Fucoidan promotes osteoblast differentiation via JNK- and ERK-dependent BMP2–Smad 1/5/8 signaling in human mesenchymal stem cells

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Fucoidan has attracted attention as a potential drug because of its biological activities, which include osteogenesis. However, the molecular mechanisms involved in the osteogenic activity of fucoidan in human alveolar bone marrow-derived mesenchymal stem cells (hABM-MSCs) remain largely unknown. We investigated the action of fucoidan on osteoblast differentiation in hABM-MSCs and its impact on signaling pathways. Its effect on proliferation was determined using the crystal violet staining assay. Osteoblast differentiation was evaluated based on alkaline phosphatase (ALP) activity and the mRNA expression of multiple osteoblast markers. Calcium accumulation was determined by Alizarin red S staining. We found that fucoidan induced hABM-MSC proliferation. It also significantly increased ALP activity, calcium accumulation and the expression of osteoblast-specific genes, such as ALP, runt-related transcription factor 2, type I collagen-α1 and osteocalcin. Moreover, fucoidan induced the expression of bone morphogenetic protein 2 (BMP2) and stimulated the activation of extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase by increasing phosphorylation. However, the effect of fucoidan on osteogenic differentiation was inhibited by specific inhibitors of ERK (PD98059) and JNK (SP600125) but not p38 (SB203580). Fucoidan enhanced BMP2 expression and Smad 1/5/8, ERK and JNK phosphorylation. Moreover, the effect of fucoidan on osteoblast differentiation was diminished by BMP2 knockdown. These results indicate that fucoidan induces osteoblast differentiation through BMP2–Smad 1/5/8 signaling by activating ERK and JNK, elucidating the molecular basis of the osteogenic effects of fucoidan in hABM-MSCs.

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INTRODUCTION

Bone development involves a balance between resorption and formation, and it is performed by osteoclasts and osteoblasts, respectively.1 Osteoporosis is one of the most common bone diseases characterized by a systemic reduction in bone mass.2 Current osteoporosis treatment strategies depend on the use of anti-resorptive and bone-forming drugs. Unfortunately, the long-term use of anti-osteoporotic medications is associated with serious side effects.3 Therefore, effective treatments that are devoid of side effects and focus on osteoblast activation are urgently needed.

Osteoblasts arise from a mesenchymal stem cell (MSC) precursor, and their differentiation is regulated by a number of growth factors, cytokines, and environmental factors.4,5 As a result, numerous researchers in the field of bone biology have studied the osteogenic-enhancing effects of compounds derived from natural products.6,7 Recently, several studies have reported that sulfated polysaccharides influence osteoblast differentiation.8 Fucoidan is a polysaccharide containing substantial proportions of L-fucose and sulfate ester groups that is mainly derived from brown algae and seaweed.9,10 For the past decade, fucoidan has been extensively studied because of its numerous biological activities, which include anti-coagulant,11 anti-inflammatory12 and anti-cancer13 properties. In addition, it has been reported to induce osteogenic differentiation in human adipose-derived stem cells14 and MG-63 cells.15 However, the mechanism by which fucoidan induces this process is poorly understood.

Because there are no reports on the molecular mechanism of fucoidan in osteoblast differentiation, in this study, we aimed to determine whether it affects osteogenic differentiation via mitogen-activated protein kinases (MAPKs), several of which are essential components of the signal transduction machinery that occupy central positions in this differentiation process.16 Several MAPKs have been identified, including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal protein...
kinase (JNK) and p38 MAPK. These three types of MAPKs regulate key transcriptional events that mediate osteoblast differentiation. Following activation of MAPK signaling during this differentiation process, bone morphogenetic protein-2 (BMP2)/Smad signaling is activated.

**MATERIALS AND METHODS**

**Fucoidan**

Fucoidan (a broad-range molecular weight polysaccharide) was purchased from Haewon Biotech (Seoul, Korea). Fucoidan, which is composed of 61.5% polysaccharides and 23.5% sulfate, was extracted from the brown seaweed *Laminaria japonica*.

**Cell culture**

We used MSCs from human alveolar bone marrow (hABM-MSCs), which was isolated and characterized as described previously. The cells were cultured in α-Minimum Essential Medium (Gibco, Gaithersbug, MD, USA) containing 10% fetal bovine serum (Gibco) and 1% antibiotics (10 000 U ml⁻¹ penicillin G and 25 μg ml⁻¹ amphotericin B; Gibco) in a humidified CO₂ incubator at 37 °C. The cells were used at passages 3–6 in this study.

**Cell proliferation assay**

To assess the effects of fucoidan on cell proliferation, cells were seeded in a 96-well plate at a density of 5 × 10³ cells per well. The cells were incubated in growth medium for the indicated durations at various concentrations of fucoidan (0.1, 0.5, 1, 5 and 10 μg ml⁻¹). They were cultured for 1, 2 and 3 days before crystal violet assays were performed. Next, the medium was removed, and the cells were washed twice with phosphate-buffered saline, fixed with 10% formaldehyde and stained with 0.5% (w/v) crystal violet solution for 10 min. After washing with water, the crystal violet was solubilized with 200 μl of 33% acetic acid solution, and absorbance was measured at 570 nm using a microplate reader (SpectraMAX M3; Molecular Devices, Sunnyvale, CA, USA).

**Alkaline phosphatase (ALP) staining and activity**

For ALP staining, cells were seeded in a 96-well plate at a density of 3 × 10³ cells per well. The cells were incubated in maintenance medium until confluence. They were then treated with fucoidan in the presence or absence of MAPK inhibitors. Cell cultures were harvested at the indicated times and extracted at 4 °C in PRO-PREP solution (Intron Biotechnology, Inc., Sungnam, Korea). Extracts were cleared by centrifugation, and protein concentrations were determined using a bicinchoninic acid protein assay kit (Sigma-Aldrich Co.). The quantity of ALP activity was measured using a bicinchoninic acid protein assay kit (Sigma-Aldrich Co.). The activity of ALP was calculated as protein.

**Calcium accumulation assay**

Calcium accumulation was evaluated by alizarin red-sulfate (AR-S; Sigma-Aldrich Co.) staining. Cells were seeded in a 96-well plate at a density of 5 × 10³ cells per well and cultured with continuous exposure to fucoidan for 20 days. After 20 days of fucoidan exposure, the cells were fixed with 70% (v/v) ice-cold ethanol for 1 h at 4 °C, stained with 40 μl AR-S solution, and imaged using a microscope (DM II LED Fluo). The cells were solubilized using 10% (w/v) cetylpyridinium chloride in 10 mM sodium phosphate (pH 7.0), and absorbance was measured at 562 nm.

**Quantitative real-time PCR analysis**

To evaluate the effects of fucoidan on osteoblast differentiation, mRNA expression levels of several osteoblast marker genes were determined by quantitative real-time PCR using TaqMan Universal PCR Master Mix and TaqMan primers and probe sets specifically targeting runt-related transcription factor 2 (*Runx2*, Hs00231692_m1), collagen type I α1 (*Col1α1*, Hs00164004_m1), osteocalcin (OC, Hs01587814_g1), ALP (*ALP*, Hs01029144_m1), BMP2 (*BMP2*, Hs01055564_m1) and 18S ribosomal rRNA (Hs99999901_s1; Applied Biosystems, Carlsbad, CA, USA). All TaqMan PCR assays were performed using a StepOne Plus Real-Time PCR System (Applied Biosystems). 18S rRNA was co-amplified as an internal standard. Relative mRNA expression levels were normalized to those of the non-target shRNA sample.

**Western blot**

Cells were seeded at a density of 1 × 10⁵ cells per 6-cm cell culture dish and grown in maintenance medium until confluence. They were then treated with fucoidan in the presence or absence of MAPK inhibitors. Cell cultures were harvested at the indicated times and extracted at 4 °C in PRO-PREP solution (Intron Biotechnology, Inc., Sungnam, Korea). Extracts were cleared by centrifugation, and protein concentrations were determined using a bicinchoninic acid protein assay kit (Bio-Rad, Hercules, CA, USA). For western blots, 10 μg of each protein sample was separated by 10% SDS-polyacrylamide gel electrophoresis and electro-transferred onto nitrocellulose membranes. The blots were blocked with a buffer containing 0.05% Tween-20 and 3% bovine serum albumin and reacted sequentially with primary and secondary antibodies. Primary antibodies against phosphorylated JNK, BMP2 (Cell Signaling Technology, Beverly, MA, USA), ERK (Genetex, Inc., Irvine, CA, USA), p38 MAP kinase and Smad 1/5/8 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were diluted 1:1000. Horseradish peroxidase-conjugated secondary antibodies were diluted 1:7000. Finally, the membranes were developed with enhanced chemiluminescence solution and exposed to X-ray film.

**Knockdown of BMP2 using short hairpin RNA**

To knockdown BMP2, human BMP2 shRNA lentiviral particle (Santa Cruz Biotechnology) transduction was performed. Control transduction with non-target shRNA was also performed. Transduction of BMP2 shRNA and non-target shRNA (control shRNA) was conducted following the manufacturer’s protocol. To confirm the efficiency of shRNA-mediated knockdown, BMP2 protein levels were evaluated by western blotting.

**Statistical analysis**

The values are expressed as the mean ± standard deviation (s.d.), and statistical analysis was performed by one-way analysis of variance followed by the Student–Newman–Keuls multiple comparisons test for comparisons between different groups.
RESULTS
Fucoidan promotes cell proliferation in a dose-dependent manner
To estimate the effect of fucoidan on hABM-MSCs, cell proliferation was determined. hABM-MSCs were treated with various concentrations of fucoidan (0.1–10 μg ml⁻¹), and cell proliferation was analyzed using the crystal violet assay. On day 1, hABM-MSCs incubated with fucoidan at concentrations of 5 and 10 μg ml⁻¹ showed significantly increased proliferation in comparison with untreated (control) cells. After 2 days of culture, fucoidan induced cell proliferation in a dose-dependent manner at all tested concentrations (0.1–1.0 μg ml⁻¹; Figure 1).

Fucoidan promotes osteoblast differentiation
We examined the effect of fucoidan on osteoblast differentiation using hABM-MSCs. To assess the ability of fucoidan to promote osteoblast differentiation, ALP activity was determined by staining and activity measurement. The results from the ALP staining assay demonstrated that fucoidan significantly (P<0.01) induced ALP activity in the concentration range of 0.1–1.0 μg ml⁻¹. Of the tested concentrations, the strongest effect on ALP activity was observed following the 1.0 μg ml⁻¹ fucoidan treatment. ALP activity was reduced at 5 and 10 μg ml⁻¹ fucoidan (Figures 2a and b). In addition, quantitative real-time PCR revealed that mRNA expression levels of the osteoblast marker genes Runx2, Col1α1, OC and ALP were significantly increased following treatment with 1 μg ml⁻¹ fucoidan (Figure 2c).

Fucoidan promotes calcium accumulation
Calcium accumulation in the extracellular matrix is a phenotypic marker of the final stages of osteoblast differentiation. Therefore, to examine whether fucoidan promotes calcium accumulation, hABM-MSCs were treated with various concentrations of fucoidan (0.1–10 μg ml⁻¹) and subjected to AR-S staining assays. Calcium accumulation in hABM-MSCs cultured for 15 days in the presence of fucoidan is shown in Figures 3a and b. Calcium deposition was significantly increased by the fucoidan treatment at concentrations ranging from 0.1 to 10 μg ml⁻¹. The greatest calcium deposition was observed following treatment with 5 μg ml⁻¹ fucoidan. Calcium deposition was slightly reduced by the treatment with 10 μg ml⁻¹ fucoidan.

Effects of fucoidan MAPK and Smad 1/5/8 phosphorylation and BMP2 mRNA expression
To explore the involvement of MAPKs in fucoidan-induced osteoblast differentiation, activation of MAPKs was examined by western blotting with specific antibodies. We also explored the mRNA expression of BMP2, which is a key component of mitogenic signaling pathways. As shown in Figure 4a, fucoidan (1 μg ml⁻¹) treatment activated JNK, ERK and p38 within 30 min, as evidenced by increased protein phosphorylation. Furthermore, the level of phosphorylated Smad 1/5/8 was strongly increased after 30 min of treatment with fucoidan (Figure 4b). BMP2 mRNA expression was increased approximately twofold by treatment with fucoidan for 3 days (Figures 4c and d).

Effects of MAPK inhibitors on fucoidan-induced osteoblast differentiation
To determine whether MAPKs play a role in fucoidan-induced osteoblast differentiation in hABM-MSCs, cells treated with specific inhibitors of JNK, ERK and p38 were subjected to ALP staining analysis. As shown in Figure 5, the JNK inhibitor SP600125 and ERK inhibitor PD98059 reversed the effect of fucoidan on osteoblast differentiation. However, the p38 inhibitor SB203580 had no effect on fucoidan-induced osteoblast differentiation.

MAPK inhibitors prevent mRNA expression of fucoidan-induced osteoblast marker genes
Next, we examined whether the effects of fucoidan on several osteoblast markers were mediated via MAPK signaling. The mRNA expression levels of Runx2, Col1α1, OC and ALP were evaluated in hABM-MSCs treated with specific inhibitors of JNK, ERK and p38 MAPKs. As shown in Figure 6, fucoidan-induced increases in Runx2, Col1α1 and ALP gene expression were attenuated by the JNK inhibitor SP600125 and ERK inhibitor PD98059. In contrast, the p38 inhibitor SB203580 did not affect fucoidan-induced gene expression. These observations strongly suggest that the stimulatory effect of fucoidan on hABM-MSC osteoblast differentiation is mediated at least in part through JNK and ERK signaling.
Activation of JNK and ERK occurs upstream of Smad 1/5/8 and BMP2 signaling

In the present study, we observed that fucoidan increased Smad 1/5/8 phosphorylation (Figure 4b) and enhanced BMP2 mRNA expression (Figures 4c and d). To evaluate whether MAPKs interact with Smad 1/5/8 or BMP2 signaling, we assessed Smad 1/5/8 phosphorylation and BMP2 mRNA expression in cells treated with specific inhibitors of JNK, ERK and p38 MAPKs. Interestingly, inhibition of the JNK-ERK signaling pathway prevented the fucoidan-induced phosphorylation of Smad 1/5/8 and BMP2 gene expression, whereas these effects were not observed in the presence of the p38 MAPK inhibitor (Figures 7a–c). These data clearly indicate that JNK and ERK are involved in signaling events that mediate Smad 1/5/8 and BMP2 signaling in fucoidan-induced osteoblast differentiation in hABM-MSCs.

BMP2 knockdown inhibits fucoidan-induced osteoblast differentiation

To understand the influence of BMP2 knockdown on fucoidan-induced osteoblast differentiation in hABM-MSCs, cells were transfected with BMP2-specific shRNA lentiviral particles. The western blot data showed that BMP2 expression was reduced in the BMP2 shRNA-transfected cells by approximately 78% in comparison with the cells treated with control shRNA (Figures 8a and b). BMP2 expression was significantly

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**Figure 2** Effects of fucoidan on osteogenic differentiation. Confirmation of osteogenic differentiation by (a) alkaline phosphatase (ALP) staining and (b) ALP activity in human alveolar bone marrow-derived mesenchymal stem cells cultured for 5 days with fucoidan (0.1–10 μg ml⁻¹). Fucoidan at concentrations of 0.1, 0.5 and 1 μg ml⁻¹ strongly induced ALP activity. (c) Osteoblast differentiation was confirmed by measuring mRNA expression using real-time quantitative PCR on RNA isolated from cells treated with 1 μg ml⁻¹ fucoidan. Scale bar = 500 μm. All data represent the mean ± s.d. of three independent experiments. *P < 0.05 and **P < 0.01 compared with control (Con) cells.

**Figure 3** Effects of fucoidan on osteogenic differentiation. (a) Determination of calcium accumulation using AR-S in human alveolar bone marrow-derived mesenchymal stem cells cultured for 21 days with fucoidan (1 μg ml⁻¹). (b) Optical density measured at 562 nm of stained sections solubilized with cetylpyridinium chloride solution. Scale bar = 200 μm. All data represent the mean ± s.d. of five independent experiments. **P < 0.01 compared with control cells.
increased by fucoidan treatment and significantly (approximately 2.8-fold) reduced by the BMP2-specific shRNA. The influence of BMP2 knockdown on ALP activity in hABM-MSCs was determined after 5 days of cultivation in the presence or absence of fucoidan. ALP expression in BMP2-specific shRNA-transfected cells was significantly reduced in comparison with that of control shRNA-transfected cells. Furthermore, pre-treatment of hABM-MSCs with the JNK inhibitor SP600125 or ERK inhibitor PD98059 led to a synergistic reduction in ALP expression following the administration of BMP2-specific shRNA (Figure 8c).

**DISCUSSION**

Fucoidan is a vegetal sulfated polysaccharide extracted from brown seaweed that has attracted significant research attention because of its extensive bioactivity.11–13 Recently, a small number of studies have reported the effects of fucoidan osteoblast differentiation in human osteoblast,6 osteoblast-like (MG-63)15 and adipose-derived stem cells.14 However, the effects of fucoidan on osteoblast differentiation in hABM-MSCs and the detailed signaling mechanisms involved in these effects are not well understood. In this study, we focused on the effects of fucoidan on osteoblast differentiation and associated bone formation signaling via MAPKs, Smads and BMP2. We found that fucoidan promoted proliferation and osteoblast differentiation via JNK and ERK signaling in hABM-MSCs. Our data also revealed that fucoidan stimulated osteoblast differentiation through Smad 1/5/8 and BMP2 signaling.

A number of studies have reported conflicting effects of fucoidan on in vitro cell growth. For example, several reports have demonstrated an anti-proliferative effect of fucoidan in several tumor cell lines, including human hepatoma cells20 and lung carcinoma cells.21 In contrast, other studies have reported a proliferative effect of fucoidan in human endothelial cells.22 According to Cho et al., fucoidan does not significantly affect MG-63 cell proliferation.15 Taken together, these findings reveal that the effects of fucoidan on proliferation vary with concentration and with the tested cell line. Our findings indicated that fucoidan enhanced hABM-MSC proliferation in a dose-dependent manner, demonstrating its ability to promote proliferation in hABM-MSCs. Although we did not
explore the exact mechanism through which fucoidan enhances proliferation, this proliferative effect may be explained by the heparin-like properties of this polysaccharide, which include growth factor binding, protection and promotion. 23,24

We also determined whether fucoidan promoted osteoblast differentiation in hABM-MSCs. Changotade et al.24 have demonstrated that low-molecular-weight fucoidan has a synergistic effect on osteoblast differentiation in adipose-derived...
stem cells co-treated with osteoblast reagents. Cho et al. have revealed that fucoidan promotes ALP activity and mineralization, increases OC mRNA expression and increases BMP2 expression in MG-63 cells. In the present study, we confirmed that fucoidan treatment increased ALP activity. Although cell proliferation was increased by fucoidan in a dose-dependent manner, ALP expression and activity were decreased at high doses (5 and 10 μg ml⁻¹). Although this study did not evaluate the reason for the dose-dependent discrepancy between the effects of fucoidan on proliferation and differentiation, this discrepancy may have been caused by complex effects on cellular signaling mechanisms that vary with fucoidan concentration. Further studies are required to clarify the mechanisms underlying the effects of fucoidan on hABM-MSC proliferation.

In addition, we found that fucoidan markedly increased calcium accumulation and the mRNA transcription of genes involved in osteoblast differentiation, such as Runx2, Colla1, OC and ALP. Our findings strongly suggest that fucoidan stimulates osteoblast differentiation in hABM-MSCs.

To gain further insights into the mechanisms by which fucoidan promotes osteoblast differentiation, we first evaluated MAPK signaling because the JNK-ERK-p38 MAPK pathway is known to be involved in osteogenesis. It has been reported that JNK and ERK are involved in osteoblast differentiation induced by fibroblast growth factor. In addition, p38 MAPK is required for osteoblast differentiation, osteogenic marker gene induction and ALP activity. However, there is evidence that osteoblast differentiation is stimulated by activation of ERK and JNK but not by activation of p38 MAPKs. In the present study, we demonstrated that the JNK and ERK MAPK signaling pathways were phosphorylated during fucoidan-induced osteoblast differentiation. Furthermore, we demonstrated that the pretreatment of cells with specific JNK and ERK inhibitors, but not with a specific p38 inhibitor, abolished fucoidan-induced osteoblast differentiation. Taken together, these data indicate that fucoidan-induced osteoblast differentiation is mediated by the JNK and ERK signaling pathways.

In addition, we examined whether MAPK inhibitors abolished the effects of fucoidan on the mRNA expression of osteogenesis-related genes (Runx2, Colla1, OC and ALP). Our results demonstrated that inhibitors of JNK and ERK, but not of p38 MAPK, led to significantly decreased expression of Runx2, Colla1 and ALP. Of particular interest, OC is a late marker of osteoblast differentiation that is closely related to osteoblast maturation and matrix mineralization. Numerous studies have reported that OC expression is regulated by JNK, ERK and/or p38 MAPK. However, we did not observe a significant alteration of OC gene expression in the presence of any of the MAPK inhibitors used. Although we were unable to determine the manner by which OC was stimulated by
fucoidan independent of MAPKs, our observations suggest that it promotes osteoblast differentiation partially through JNK- and ERK-dependent pathways involving Runx2, Col1α1 and ALP transcript expression, as well as through MAPK-independent pathways involving OC transcript expression. We found that fucoidan markedly increased phosphorylation of Smad 1/5/8 and BMP2 mRNA expression. BMPs are known to play an instrumental role in bone formation signaling pathways, and their effects are mediated through Smad signaling. During fucoidan-induced osteoblast differentiation, we found that Smad 1/5/8 phosphorylation was enhanced and BMP2 transcript expression was increased. Furthermore, fucoidan-induced Smad 1/5/8 phosphorylation and BMP2 mRNA expression were reduced by JNK and ERK inhibitors. These results suggest that the fucoidan-induced activation of JNKs and ERKs in osteoblasts is mediated by Smad 1/5/8 and BMP2 signaling. We found that osteoblast differentiation was significantly reduced by BMP2 knockdown. Furthermore, osteoblast differentiation was synergistically inhibited by BMP2 knockdown and the inhibition of JNK and ERK. These results indicate that BMP2 upregulation is important for fucoidan-induced osteoblast differentiation in hABM-MSCs.

In summary, our study demonstrated that fucoidan induced osteoblast differentiation in hABM-MSCs. We propose that fucoidan-induced osteoblast differentiation is mediated by Smad 1/5/8 and BMP2 signaling via the JNK and ERK signaling pathways. Our findings suggest that fucoidan could represent a promising lead in the development of drugs for bone healing and osteoporosis treatment. In addition, further in vivo studies are necessary to identify the pharmacological effects of fucoidan in animal models.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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1. Eker BN. Bone remodeling and balance and the development of osteoporosis. Parodontol Acad Rev 1968; 2: 125–135.

2. Ross PD, Davis JW, Vogel JM, Wanshich RD. A critical review of bone mass and the risk of fractures in osteoporosis. Calcif Tissue Int 1990; 46: 149–161.

3. Yu SF, Yang TS, Chiu WC, Hsu CY, Chou CL, Su YJ et al. Non-adherence to anti-osteoporotic medications in Taiwan: physician specialty makes a difference. J Bone Miner Metab 2013; 31: 351–359.

4. Hughes FJ, Turner W, Belibasakis G, Martuscelli G. Effects of growth factors and cytokines on osteoblast differentiation. Periodontology 2006; 41: 48–72.

5. Panetta NJ, Gupta DM, Longaker MT. Bone regeneration and repair. Curr Stem Cell Res Ther 2010; 5: 122–128.

6. Jang HO, Park YS, Lee JH, Seo JB, Koo KL, Jeong SC et al. Effects of extracts from safflower seeds on osteoblast differentiation and intracellular calcium ion concentration in MC3T3-E1 cells. Nat Prod Res 2007; 21: 787–797.

7. Kim BS, Kim YC, Zadeh H, Park YJ, Pi SH, Shin HS et al. Effects of the dichloromethane fraction of Dipsaci Radix on the osteoblastic differentiation of human alveolar bone marrow mesenchymal stem cells. Boscii Biotechnol Biochem 2011; 75: 13–19.

8. Manton KJ, Leong DF, Cool SM, Nurcombe V. Disruption of heparan sulfate proteoglycans (HSPGs) modulate BMP2 osteogenic activity in C2C12 cells. The J Biol Chem 2007; 282: 1080–1086.

9. Changotade S, Korb G, Bassil J, Banoukou B, Willig C, Colliec-Jouault S et al. Potential effects of a low-molecular-weight fucoidan extracted from brown algae on bone biomaterial osteoconductive properties. J Biomater Res A 2008; 87: 666–675.

10. Hata K, Ikekbe K, Wada M, Nokubi T. Osteoblast response to titanium regulates transcriptional activity of Runx2 through MAPK pathway. J Biomater Res A 2007; 81: 446–451.

11. Xiao G, Jiang D, Thomas P, Benson MD, Guan K, Karsenty G et al. MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbf1. J Biol Chem 2000; 275: 4453–4459.

12. Kim BS, Kim YC, Zadeh H, Park YJ, Pi SH, Shin HS et al. Secretion of osteocalcin and its propeptide from human osteoblastic cells: dissociation of the secretory patterns of osteocalcin and its propeptide. J Bone Miner Res 1993; 8: 553–565.

13. Kondo A, Tokuda H, Matsushima-Nishiihi K, Kuroyanagi M, Yamamoto N, Mizutani J et al. Rho-kinase limits BMP-4-stimulated osteoclast synthesis in osteoblasts: regulation of the p38 MAP kinase pathway. Life Sci 2014; 96: 18–25.

14. Yasuda E, Tokuda H, Ishisaki A, Hirade K, Kann Y, Hanai Y et al. PPAR-gamma ligands up-regulate basic fibroblast growth factor-induced VEGF release through amplifying SAPK/JNK activation in osteoblasts. Biochem Biophys Res Commun 2005; 328: 137–143.

15. Wang H, Harimoto K, Liu J, Guo J, Hinshaw S, Chang Z et al. Spata4 promotes osteoblast differentiation through Erk-activated Runx2 pathway. J Bone Miner Res 2011; 26: 1964–1973.

16. Phimphilai M, Zhao Z, Boules H, Roca H, Franceschi RT. BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype. J Bone Miner Res 2006; 21: 637–646.

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