Fucoidan, a sulfated polysaccharide, inhibits osteoclast differentiation and function in vitro

+1,2Shin-Yoon Kim; 1Mi-Hyung Kim; 1Ki-Young Kim; 1Hyun-Ju Kim; 1Tae-Ho Kim

INTRODUCTION
Adult bone is continuously remodeled through the formation of new bone tissue by osteoblasts and the resorption of old bone tissue by osteoclasts [1]. In general, an imbalance in bone remodeling that is caused by increased bone resorption over bone formation leads to most adult skeletal diseases including osteoporosis and rheumatoid arthritis [2]. Therefore, the enhanced activation of osteoclasts is the target for the therapeutic intervention of pathological bone loss. Current drugs for bone health include bisphosphonates, calcitonin and estrogen, which prevent osteoclastic bone resorption, resulting in the maintenance of bone mass and a reduction of fracture [3, 4]. Hence, growing evidence of the benefits of natural foods for bone health provide an alternative approach for managing osteoporosis. Fucoidan is a sulfated polysaccharide that is primarily extracted from brown seaweeds such as Fucus vesiculosus, Ecklonia kurome and Undaria pinnatifida used as commercial seafood in the East Asian countries. Fucoidan has a variety of biological actions, including anticoagulant, antiinhibitory, antitumor, antiviral, antioxidant and anti-inflammatory effects. Recent studies have shown that fucoidan has beneficial effects on osteoclastic cell differentiation and bone biomaterial osteoconductive properties. In this study, we were conducted to investigate the effect of fucoidan on osteoclast differentiation and function in vitro.

METHODS
Osteoclast cultures: Bone marrow mononuclear (BMM) cells were plated in a 96-well culture plate at a density of 4X10^4 cells/well and cultured in α-MEM containing 10% FBS in the presence of 20 ng/ml RANKL (Receptor activator of nuclear factor kappa-B ligand) and 10 ng/ml M-CSF, with or without fucoidan. Osteoclasts were stained for tartrate-resistant acid phosphatase activity.

Translocation of NF-κB : Mouse BMM cells were seeded at 1x10^5 cells per well into 8-well plate chambered glass slide. Adherent cells were transfected with 1.5 μg of the LigandLink™ pLL-1-NF-κB p65 GFP vector (Active motif, Carlsbad, CA), then stained with fluorescein. BMMs were pretreated with 5 μg/ml fucoidan for 30 min and then incubated with RANKL 20 ng/ml for 30 min further. The distribution of localization of NF-κB GFP fluorescent images was visualized and detected under a LSM5 confocal microscope (Carl Zeiss, Germany).

RESULTS
Fucoidan markedly decreased the RANKL-induced osteoclast differentiation and bone resorptive activity of mature osteoclasts in dose dependent manner. To determine at which stage of differentiation fucoidan is able to inhibit osteoclast differentiation, fucoidan (0.5 μg/ml) was added into the BMM cells already treated with RANKL and M-CSF with a time interval. When fucoidan was added to the cells simultaneously with RANKL and M-CSF, osteoclast formation was completely abolished (Fig. 3). By contrast, treatment with fucoidan on the second or on the third day failed to inhibit formation of TRAP-positive MNCs. The expression of genes that are involved in osteoclast differentiation such as NFATc1, c-Src, DC-STAMP and TRAP was inhibited by fucoidan at differentiation stage. To define the molecular mechanisms by which fucoidan directly inhibits osteoclast differentiation, the activity of MAP kinases was examined. Fucoidan strongly inhibited the RANKL-induced phosphorylation of ERK, p38 and JNK in mouse BMMs (Fig. 5A). We then examined the effects of fucoidan on RANKL-induced phosphorylation of NF-κB and degradation of IκB. Confocal microscopy showed that GFP-p65 is expressed diffusely in the cytoplasm of unstimulated osteoclasts. Redistribution of GFP-p65 into the nuclei was observed after RANKL treatment. Pretreatment with 5 μg/ml of fucoidan before RANKL stimulation completely prevented GFP-p65 translocation (Fig 5D). These results suggest that fucoidan inhibited the RANKL-induced activation of p38MAPK, JNK, ERK, and NF-κB, and thereby suppressed osteoclast differentiation.

DISCUSSION
It is well established that RANKL/RANK plays a central role in regulating both osteoclast differentiation and function. Down regulation of RANKL expression or its downstream signals may be a valuable approach to the treatment of pathological bone loss. Blocking the RANKL and/or NF-κB signaling pathway could prevent bone loss. In this study, we found that fucoidan reduced the RANKL-induced phosphorylation of p38MAPK, ERK, and JNK. Thus, Fucoidan may target signaling pathways that lead to the activation of MAP kinases, such as p38MAPK, ERK, and JNK. Fucoidan also inhibited the transactivation of NF-κB by RANKL. Therefore, we determined that fucoidan inhibits both MAP kinases and NF-κB, and thereby suppresses osteoclast differentiation and function.

In conclusion, the results of the present study demonstrated that fucoidan had an anti-osteoclastogenic effect in vitro by inhibiting MAP kinase and NF-κB, and down-regulating the expression of genes that were involved in both osteoclast differentiation and function. Therefore, we suggest that fucoidan might be useful for the prevention or treatment of various disorders related to bone loss.

REFERENCES

ACKNOWLEDGEMENTS
This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (Project No.: A010252).

Figure 3. Fucoidan targets at the early stage of the RANKL-induce osteoclast formation in mouse BMM cells.

Figure 5. Fucoidan down-regulates the MAP kinase signalin pathway in BMMs and inhibits osteoclastic differentiation via suppression of NF-κB transcriptive activity and translocation.