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Journal of Cellular Biochemistry 116:1144–1152 (2015)

Journal of Cellular Biochemistry

β_2Adrenergic Receptor Activation Suppresses Bone Morphogenetic Protein (BMP)-Induced Alkaline Phosphatase Expression in Osteoblast-Like MC3T3E1 Cells

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ABSTRACT

β adrenergic stimulation suppresses bone formation in vivo while its actions in osteoblastic differentiation are still incompletely understood. We therefore examined the effects of β_2 adrenergic stimulation on osteoblast-like MC3T3-E1 cells focusing on BMP-induced alkaline phosphatase expression. Morphologically, isoproterenol treatment suppresses BMP-induced increase in the numbers of alkaline phosphatase-positive small foci in the cultures of MC3T3-E1 cells. Biochemically, isoproterenol treatment suppresses BMP-induced enzymatic activity of alkaline phosphatase in a dose-dependent manner. Isoproterenol suppression of alkaline phosphatase activity is observed even when the cells are treated with high concentrations of BMP. With respect to cell density, isoproterenol treatment tends to suppress BMP-induced increase in alkaline phosphatase expression more in osteoblasts cultured at higher cell density. In terms of treatment protocol, continuous isoproterenol treatment is compared to cyclic treatment. Continuous isoproterenol treatment is more suppressive against BMP-induced increase in alkaline phosphatase expression than cyclic regimen. At molecular level, isoproterenol treatment suppresses BMP-induced enhancement of alkaline phosphatase mRNA expression. Regarding the mode of isoproterenol action, isoproterenol suppresses BMP-induced BRE-luciferase activity. These data indicate that isoproterenol regulates BMP-induced alkaline phosphatase expression in osteoblast-like MC3T3E1 cells. J. Cell. Biochem. 116: 1144–1152, 2015.

KEY WORDS: ISOPROTERENOL; BMP; OSTEOBLAST; ALKALINE PHOSPHATASE

Osteoporosis affects approximately 20 million patients in the United States and it is a life-threatening disease with poor prognosis after the fractures in the femoral neck, especially in highly aged patients. Bone mass determination is based on the balance between bone formation and bone resorption. Each of these two arms is under the control of positive regulators as well as negative regulators. Therefore, the balances between these positive and negative regulators for bone formation and another set of positive and negative regulators for bone resorption constitute a hierarchical regulatory system to determine the bone mass. Accordingly, long-term existence of the imbalance in the complex system would result in a reduction in bone mass and an increase in the risk of fractures [Harada and Rodan, 2003; Armas and Recker, 2012; Lewiecki et al., 2014; Martin, 2014].

Sympathetic tone regulates both bone formation and bone resorption locally as well as centrally [Elefteriou et al., 2005; Nagao et al., 2011; Bajayo et al., 2012; D′Amelio et al., 2012]. We have reported that unloading-induced bone loss in disuse osteoporosis is

Conflict of interest: Any of the authors have no conflict of interest to declare.

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Manuscript Received: 16 December 2014; Manuscript Accepted: 18 December 2014
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 23 December 2014
DOI 10.1002/jcb.25071 • © 2014 Wiley Periodicals, Inc.

under the control of the sympathetic tone [Kondo et al., 2005]. In this report, propranolol treatment suppresses unloading-induced bone loss in animals subjected to tail suspension. Analysis on the components underlying the regulation of bone formation and bone resorption indicates that propranolol treatment suppresses the reduction of bone formation due to unloading and also suppresses the increase of bone resorption due to unloading. These observations suggest that sympathetic tone is enhanced in the case of disuse osteoporosis.

In fact, isoproterenol that stimulates β_2 adrenergic receptor suppresses bone formation when injected into the animals and it stimulates bone resorption [Kondo et al., 2005]. This combination of suppression of bone formation and enhancement of bone resorption is observed in unloading-induced disuse osteoporosis [Ishijima et al., 2001]. Isoproterenol treatment alone increases bone resorption in animals but it does not further increase bone resorption induced by unloading when it is combined with unloading condition [Kondo et al., 2005]. Similar interaction is also seen in terms of isoproterenol and unloading-induced suppression of bone formation. These observations suggest that isoproterenol suppresses bone formation and increases bone resorption in a similar pathway shared with that in disuse osteoporosis. Thus, unloading-induced bone loss is at least in part due to an increase in sympathetic tone. However, how the sympathetic tone affects bone mass has been still incompletely understood.

Osteoblastic cells express β_2 adrenergic receptor that is one of the dominant forms of the adrenergic receptor system [Elefteriou et al., 2005; Togari et al., 2005]. Isoproterenol has been shown to bind to the β_2 adrenergic receptor and to modulate the cyclic AMP systems inside the cells. MC3T3-E1 cells are derived from calvariae of normal mice and have been used to examine direct actions of the hormones and cytokines as an osteoblast-like cell line [Sudo et al., 1983]. To this end, we examine the effects of isoproterenol on the BMP2-induced alkaline phosphatase expression in MC3T3-E1 cell.

MATERIALS AND METHODS

CELL CULTURES

MC3T3-E1 cells were obtained from RIKEN (Saitama, Japan). The cells were plated in 24-well plates (2×10^4 cells/well) and were cultured in alpha-minimum essential medium (aMEM) supplemented with 10% fetal bovine serum (FBS). For BMP-induced differentiation of MC3T3-E1 osteoblast like cells, the medium was replaced with fresh aMEM supplemented with 10% FBS and human BMP2 (from hereon we simply refer to BMP). For isoproterenol treatment, the cells were cultured in aMEM in the continuous presence or the absence of isopreoternol or vehcle. Some of the cells were subjected to isoproterenol treatment in a cyclic regimen. Briefly, the cells were subjected to ten cycles of treatment with isoproterenol at 3-min intervals as reported previously [Uemura et al., 2010]. This cyclic treatment resulted in a final cumulative concentration (tenfold concentration). The cells were then cultured for additional 3 days. Cell cultures were conducted in aMEM in a humidified atmosphere at 37°C (5% CO_2 95% air). This term of "cyclic" administration was described in the reference [Uemura et al., 2010] where the epinephrine-elevated cAMP level was maintained for a longer time when epinephrine was added by 10 cyclic additions at a low dose (1nM) using ST2 cells, rather than by addition using a single high dose (10nM). According to these observations, epinephrine stimulation was conducted in this paper by cyclic additions of the low dose (10 cycles of 1nM at 3-min time intervals, resulting in a final cumulative concentration of 10nM). In our current study, we applied this regimen using isoproterenol.

ALKALINE PHOSPHATASE (ALP) ASSAY

Alkaline phosphatase (ALP) activity measurement and protein determination were performed according to PNPP method [Kamolratanakul et al., 2011]. The cells were rinsed twice with PBS and scraped into 10 mM Tris-HCl containing 2 mM $MgCl_2$ and 0.05% Triton X-100 at pH 8.2. The cell lysates were frozen and stored. Aliquots of supernatants were subjected to ALP activity measurement and protein determination. In brief, aliquots of cell lysates were mixed with an aliquot of assay buffer containing 10 mM p-nitrophenyl phosphate in 0.1 M sodium carbonate, pH10, supplemented with 1 mM $MgCl_2$ followed by incubation at 37°C for 30 min. After adding 1 M NaOH, amounts of p-nitrophenol liberated in the assay mixtures were measured using a spectrophotometer.

ALKALINE PHOSPHATASE STAINING

Alkaline phosphatase staining was performed using BM purple ALP substrate precipitating kit (Roche Diagnostics, Mannheim, Germany). MC3T3-E1 cells were fixed with 4% paraformaldehyde for 1 min at room temperature, and then incubated with BM purple at 37°C for more than 1 h. After rinsing with water, plates were dried up and the cells were subjected to microscopic analysis. The number and size of alkaline phosphatase positive foci were quantified by using an image analysis software (Image-J: http://imagej.nih.gov/ij/).

GENE EXPRESSION ANALYSIS

MC3T3-E1 cells were seeded at 2.5×10^4 cells/cm^2 in aMEM supplemented with 10% FBS. After overnight culture, these cells were treated with 10^{-6} M isoproterenol and 50 ng/ml BMP for 3 days. RNAs were extracted using TRIzol (Molecular Research Center) according to the manufacturer's instruction [Morinobu et al., 2001; Hanyu et al., 2012; Miyajima et al., 2012]. Nanodrop was used to assess the quantity and quality of RNA. These RNAs were subjected to reverse transcriptase reaction using High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). Quantitative real-time PCR was performed by using SYBR Green Super Mix.

TRANSFECTION AND LUCIFERASE ASSAY

MC3T3-E1 cells were plated at a density of 2.5×10^4 cells/cm^2 in aMEM supplemented with 10% FBS. After overnight culture, these cells were transfected with reporter plasmid constructs containing a BRE (BMP response element) linked to luciferase [von Bubnuff et al., 2005] using Lipofectamine 2000 according to the manufacturer's

instructions (Invitrogen) [Watanabe et al., 2014]. After 4 h of transfection, the cells were treated with isoproterenol (100uM) and BMP (50 ng/ml) for three more days. Renilla expression vector was also transfected to monitor transfection efficiency. The cells were then harvested, and luciferase activities in the cell extracts were measured based on light using LUMAT LB9507 (Berthold).

MTT ASSAY

To estimate the levels of cell proliferation, we conducted assay for 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) activity. MTT activity measurements were performed by using MC3T3-E1 cells. These cells were plated in 24-well plates (5×10^4 cells/well) and were cultured in medium containing 5 mg/ml MTT (DOJINDO) for 1 h. After incubation, formazan dyes were extracted from cells in each group and the absorbance levels were measured at 570 nm wavelength.

STATISTICAL ANALYSIS

The data were expressed as mean ± SEM (standard error mean) for each group. Differences between the groups were analyzed based on Student's *t*-test. *P* values less than 0.05 or 0.01 were considered to be significant.

RESULTS

ISOPROTERENOL TREATMENT SUPPRESSES BMP-INDUCED INCREASE IN THE NUMBER OF SMALL FOCI OF ALKALINE PHOSPHATASE POSITIVE CELLS IN CULTURE

To first examine the effects of adrenergic stimulation on BMP-induced expression of alkaline phosphatase in osteoblastic MC3T3-E1 cells, we analyzed the alkaline phosphatase staining of these cells. As for a beta adrenergic agonist, we used isoproterenol. Isoproterenol treatment suppressed the number of foci increased by BMP treatment (Fig. 1A). As this isoproterenol effects appeared to be more in small foci, we compared the small foci with the size range between 50–100 μm^2. BMP treatment increased the number about three fold and isoproterenol treatment suppressed the BMP-induced increase by about 50% (Fig. 1B, right side two columns). Isoproterenol alone also suppressed the number of baseline levels by about 50% (Fig. 1B, left side two columns). In contrast to small foci, BMP as well as isoproterenol did not alter the number of larger (over 1000 μm^2) foci (Fig. 1A and C).

ISOPROTERENOL SUPPRESSES BMP-INDUCED ALKALINE PHOSPHATASE ACTIVITY IN MC3T3-E1 OSTEOBLAST-LIKE CELLS IN A DOSE-DEPENDENT MANNER

Although, we observed that isoproterenol treatment alters BMP-induced increase in the number of alkaline phosphatase-positive foci, effects of isoproterenol on the overall averaged values for biochemical alkaline phosphatase activity per well including both smaller and larger foci were needed to be evaluated. Therefore, we next examined the effects of isoproterenol on the averaged biochemical activity of alkaline phosphatase in the extracts of the cells per culture. Isoproterenol suppressed BMP-induced alkaline phosphatase activity in MC3T3-E1 osteoblast-like cells in a dose-dependent manner (Fig. 2).

ISOPROTERENOL SUPPRESSES ALKALINE PHOSPHATASE ACTIVITY INDUCED BY BMP AT EVEN HIGHER DOSAGE IN MC3T3-E1 OSTEOBLAST-LIKE CELLS

As higher dosage of BMP increases alkaline phosphatase activity more, we examined whether isoproterenol suppression may be

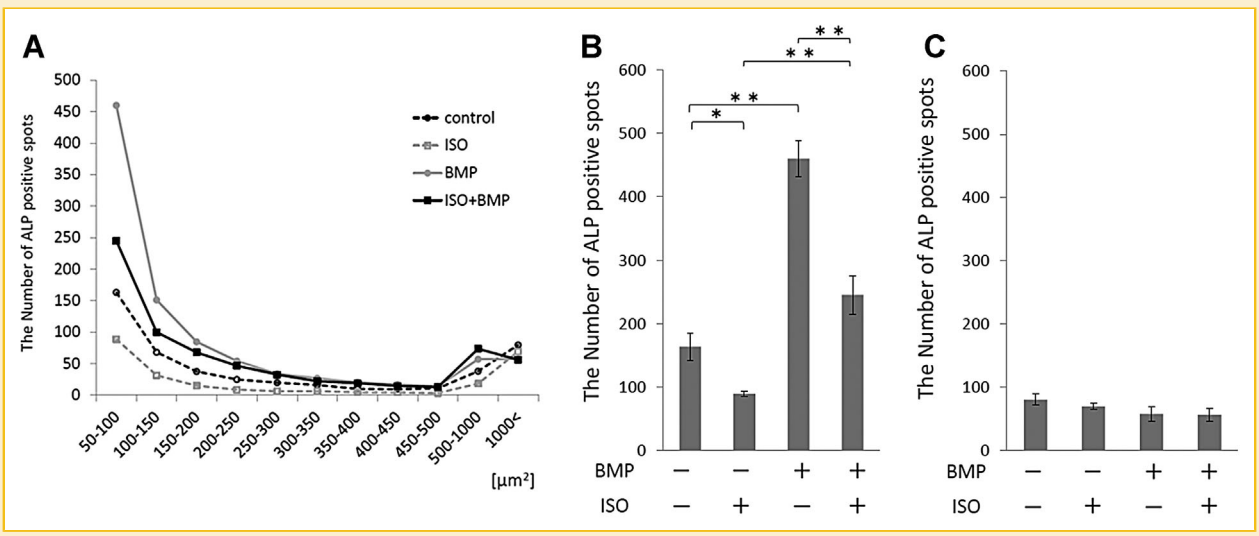

Fig. 1. Isoproterenol treatment suppresses BMP-induced increase in the number of small foci of alkaline phosphatase positive cells in culture. MC3T3-E1 cells were cultured in the absence or the presence of isoproterenol (10^{-7} M) and/or BMP2 (100 ng/ml) for 3 days. Then, the cells were subjected to cytochemical staining for alkaline phosphatase. The sizes of alkaline phosphatase positive foci were analyzed based on image analyzing software (Image-J) (A). Comparison of the numbers of alkaline phosphatase positive foci (foci) within the size range of 50–100 μm^2 (B) and over 1000 μm^2. (C). Data are expressed as mean ± SEM. n = 4 wells. $^{*}P < 0.05$, $^{**}P < 0.01$. The experiments were repeated twice with similar results.

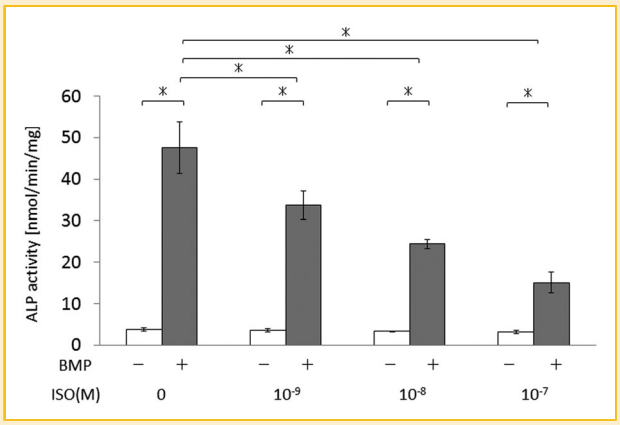

Fig. 2. Isoproterenol suppresses BMP-induced alkaline phosphatase activity in MC3T3-E1 osteoblast-like cells in a dose-dependent manner. MC3T3-E1 cells were subjected to treatment with isoproterenol at the indicated doses in the presence right side gray columns for each dose) or the absence (left side dark blue columns for each dose) of 50 ng/ml BMP2 for 3 days. Data are expressed as mean ± SEM. n = 4 wells. $^{*}P < 0.05$ compared to control (BMP2 alone without isoproterenol, right gray column). The experiments were repeated twice with similar results.

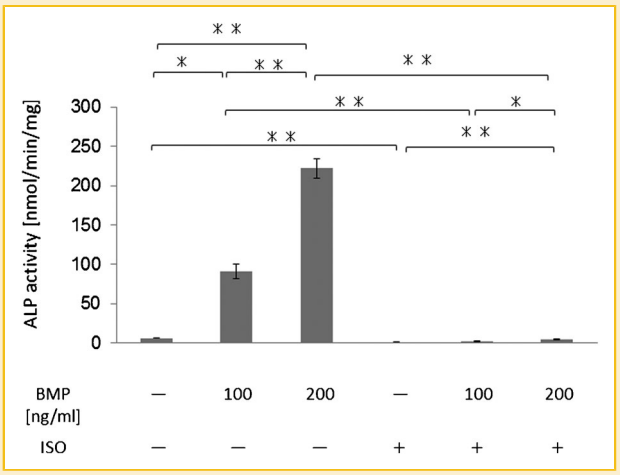

Fig. 3. Isoproterenol suppresses both high- and low-dose BMP-induced alkaline phosphatase activity in MC3T3-E1 osteoblast-like cells. MC3T3-E1 cells were subjected to treatment with isoproterenol (10^{-5} M) at the indicated doses and/or BMP for 3 days. The data are expressed as mean ± SEM. n = 4 wells. $^{*}P < 0.05$, $^{**}P < 0.01$. The experiments were repeated three times with similar results.

rescued by higher dosage of BMP. BMP at higher dosage (200 ng/ml) increased alkaline phosphatase levels two fold more compared to lower (100 ng/ml) dosage (Fig. 3). Isoproterenol co-treatment suppressed BMP-induced alkaline phosphatase activity even at higher dosage of BMP (200 ng/ml) was used (Fig. 3). Thus, higher dosage of BMP does not relieve isoproterenol suppression of BMP action.).

ISOPROTERENOL SUPPRESSES BMP-INDUCED ALKALINE PHOSPHATASE ACTIVITY IN MC3T3-E1 OSTEOBLAST-LIKE CELLS PLATED AT HIGHER CELL DENSITY

BMP actions on osteoblasts are positively affected by cell density. Therefore, we next examined the effects of cell density on the isoproterenol regulation of BMP-induced differentiation of MC3T3-E1 cells to see if cell density dependent enhancement of BMP action on osteoblastic differentiation may interfere isoproterenol suppression. BMP effects on alkaline phosphatase activity were enhanced in the cultures at higher cell density compared to those at lower cell density (Fig. 4A, open columns). Isoproterenol treatment suppressed BMP-induced alkaline phosphatase expression even at higher cell density (Fig. 4A, closed columns).

As we observed that cell density affects cellular response to BMP, it may be possible that the isoproterenol treatments taken place for more than 3 days may affect the data via proliferation. Therefore, we asked if there is any effects of isoproterenol on cell proliferation, which would result in changes in cell density. To examine this point, we conducted MTT assay. MC3T3-E1 cells were plated at low cell density and the effects of isoproterenol was evaluated. We observed that isoproterenol treatment did not affect cell proliferation (Fig. 4B).

CONTINUOUS TREATMENT WITH ISOPROTERENOL IS REQUIRED FOR SUPPRESSION OF BMP-INDUCED ALKALINE PHOSPHATASE EXPRESSION IN MC3T3-E1 CELLS

Up to this point, isoproterenol treatment was continuous as it was added to the medium only once when stimulation was initiated and then the cells were exposed to isoproterenol till the termination of culture. As nervous system may affect cells in a pulsatile manner rather than continuous one, we examined the effects of cyclic treatment with isoproterenol on BMP-induced differentiation of osteoblasts. In these experiments, 1/10 amount of isoproterenol (1 nM) was added to the culture of MC3T3-E1 cells cyclically every 3 min for 10 times. The final isoproterenol concentration was reaching at 10 nM and then the culture was continued for 3 days according to the previously published protocol [Uemura et al. 2010]. However, under this cyclic protocol condition, isoproterenol did not suppress BMP-induced enhancement of alkaline phosphatase activity (Fig. 5). Thus, continuous presence of isoproterenol is necessary for the suppression of BMP-induced enhancement alkaline phosphatase expression.

ISOPROTERENOL SUPPRESSES BMP-INDUCED ENHANCEMENT OF ALKALINE PHOSPHATASE mRNA EXPRESSION IN MC3T3-E1 CELLS

To examine the levels of isoproterenol suppression of BMP-induced event, we determined the mRNA expression level. Isoproterenol suppressed the BMP-induced increase in the levels of alkaline phosphatase mRNA in these cells (Fig. 6A). This isoproterenol suppression on alkaline phosphatase mRNA expression was specific to alkaline phosphatase as ATF4 mRNA expression was not suppressed by isoproterenol either in the presence or the absence of BMP treatment (Fig. 6B). As ATF4 is expressed in osteoblasts and is involved in ER stress [Duan et al., 2013] and glucose homeostasis

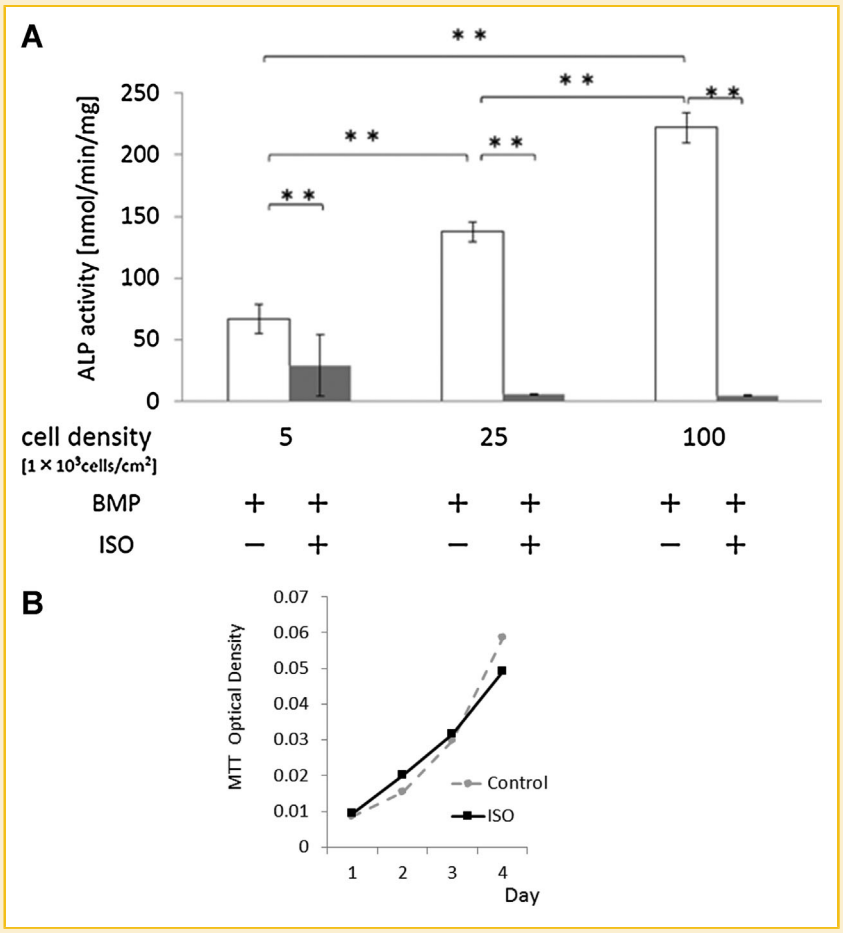

Fig. 4. Isoproterenol suppresses BMP-induced alkaline phosphatase activity in MC3T3-E1 osteoblast-like cells plated at both high and low cell density. (A) MC3T3-E1 cells were plated at indicated cell density and were subjected to treatment with isoproterenol (10^{-5} M) and/or BMP (200 ng/ml) for 3 days. Data are expressed as mean ± SEM. n = 4 wells. $^{*}P < 0.05$, $^{**}P < 0.01$. (B) MC3T3-E1 cells were plated at 5000 cells/cm^2 and were cultured in the presence or the absence of isoproterenol for 4 days. At each time point, cells were subjected to MTT assay to estimate the effects of isoproterenol on proliferation of these cells. The experiments were repeated twice with similar results.

[Kode et al., 2012], isoproterenol would not be involved in these aspects of osteoblastic function. In terms of another gene expression that is induced by BMP, Osterix was also induced by BMP and this expression was suppressed by isoproterenol treatment (Fig. 6C).

ISOPROTERENOL SUPPRESSES BMP-INDUCED ENHANCEMENT IN BRE-LUCIFERASE CONSTRUCT ACTIVATION IN MC3T3-E1 CELLS

We further examined whether isoproterenol treatment interferes BMP activation of transcription. For this purpose, we transfected MC3T3-E1 cells with a plasmid containing luciferase linked to BMP-response element (BRE). BMP binds to its receptor and subsequently activates Smad proteins that bind to BMP response element to promote transcription of target genes. Smad proteins consist of heterotrimers including BMP-specific Smads such as Smad1, Smad5 and Smad8 in conjunction with common Smad protein, Smad4. This heterotrimer Smad complex binds to BRE and activate transcription of the reporter gene, luciferase upon treatment with BMP. In our system, BMP treatment enhanced BRE luciferase activity as known before (Fig. 7). Under this condition, isoproterenol suppressed the levels of luciferase that was enhanced by BMP (Fig. 7).

DISCUSSION

Disuse osteoporosis is currently getting to be more critical situation as aged population is soaring in the modern society. However, the mechanism underlying disuse osteoporosis is still incompletely understood. We have previously reported that sympathetic tone is involved in disuse osteoporosis model of tail suspension and that β adrenergic stimulation using isoproterenol suppresses in vivo osteoblastic activity in mice in a similar manner to the tail suspension-induced disuse osteoporosis. However, isoproterenol actions on the differentiation of osteoblasts are not fully understood. Here, we found that isoproterenol treatment suppresses BMP-induced differentiation of osteoblastic MC3T3-E1 cells based on the monitoring of alkaline phosphatase activity. Isoproterenol suppression of BMP-induced alkaline phosphatase expression was not only

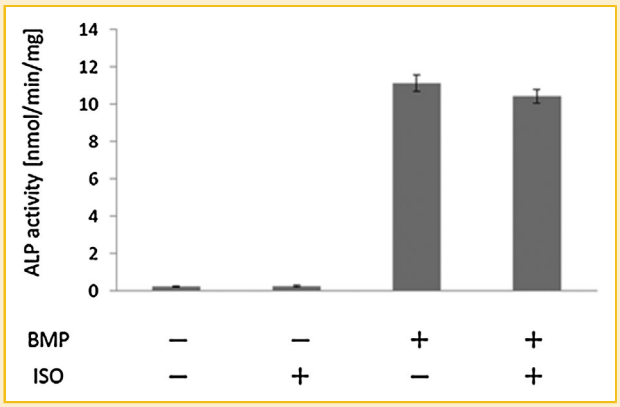

Fig. 5. Cyclic treatment with isoproterenol does not suppress BMP-induced osteoblastic differentiation in MC3T3-E1 cells. MC3T3-E1 cells were subjected to treatment with isoproterenol (10 nM) according to cyclic application protocol as described in Materials and Methods and/or BMP (50 ng/ml) for 3 days. Data are expressed as mean ± SEM. n = 4 wells. $^{*}P < 0.05$, $^{**}P < 0.01$. The experiments were repeated twice with similar results.

observed at a suboptimal dose of BMP (50 ng/ml) but also at higher dosages including 100 and 200 ng/ml. Osteoblasts produce BMP as an autocrine and paracrine cytokine to support the differentiation of themselves. Though β adrenergic stimulation by isoproterenol suppresses osteoblastic activity in vivo by suppressing mineral apposition rate, mineralizing surface and bone formation rate [Kondo et al., 2005], details of isoproterenol action in vivo have not been fully understood. Our observations suggest that isoproterenol suppresses osteoblastic differentiation at least in part via suppression of BMP actions on these cells. Such BMP may be from the osteoblasts themselves in a manner known as autocrine or paracrine system or from other sources surrounding osteoblasts, including stromal cells or bone matrix.

BMP induces differentiation of osteoblasts as shown by its enhancement of alkaline phosphatase activity via its up-regulation of alkaline phosphatase messenger RNA. Therefore, BMP action on osteoblasts would be largely via its regulation of the expression of the genes related to osteoblastic phenotypes. BMP may also regulate posttranscriptional regulation of gene expression. As isoproterenol suppresses BMP-induced increase in the steady state levels of alkaline phosphatase mRNA, both transcriptional and/or posttranscriptional regulation may be the point of isoproterenol interference of BMP actions. Since BMP-enhancement of BRE-luciferase expression was suppressed by isoproterenol, at least BMP-induced transcriptional regulation could be the event where isoproterenol may interfere the pathway. However, posttranscriptional part is still a possibility in addition to such transcriptional events as isoproterenol was shown to decrease leptin release from rat and human adipose tissue through posttranscriptional mechanisms [Ricci et al., 2005].

Our data also revealed that isoproterenol effects on osteoblastic differentiation are dependent on administration protocol. We showed that continuous isoproterenol treatment suppresses BMP-induced enhancement in alkaline phosphatase activity in osteoblast-like MC3T3-E1 cells. In contrast, cyclic stimulation with isoproterenol does not largely affect BMP-induced alkaline phosphatase activity. This is in contrast to the previous report in that epinephrine treatment was effective in the cyclic regimen to enhance BMP actions in culture [Uemura et al., 2010]. These observations suggest that isoproterenol actions on BMP-induced alkaline phosphatase expression in osteoblasts require continuous presence to the cells but no cyclic treatment.

Isoproterenol treatment affects the number of small alkaline phosphatase positive foci that was increased by BMP treatment. Interestingly, the number of larger foci positive for alkaline phosphatase was not largely affected by BMP treatment as well as isoproterenol treatment. As control cultures of MC3T3-E1 cells exhibit some alkaline phosphatase positive foci even before treatment with BMP, there may be some flow of immature cells to become alkaline phosphatase positive foci in a stochastic manner. Since BMP is known to be less potent in regulation of cell proliferation, isoproterenol action may be to modulate such stochastic rate of the flow of immature cells to mature alkaline phosphatase positive foci forming cells [MacArthur et al., 2008]. The molecular bases of such isoproterenol interaction with BMP are still to be elucidated.

It is intriguing that one report indicated unresponsiveness to isoproterenol in primary cultures of osteoblast (Aitken SJ et al., 2009) as we have also observed such unresponsiveness to isoproterenol with respect to the number of large foci formation in MC3T3-E1 cells (Fig. 1C). On top of such similarity, we have further shown isoproterenol suppression on the BMP-induced small foci formation in MC3T3-E1 cells. These observations on the diversity in terms of the responsiveness to isoproterenol suggest the presence of response variations in osteoblastic cell populations. Whether such variations are due to the presence of subpopulations of osteoblasts or not is still to be determined. Regardless of such heterogeneity of osteoblasts, our in vivo observation indicated clearly that isoproterenol suppresses osteoblastic bone formation activity such as bone formation rate (BFR) and mineral apposition rate (MAR) [Kondo et al., 2005].

Disuse osteoporosis occurs in patients suffering from spinal cord injury [Alizadeh-Meghrazi et al., 2014] or spina bifida [Marreiros et al., 2012] and is also observed in patients with bedridden condition due to neuronal and cardiovascular diseases [Jaul et al., 2009]. Osteoporosis during space flight has been another major issue in addition to the pathological conditions on the ground levels [Leblanc et al., 2013]. All these disuse conditions are ending up with or in conjunction with impairment of motor function of the body especially in association with ageing and therefore they are considered to be important bases for locomotive syndrome [Yoshimura et al., 2011]. Our observation that isoproterenol suppresses BMP-induced differentiation of osteoblastic MC3T3-E1 cells is still one of the many basic events underlying multistep pathogenesis of the locomotive syndrome. Further investigation would be required to fully understand the mechanism of disuse osteoporosis induced by bedridden conditions due to systemic diseases.

Aging causes not only impairment in bone but also in many other organs including lung. Chronic obstructive pulmonary syndrome (COPD) is a major lung disease where proliferation and myofibroblastic transformation induces pathologic airway

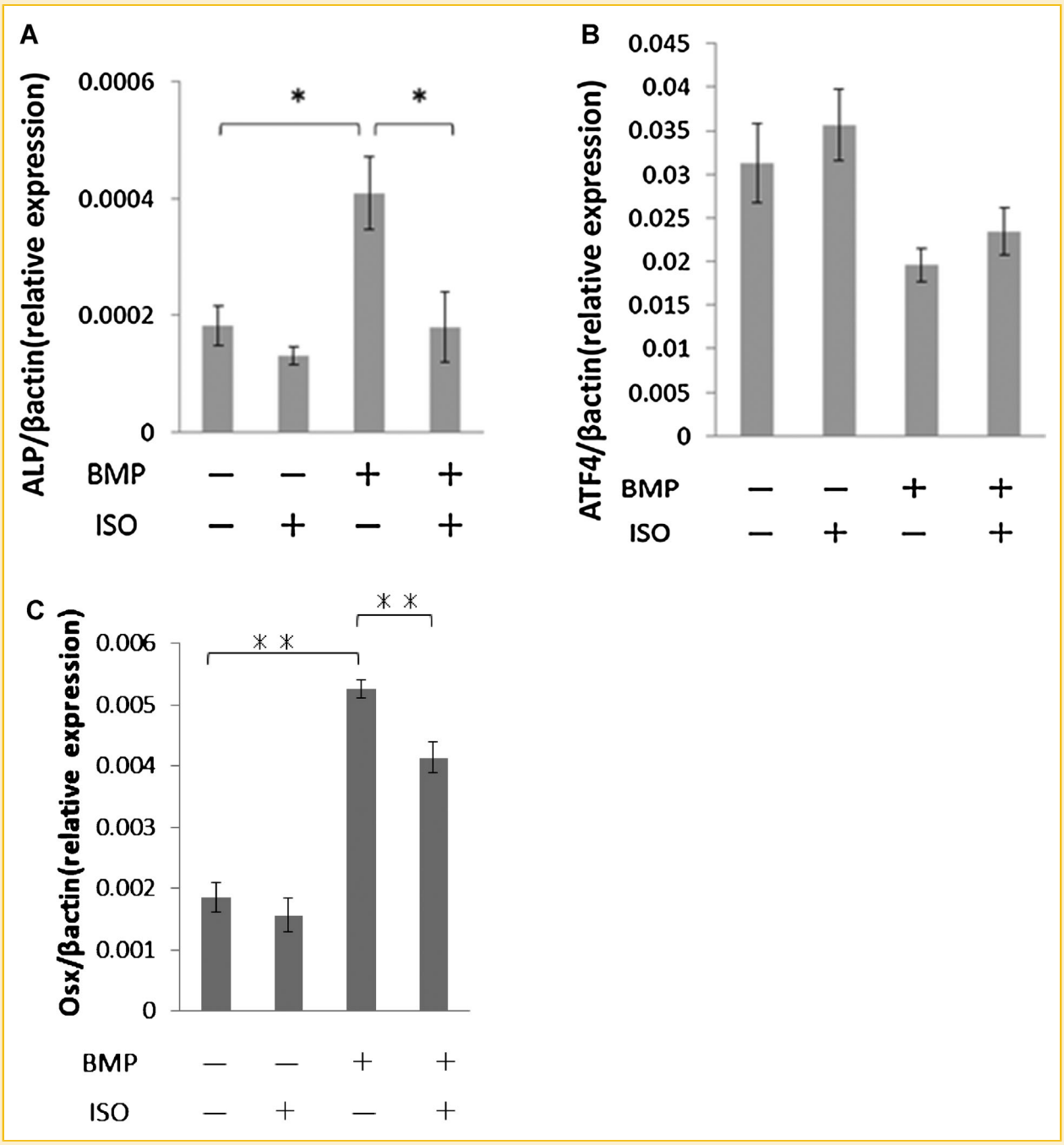

Fig. 6. Isoproterenol suppresses BMP-induced enhancement in alkaline phosphatase mRNA expression. MC3T3-E1 cells were plated and were subjected to treatment with isoproterenol (10^{-5} M) and/or BMP (50 ng/ml) for 3 days. RNAs were extracted and were subjected to quantitative RT-PCR. All the values were normalized against beta actin mRNA levels. (A) alkaline phosphatase, (B) ATF4, (C) Osterix. The data are expressed as mean ± SEM. n = 4–5. $^{*}P < 0.05$. The experiments were repeated twice with similar results.

obstruction. It was reported that isoproterenol inhibits differentiation of lung fibroblasts into myofibroblasts induced by TGF-beta [Franke et al., 2014]. In these COPD patients, osteoporosis is frequently associated and reduces quality of patient life [Evans and Morgan, 2014]. In this case, the link between the COPD and osteoporosis [Bai et al., 2011] may not be only based on the loss of exercise but also the underlying actions of beta adrenergic receptor actions. With respect to signaling in cardiac fibroblasts, isoproterenol was reported to inhibit TGF-beta-induced collagen production via its suppression of Erk [Liu et al., 2006]. These features of the commonality of the diseases and sympathetic tone point to the importance of the understanding on systemic aspects of diagnosis and treatment in these diseases in our modern society where aged population is soaring.

In summary, we found that isoproterenol suppresses BMP-induced alkaline phosphatase expression in osteoblast-like MC3T3-E1 cells.

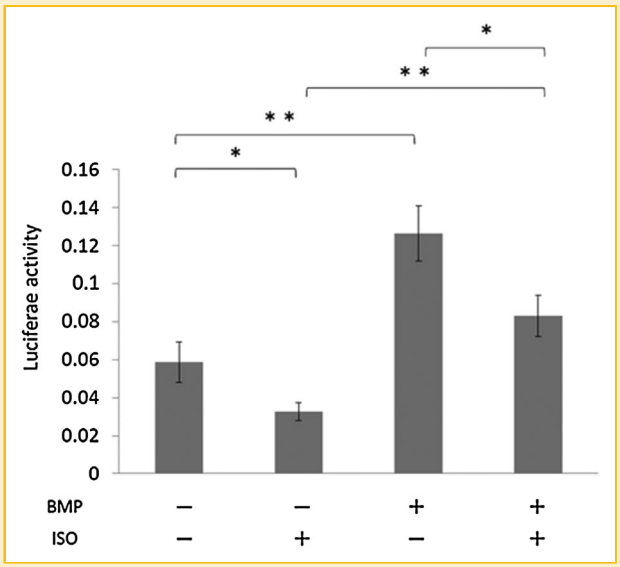

Fig. 7. Isoproterenol suppresses BMP-induced enhancement in BRE-luciferase construct activation in MC3T3-E1 cells. MC3T3-E1 cells were transfected with a reporter construct containing BMP response element (BRE) linked to luciferase. Renilla construct was also co-transfected as internal reference. Then the cells were treated with isoproterenol (10^{-4} M) and/or BMP (50 ng/ml) for 3 days followed by luciferase assay. The data are expressed as mean ± SEM. n = 6. $^{*}P < 0.05$, $^{**}P < 0.01$. The experiments were repeated twice with similar results.

Our data suggest that BMP-induced enhancement of osteoblastic differentiation may be one of the target points of sympathetic tone-dependent suppression of osteoblastic activity observed in disuse osteoporosis.

ACKNOWLEDGEMENTS

This research was supported by the grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Education, JAXA, MSD, Metabolic Abnormality Res Foundation and TBRF. We thank Dr. Ken Cho for providing us his plasmids.

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