (Oxf) 2005;63:131–138.
9 Ducy P, Amling M, Takeda S et al. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. Cell 2000;100:197–207.
10 Kadowaki T, Yamauchi T, Kubota N et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–1792.
11 Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: An adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84–89.
12 Yatagai T, Nagasaka S, Taniguchi A et al. Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism 2003;52:1274–1278.
13 Takemura Y, Ouchi N, Shibata R et al. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J Clin Invest 2007;117:375–386.
14 Yamauchi T, Kamon J, Ito Y et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–769.
15 Yoon MJ, Lee GY, Chung JJ et al. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 2006;55:2562–2570.
16 Luo XH, Guo LJ, Yuan LQ et al. Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. Exp Cell Res 2005;309:99–109.
17 Raisz LG. Prostaglandins and bone: Physiology and pathophysiology. Osteoarthritis Cartilage 1999;7:419–421.
18 Jee WS, Ma YF. The in vivo anabolic actions of prostaglandins in bone. Bone 1997;21:297–304.
19 Weinreb M, Suponitzky I, Keila S. Systemic administration of an anabolic dose of PGE2 in young rats increases the osteogenic capacity of bone marrow. Bone 1997;20:521–526.
20 Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. J Bone Miner Res 1996;11:1688–1693.
21 Zhang X, Schwarz EM, Young DA et al. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Invest 2002;109:1405–1415.
22 Berner HS, Lyngstadaas SP, Spahr A et al. Adiponectin and its receptors are expressed in bone-forming cells. Bone 2004;35:842–849.
23 Yokota T, Meka CS, Medina KL et al. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. J Clin Invest 2002;109:1303–1310.
24 Lee HW, Suh JH, Kim HN et al. Berberine promotes osteoblast differentiation by Runx2 activation with p38 MAPK. J Bone Miner Res 2008;23:1227–1237.
25 Chun KS, Surh YJ. Signal transduction pathways regulating cyclooxygenase-2 expression: Potential molecular targets for chemoprevention. Biochem Pharmacol 2004;68:1089–1100.
26 Bannister AJ, Oehler T, Wilhelm D et al. Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. Oncogene 1995;11:2509–2514.
27 Musti AM, Treier M, Bohmann D. Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. Science 1997;275:400–402.
28 Yamagishi S, Yamada M, Ishikawa Y et al. p38 mitogen-activated protein kinase regulates low potassium-induced c-Jun phosphorylation and apoptosis in cultured cerebellar granule neurons. J Biol Chem 2001;276:5129–5133.
29 Zaidi SK, Sullivan AJ, van Wijnen AJ et al. Integration of Runx and Smad regulatory signals at transcriptionally active subnuclear sites. Proc Natl Acad Sci U S A 2002;99:8048–8053.
30 Arikawa T, Omura K, Morita I. Regulation of bone morphogenetic protein-2 expression by endogenous prostaglandin E2 in human mesenchymal stem cells. J Cell Physiol 2004;200:400–406.
31 Afzal F, Pratap J, Ito K et al. Smad function and intranuclear targeting share a Runx2 motif required for osteogenic lineage induction and BMP2 responsive transcription. J Cell Physiol 2005;204:63–72.
32 Chikazu D, Li X, Kawaguchi H et al. Bone morphogenetic protein 2 induces cyclo-oxygenase 2 in osteoblasts via a Cbfal binding site: Role in effects of bone morphogenetic protein 2 in vitro and in vivo. J Bone Miner Res 2002;17:1430–1440.
33 Mehrotra M, Saegusa M, Voznesensky O et al. Role of Cbfa1/Runx2 in the fluid shear stress induction of COX-2 in osteoblasts. Biochem Biophys Res Commun 2006;341:1225–1230.
34 Shinoda Y, Yamaguchi M, Ogata N et al. Regulation of bone formation by adiponectin through autocrine/paracrine and endocrine pathways. J Cell Biochem 2006;99:196–208.
35 Lee NK, Sowa H, Hinoi E et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130:456–469.
36 Ferron M, Hinoi E, Karsenty G et al. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci U S A 2008;105:5266–5270.

37 Oshima K, Nampei A, Matsuda M et al. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Biochem Biophys Res Commun 2005;331:520–526.
38 Lenchik L, Register TC, Hsu FC et al. Adiponectin as a novel determinant of bone mineral density and visceral fat. Bone 2003;33: 646–651.
39 Paralkar VM, Grasser WA, Mansolf AL et al. Regulation of BMP-7 expression by retinoic acid and prostaglandin E(2). J Cell Physiol 2002;190:207–217.
40 Shibata R, Sato K, Pimentel DR et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. Nat Med 2005;11:1096–1103.
41 Ikeda Y, Ohashi K, Shibata R et al. Cyclooxygenase-2 induction by adiponectin is regulated by a sphingosine kinase-1 dependent mechanism in cardiac myocytes. FEBS Lett 2008;582:1147–1150.
42 Schroeder TM, Jensen ED, Westendorf JJ. Runx2: A master organizer of gene transcription in developing and maturing osteoblasts. Birth Defects Res C Embryo Today 2005;75:213–225.


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