Regulation by AMP-activated protein kinase of PGE_2-induced osteoprotegerin synthesis in osteoblasts

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Abstract. Adenosine monophosphate-activated protein kinase (AMPK) is currently recognized to act as a key sensing enzyme in the regulation of cellular energy homeostasis. It has been previously demonstrated that prostaglandin E_2 (PGE_2) stimulates the synthesis of osteoprotegerin (OPG) through the activation of p38 mitogen-activated protein (MAP) kinase, p44/p42 MAP kinase and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in osteoblast-like MC3T3-E1 cells. In the present study, it was investigated whether AMPK is implicated in the PGE_2-induced OPG synthesis in MC3T3-E1 cells. PGE_2 was observed to induce the phosphorylation of AMPKα (Thr-172) and AMPKβ (Ser-108) in a time-dependent manner. PGE_2 additionally induced the phosphorylation of acetyl-coenzyme A (CoA) carboxylase, a direct substrate of AMPK. Compound C, an inhibitor of AMPK, which attenuated the phosphorylation of acetyl-CoA carboxylase, significantly suppressed the PGE_2-stimulated OPG release and the mRNA expression level. Compound C failed to affect the PGE_2-stimulated phosphorylation of p38 MAP kinase or p44/p42 MAP kinase. On the contrary, the phosphorylation of SAPK/JNK was markedly attenuated by compound C. The results of the current study suggest that AMPK acts as a positive regulator in PGE_2-stimulated OPG synthesis via SAPK/JNK signaling in osteoblasts.

Introduction

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is widely recognized as a central regulator of cellular metabolism and energy homeostasis (1,2). AMPK has been discovered as an enzyme that catalyzes the phosphorylation of acetyl coenzyme A (CoA) carboxylase, which regulates lipid synthesis (2). AMPK activity is upregulated by the elevation of the AMP/adenosine triphosphate (ATP) ratio in response to various types of physiological and pathological stress, resulting in restoring cellular enzyme balance by ATP generating pathways (3). In addition, activated AMPK suppresses ATP utilizing pathways. Therefore, AMPK is currently known to regulate metabolic homeostasis throughout the body (2).

Bone metabolism is predominantly regulated by two types of functional cells, osteoblasts and osteoclasts (4). The former cells are responsible for bone formation, while the latter cells are responsible for bone resorption. Constant bone mass is maintained by bone remodeling, which comprises osteoclastic bone resorption followed by osteoblastic bone formation. Disruption to bone remodeling can result in metabolic bone disease, including osteoporosis. Concerning the association between AMPK and bone metabolism, it has been demonstrated that AMPK activation stimulates osteoblast differentiation and bone formation, resulting in increased bone mass (5). It has been previously reported that vascular endothelial growth factor synthesis induced by basic fibroblast growth factor is regulated by AMPK in osteoblast-like MC3T3-E1 cells (6). However, the precise role of AMPK in osteoblasts remains to be fully elucidated.

Osteoprotegerin (OPG) is an essential protein secreted from osteoblasts, which inhibits osteoclast activation and its differentiation, and a member of the tumor necrosis factor receptor family along with receptor activator of nuclear factor-κB (RANK) (7). OPG binds to RANK ligand (RANKL) as a decoy receptor, and prevents RANKL from binding to RANK, resulting in the suppression of bone resorption (7). It has been demonstrated that RANKL knockout mice suffer from severe osteopetrosis (8), suggesting that RANKL is a key regulator of osteoclastogenesis. The RANK/RANKL/OPG axis is currently recognized as a major regulatory system for osteoclast formation and activity (9).

Prostaglandins (PGs) are autocrine/paracrine modulators in the bone metabolism (10). Among them, PGE_2 has been recognized as an important mediator of bone remodeling (11). It has been previously reported that PGE_2 stimulates OPG synthesis via the activation of p38 mitogen-activated protein

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(MAP) kinase, p44/p42 MAP kinase and stress activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in osteoblast-like MC3T3-E1 cells (12). However, the detailed mechanism underlying PGE_2-stimulated OPG synthesis in osteoblasts remains to be fully elucidated.

In the present study, the involvement of AMPK in the synthesis of OPG induced by PGE_2 in osteoblast-like MC3T3-E1 cells was investigated. The current study aimed to investigate whether AMPK positively regulates the PGE_2-stimulated OPG synthesis via the SAPK/JNK pathway in these cells.

Materials and methods

Materials. PGE_2 was obtained from Sigma-Aldrich (St. Louis, MO, USA). The mouse OPG enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Compound C was obtained from Calbiochem; EMD Millipore (Billerica, MA, USA). Polyclonal rabbit phosphorylated (p)-AMPKα (Thr-172; cat. no. 2531), p-AMPKβ (Ser-108; cat. no. 4181), p-acetyl-CoA carboxylase (cat. no. 3661), p38 MAP kinase (cat. no. 9212), p-p44/p42 MAP kinase (cat. no. 9101), p44/p42 MAP kinase (cat. no. 9102), SAPK/JNK (cat. no. 9252) antibodies, and monoclonal rabbit p-p38 MAP kinase (cat. no. 4511) and p-SAPK/JNK (cat. no. 4668) antibodies, were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (sc-25778) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The Enhanced Chemiluminescence (ECL) Western Blotting Detection System was purchased from GE Healthcare Life Sciences (Chalfont, UK). Acrylamide monomer, Tris(hydroxymethyl)aminomethane, sodium dodecyl sulfate (SDS), dithiothreitol and glycerol were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). PGE_2 was dissolved in ethanol. Compound C was dissolved in dimethyl sulfoxide (Sigma-Aldrich). The maximum concentration of ethanol or dimethyl sulfoxide was 0.1%, which did not affect either the assay for OPG or the detection of protein levels using western blot analysis.

Cell culture. Cloned osteoblast-like MC3T3-E1 cells, which were originally derived from newborn mouse calvaria (13), were maintained as described previously (14). Briefly, the cells were cultured in α-minimum essential medium (α-MEM; Sigma-Aldrich) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C in a humidified atmosphere of 5% CO_2/95% air. The cells were seeded into 35-mm diameter dishes ($5x10^4$ cells/dish) or 90-mm diameter dishes ($2x10^5$ cells/dish) in α-MEM containing 10% FBS. Following culture for five days, the medium was exchanged for α-MEM containing 0.3% FBS. The cells were then used for experiments after 48 h.

Assay for OPG. The cultured cells were pretreated with 0.3, 1, 3 or 10 μM compound C for 60 min, then stimulated with 10 μM of PGE_2 or vehicle in 1 ml α-MEM containing 0.3% FBS, and were incubated for 48 h at 37˚C. The conditioned medium was collected, and the OPG concentration in the medium was measured using the mouse OPG ELISA kit according to the manufacturer's protocol.

Western blot analysis. The cultured cells were pretreated with 1, 3 or 10 μM compound C for 60 min, and then were stimulated with 10 μM PGE_2 or vehicle for 1, 3, 5, 10, 20, 30 or 60 min. The cells were then washed twice with phosphate-buffered saline (Sigma-Aldrich) and then lysed, homogenized and sonicated 900 μl lysis buffer containing 62.5 mM Tris/HCl, pH 6.8, 2% SDS, 50 mM dithiothreitol and 10% glycerol. SDS-polyacrylamide gel electrophoresis was performed by the method described by Laemmli (15) in 10% polyacrylamide gels. The protein was fractionated and transferred onto an Immun-Blot polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Western blot analysis was performed as described previously (16) using p-AMPKα (Thr-172), p-AMPKβ (Ser-108), p-acetyl-CoA carboxylase, p-p38 MAP kinase, p38 MAP kinase, p-p44/p42 MAP kinase, p44/p42 MAP kinase, p-SAPK/JNK, SAPK/JNK and GAPDH antibodies as the primary antibodies at a dilution of 1:1,000 in 5% milk in Tris-buffered saline (20 mM Tris/HCl, pH 7.6, 137 mM NaCl) with 0.1% Tween-20 (TBST) overnight at 4˚C. Goat-anti rabbit IgG horseradish peroxidase-labeled antibodies (074-1506; KPL, Inc., Gaithersburg, MD, USA) were used as the secondary antibodies at a dilution of 1:1.000 in 5% milk in TBST for 1 h at room temperature. Peroxidase activity on the PVDF membrane was visualized on X-ray film (Super RX; Fujifilm, Tokyo, Japan) by means of the ECL Western Blotting Detection System.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The cultured cells were pretreated with 10 μM compound C or vehicle for 60 min, then were stimulated by 10 μM PGE_2 or vehicle in α-MEM containing 0.3% FBS for 3 h. Total RNA was isolated and reverse transcribed into complementary DNA using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and the Omniscript Reverse Transcriptase kit (Qiagen Inc., Valencia, CA, USA), respectively. RT-qPCR was performed using a LightCycler system (version 2.0; Roche Diagnostics, Basel, Switzerland) in capillaries with the FastStart DNA Master SYBR Green I provided with the RightCycler FastStart DNA kit (Roche Diagnostics). Sense and antisense primers for mouse OPG or GAPDH mRNA were purchased from Takara Bio, Inc. (Otsu, Japan; primer set ID, MA026526). Amplification of the correct PCR products was confirmed by melting curve analysis according to the manufacturer's instructions. The OPG mRNA levels were normalized to those of GAPDH mRNA.

Densitometric analysis. A densitometric analysis of the protein expression was performed using a scanner (GT-F600; Seiko Epson Corporation, Nagano, Japan) and an ImageJ analysis software, version 1.48 (National Institutes of Health, Bethesda, MD, USA). The background-subtracted signal intensity of each phosphorylation signal was normalized to the respective total protein signal and plotted as the fold increase in comparison to control cells without stimulation.

Statistical analysis. The data were analyzed by an analysis of variance, followed by the Bonferroni method for multiple comparisons between pairs. $P<0.05$ was considered to indicate a statistically significant difference. All data are presented as

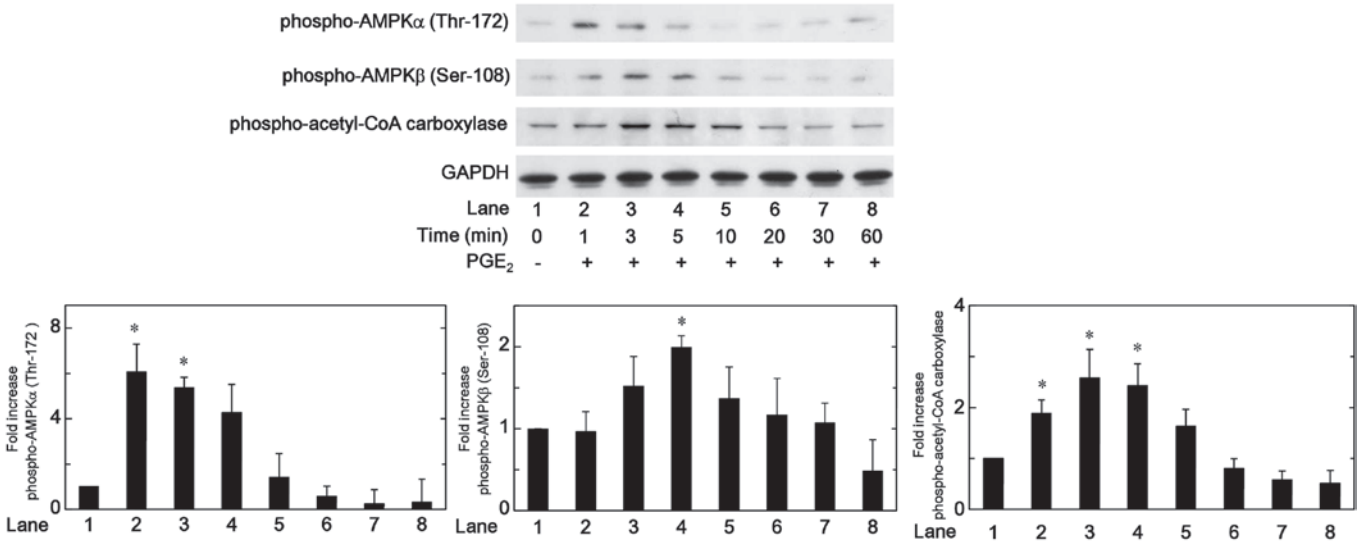

Figure 1. Effects of PGE_2 on the phosphorylation of AMPK or acetyl-CoA carboxylase in MC3T3-E1 cells. The cultured cells were stimulated by 10 μM of PGE_2 for the indicated periods. The extracts of cells were subjected to sodium dodecyl sulfate-polyacrylimide gel electrophoresis with subsequent western blotting using antibodies against phospho-specific AMPKα (Thr-172), phospho-specific AMPKβ (Ser-108), phospho-specific acetyl-CoA carboxylase or GAPDH. The histograms present quantification of the levels of the PGE_2-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were normalized to the GAPDH levels and expressed as the fold increase to the basal levels presented in lane 1. Each value represents the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control. PGE_2, prostaglandin E_2; AMPK, adenosine monophosphate-activated protein kinase; CoA, coenzyme A; phospho, phosphorylated; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

the mean ± standard error of triplicate determinations from three independent cell preparations.

Results

Effects of PGE_2 on the phosphorylation of AMPK or acetyl-CoA carboxylase in MC3T3-E1 cells. It has been previously established that the phosphorylation of AMPK is essential for its activation (17). Therefore, in order to clarify whether AMPK is activated by PGE_2 in osteoblast-like MC3T3-E1 cells, the effect of PGE_2 on the phosphorylation of AMPK was investigated by western blot analysis. PGE_2 was observed to significantly induce the phosphorylation of AMPKα (Thr-172) and AMPKβ (Ser-108). The effects of PGE_2 on the phosphorylation of AMPK α and AMPKβ reached their peak 1 min and 5 min subsequent to the stimulation, respectively, and reduced thereafter (Fig. 1). It is widely accepted that AMPK induces the phosphorylation of acetyl-CoA carboxylase as a direct substrate of AMPK, and regulates it (2). Thus, the effect of PGE_2 on the phosphorylation of acetyl-CoA carboxylase was investigated in MC3T3-E1 cells. PGE_2 significantly induced the phosphorylation of acetyl-CoA carboxylase, and the effect on the phosphorylation reached its peak 5 min subsequent to stimulation (Fig. 1).

Effect of compound C on the PGE_2-stimulated OPG release in MC3T3-E1 cells. It has been previously reported that PGE_2 stimulates OPG synthesis via the activation of p38 MAP kinase, p44/p42 MAP kinase and SAPK/JNK in osteoblast-like MC3T3-E1 cells (12). In order to elucidate whether AMPK serves a role in the PGE_2-induced synthesis of OPG in MC3T3-E1 cells, the effect of compound C, an inhibitor of AMPK (18), on the release of OPG induced by

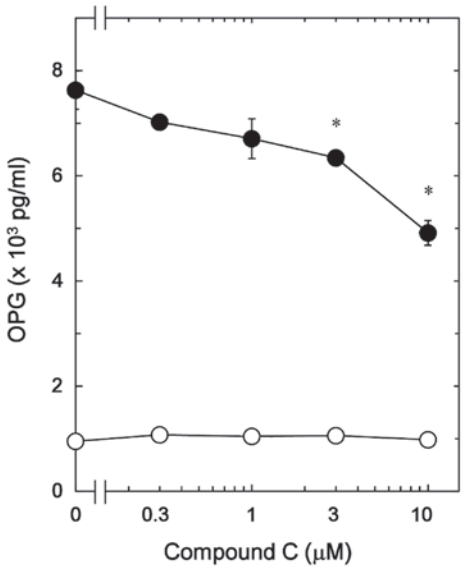

Figure 2. Effect of compound C on the PGE_2-stimulated OPG release in MC3TC-E1 cells. The cultured cells were pretreated with various concentrations of compound C for 60 min, and then stimulated by 10 μM of PGE_2 (●) or vehicle (○) for 48 h. The OPG concentrations of the culture medium were determined by the enzyme-linked immunosorbent assay. Each value is presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. PGE_2 alone. PGE_2, prostaglandin E_2; OPG, osteoprotegerin.

PGE_2 was investigated. Compound C, which by itself had minimal effect on the levels of OPG, significantly reduced the PGE_2-stimulated OPG release in a dose-dependent manner in the range between 0.3 and 10 μM (Fig. 2). A 10 μM dose of compound C resulted in an approximately 40% inhibition in the PGE_2-effect.

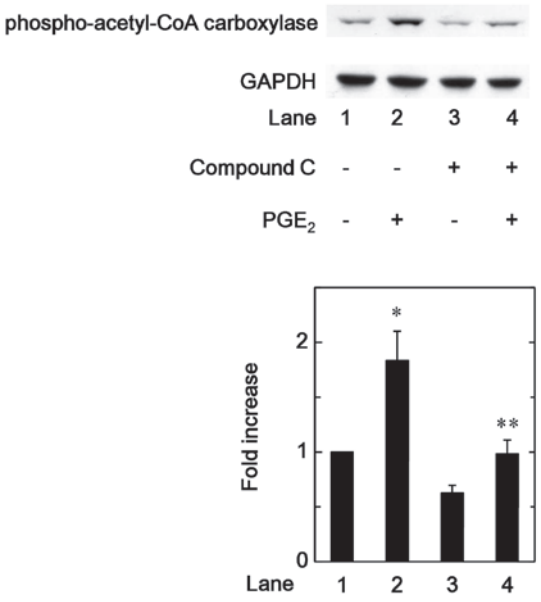

Figure 3. Effect of compound C on the PGE_2-stimulated phosphorylation of acetyl-CoA carboxylase in MC3T3-E1 cells. The cultured cells were pretreated with 10 μM compound C or vehicle for 60 min, then were stimulated by 10 μM PGE_2 or vehicle for 5 min. The extracts of cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent western blot analysis using antibodies against phospho-specific acetyl-CoA carboxylase or GAPDH. The histogram presents quantification of the levels of the PGE_2-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were normalized to GAPDH levels and expressed as the fold increase vs. basal levels presented in lane 1. Values are presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control; **P<0.05 vs. PGE_2 alone. PGE_2, prostaglandin E_2; CoA, coenzyme A; phospho, phosphorylated; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

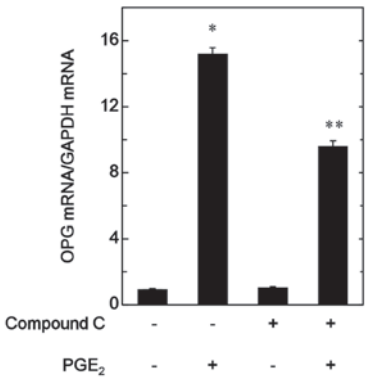

Figure 4. Effect of compound C on the PGE_2-induced OPG mRNA expression in MC3T3-E1 cells. The cultured cells were pretreated with 10 μM compound C or vehicle for 60 min, then were stimulated by 10 μM PGE_2 or vehicle for 3 h. The respective total RNA was then isolated and quantified by reverse transcription-quantitative polymerase chain reaction. Values are presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control; **P<0.05 vs. PGE_2 alone. PGE_2, prostaglandin E_2; OPG, osteoprotegerin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Effect of compound C on the PGE_2-stimulated phosphorylation of acetyl-CoA carboxylase in MC3T3-E1 cells. In order to investigate whether compound C functions as an inhibitor of AMPK in osteoblast-like MC3T3-E1 cells, the effect of compound C on the PGE_2-induced phosphorylation of acetyl-CoA carboxylase was examined. Compound C significantly suppressed the PGE_2-induced phosphorylation of acetyl-CoA carboxylase (Fig. 3).

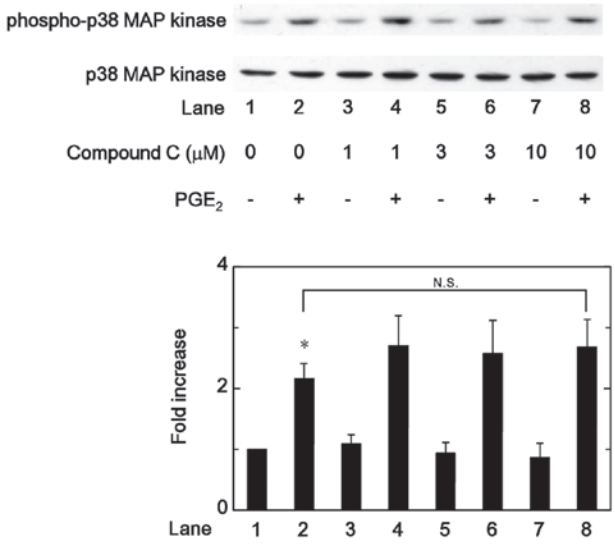

Figure 5. Effect of compound C on the PGE_2-stimulated phosphorylation of p38 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with various concentrations of compound C for 60 min, then were stimulated by 10 μM PGE_2 or vehicle for 3 min. The cell extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent western blot analysis using antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. The histogram presents quantification of the levels of the PGE_2-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were corrected by the p38 MAP kinase levels and expressed as the fold increase compared with the basal levels presented in lane 1. Values are presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control. PGE_2, prostaglandin E_2; MAP, mitogen-activated protein; phospho, phosphorylated; N.S., non-significant.

Effect of compound C on the PGE_2-induced OPG mRNA expression in MC3T3-E1 cells. In order to elucidate whether the suppression of the PGE_2-stimulated OPG synthesis by compound C is mediated via transcriptional events in MC3T3-E1 cells, the effect of compound C on the PGE_2-induced expression levels of OPG mRNA were investigated. Compound C was observed to significantly reduce the PGE_2-induced OPG mRNA expression (Fig. 4).

Effects of compound C on the PGE_2-stimulated phosphorylation of p38 MAP kinase, p44/p42 MAP kinase or SAPK/JNK in MC3T3-E1 cells. Regarding the intracellular signaling of PGE_2 in osteoblasts, it has been previously demonstrated that PGE_2 induces the activation of p38 MAP kinase, p44/p42 MAP kinase and SAPK/JNK in osteoblast-like MC3T3-E1 cells, and that these MAP kinases function as positive regulators in the PGE_2-stimulated OPG synthesis in these cells (12). Therefore, the association between AMPK and these MAP kinases in the PGE_2-stimulated OPG synthesis was investigated in MC3T3-E1 cells. Firstly the effects of compound C on the PGE_2-induced phosphorylation of p38 MAP kinase or p44/p42 MAP kinase were examined. However, compound C

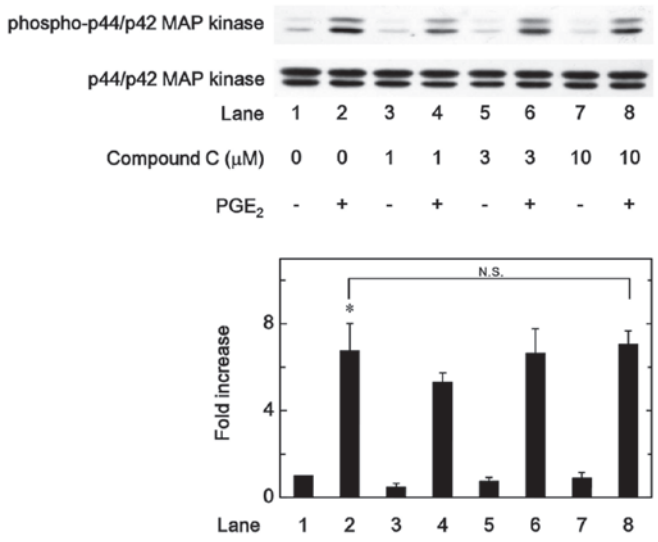

Figure 6. Effect of compound C on the PGE_2-stimulated phosphorylation of p44/p42 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with various concentrations of compound C for 60 min, then were stimulated by 10 μM PGE_2 or vehicle for 10 min. The cell extracts were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent western blotting using antibodies against phospho-specific p44/p42 MAP kinase or p44/p42 MAP kinase. The histogram presents quantification of the levels of the PGE_2-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. The phosphorylation levels were corrected by the p44/p42 MAP kinase levels and expressed as the fold increase compared with the basal levels presented in lane 1. Values are presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control. PGE_2, prostaglandin E_2; MAP, mitogen-activated protein; phospho, phosphorylated; N.S., non-significant.

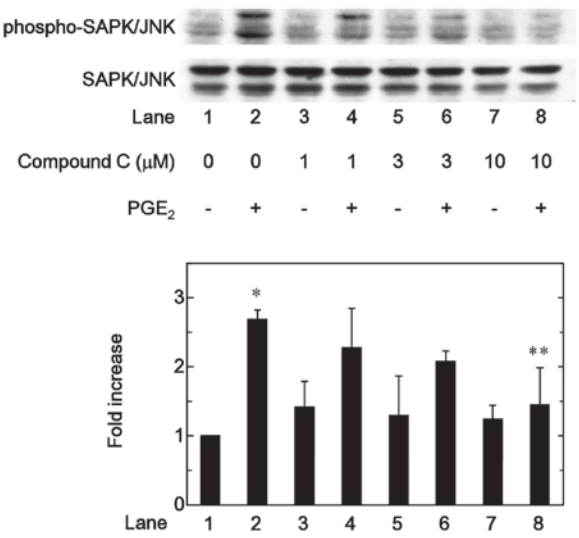

Figure 7. Effect of compound C on the PGE_2-stimulated phosphorylation of SAPK/JNK in MC3T3-E1 cells. Cultured cells were pretreated with various concentrations of compound C for 60 min, and then stimulated by 10 μM of PGE_2 or vehicle for 20 min. Cell extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis with subsequent western blotting using antibodies against phospho-specific SAPK/JNK or SAPK/JNK. The histogram presents quantification of the levels of the PGE_2-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Phosphorylation levels were corrected by the SAPK/JNK levels and expressed as the fold increase compared with the basal levels presented in lane 1. Values are presented as the mean ± standard error of triplicate determinations from three independent cell preparations. *P<0.05 vs. control; **P<0.05 vs. PGE_2 alone. PGE_2, prostaglandin E_2; SAPK, stress-activated protein kinase/c-Jun N-terminal kinase; phospho, phosphorylated.

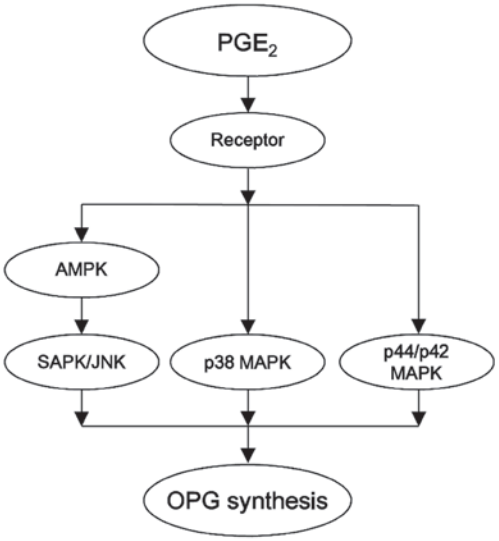

Figure 8. Schematic illustration of the involvement of AMPK in the mechanism of PGE_2-stimulated OPG synthesis in osteoblasts. AMPK, adenosine monophosphate-activated protein kinase; PGE_2, prostaglandin E_2; OPG, osteoprotegerin; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

was not observed to affect the PGE_2-induced phosphorylation of p38 MAP kinase or p44/p42 MAP kinase in the range between 1 and 10 μM (Figs. 5 and 6). By contrast, compound C significantly attenuated the phosphorylation of SAPK/JNK induced by PGE_2 in a dose-dependent manner in the range between 1 and 10 μM (Fig. 7).

Discussion

In the present study, it was demonstrated that the phosphorylation of AMPKα (Thr-172) and AMPKβ (Ser-108) was markedly induced by PGE_2 in osteoblast-like MC3T3-E1 cells. AMPK exists as a heterotrimeric complex consisting of three subunits, which are designated α, β and γ (1). Among the three AMPK subunits, the α-subunit is recognized as a catalytic site, whereas the β- and γ-subunits are regulatory sites (17). It is currently recognized that the phosphorylation of the α-subunit is essential for AMPK activation, while the phosphorylation of the β-subunit is required for correct activation of AMPK (17). In the current study, it was demonstrated that PGE_2 significantly stimulated the phosphorylation of acetyl-CoA carboxylase, a direct substrate of AMPK, in MC3T3-E1 cells. The time course of PGE_2-induced AMPKα phosphorylation was more rapid than that of acetyl-CoA carboxylase phosphorylation. Based on these observations, it is suggested that PGE_2 induces AMPK activation in osteoblast-like MC3T3-E1 cells.

In a previous study (12), it was reported that PGE_2 stimulates OPG synthesis in osteoblast-like MC3T3-E1 cells. In the present study, the involvement of AMPK in the PGE_2-stimulated OPG synthesis in MC3T3-E1 cells was investigated, and it was demonstrated that compound C, an inhibitor of AMPK (18), reduced the release of OPG induced by PGE_2. It was additionally observed that compound C significantly attenuated the phosphorylation of acetyl-CoA carboxylase stimulated by PGE_2. Therefore, the observations of the current study suggest that PGE_2-activated AMPK acts as a positive regulator in OPG

release. Furthermore, it was demonstrated that compound C significantly reduced the PGE_2-mRNA expression levels of OPG. Taking these observations into account, it is suggested that PGE_2 stimulates the synthesis of OPG, at least in part, via AMPK activation in osteoblast-like MC3T3-E1 cells.

It is widely accepted that the MAP kinase superfamily serves a central role in a variety of cellular functions, including proliferation, differentiation and survival (19). Three major MAP kinases, p38 MAP kinase, p44/p42 MAP kinase and SAPK/JNK, are recognized as central elements used by mammalian cells to transduce diverse types of messages (20). Regarding the signaling mechanism of the PGE_2-stimulated OPG synthesis in osteoblasts, it has been previously demonstrated that PGE_2 stimulates the activation of p38 MAP kinase, p44/p42 MAP kinase and SAPK/JNK in osteoblast-like MC3T3-E1 cells, and that three MAP kinases are implicated in the PGE_2-stimulated OPG synthesis (12). In order to establish how AMPK functions in PGE_2-stimulated OPG synthesis in MC3T3-E1 cells, the association between AMPK and three MAP kinases was investigated in the current study. It was demonstrated that compound C suppressed the PGE_2-induced phosphorylation of SAPK/JNK without affecting the phosphorylation of p38 MAP kinase or p44/p42 MAP kinase. Based on the observations of the present study, it is suggested that PGE_2 induces the activation of SAPK/JNK via AMPK in osteoblast-like MC3T3-E1 cells. In the present study, it was demonstrated that the maximum effect of PGE_2 on AMPKα phosphorylation was observed at 1 min subsequent to stimulation. By contrast, a previously study demonstrated that PGE_2-induced SAPK/JNK phosphorylation reached its peak at 20 min subsequent to stimulation (12). The time course of the PGE_2-induced AMPK phosphorylation appears to be more rapid than that of SAPK/JNK phosphorylation, suggesting that the PGE_2-induced activation of SAPK/JNK occurs subsequent to AMPK activation. Taking the current and previous studies into account, it is suggested that AMPK acts upstream of SAPK/JNK and positively regulates the PGE_2-stimulated OPG synthesis in osteoblast-like MC3T3-E1 cells. The potential mechanism of AMPK in PGE_2-induced OPG synthesis in osteoblasts investigated here is summarized in Fig. 8.

AMPK is generally recognized as a key sensor in cellular energy homeostasis (1). Regarding AMPK in osteoblasts, it has been demonstrated that metformin, an activator of AMPK, increases collagen-1 and osteocalcin mRNA expression, stimulates alkaline phosphatase activity and enhances cell mineralization in osteoblast-like MC3T3-E1 cells (21). In addition, AMPK activation reportedly inhibits palmitate-induced apoptosis in osteoblasts (22). Furthermore, AMPK has been observed to stimulate osteoblast differentiation via induction of runt-related transcription factor 2 expression (23). Thus, these observations lead to the hypothesis that AMPK activation directs osteoblasts toward stimulating bone formation. PGE_2 is a well-known autocrine/paracrine regulator of osteoblasts and acts as an important mediator of bone remodeling (10). By contrast, OPG, which prevents the biological effects of RANKL as a decoy receptor, negatively regulates RANKL-mediated osteoclastic bone resorption (7). Therefore, the results of the present study indicate that PGE_2-activated AMPK in osteoblasts functions as a modulator of bone metabolism via OPG synthesis, resulting in the upregulation of bone formation, and these results may provide a novel insight into bone metabolism. Further investigation would be necessary to clarify the exact roles of AMPK in bone metabolism.

The results of the current study indicate that AMPK functions as a positive regulator in PGE_2-stimulated OPG synthesis via SAPK/JNK activation in osteoblasts.

Acknowledgements

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