INTRODUCTION:

Osteoporosis is a reduction in skeletal mass due to an imbalance between bone resorption and bone formation. Bone morphogenetic protein (BMP) plays important roles in osteoblastic differentiation and bone formation. Therefore, components involved in BMP activation are good targets for the development of anti-osteoporosis drugs. In this study, naringin, a polymethoxylated flavonoid, was shown to enhance alkaline phosphatase activity, osteocalcin synthesis, and cell proliferation in primary cultured osteoblasts. Naringin increased mRNA and protein levels of BMP-2 using Western blot, ELISA and RT-PCR assay. The phosphatidylinositol 3-kinase (PI3K), Akt, c-Fos/c-Jun and activator protein-1 (AP-1) -dependent pathways may be involved in the increase of BMP-2 expression and osteogenic response by naringin.

METHODS:

Cell culture: The murine osteoblast cell line MC3T3-E1 was purchased from American Type Culture Collection. Cells were cultured in MEM supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). The conditionally immortalized human fetal osteoblastic cell line (hOB; CRL-11372) was maintained in a 1:1 mixture of DMEM/Ham's F-12 medium containing 10% FBS supplemented with Geneticin (300 µg/ml) and antibiotics at 37°C.

RESULTS:

To examine the effects of naringin on proliferation and differentiation of osteoblastic cells, MC3T3-E1, hOB and murine primary cultured osteoblast cells (pOB cells) were treated with various concentrations of naringin, and the fraction of cells in S-phase was determined. Treatment with naringin significantly increased the proliferation of MC3T3-E1 and MG63 cells, closely matching the increase observed in pOB cells cultured under identical conditions (Fig. 1A). In addition, naringin also induced the cells proliferation by using BrdU kit (Fig. 1B). Differentiated osteoblasts exhibit elevated ALP activity, which is correlated with high levels of enzyme expression. We then investigated the effects of naringin on the ALP activity of osteoblasts. Treatment of osteoblasts with naringin for 72 h significantly increased ALP activity (Fig. 1C). Expression of the osteoblast differentiation markers, osteocalcin and osteopontin, was measured by RT-PCR and ELISA assay. Naringin increased expression of both osteocalcin and osteopontin at the mRNA and protein levels in all three cells (Fig. 1D&E). To explore the anti-apoptotic effects of naringin in osteoblasts, osteoblasts were subjected to pro-apoptotic stimuli in the absence or presence of naringin. The naringin prevented induction of apoptosis in osteoblast in response to etoposide or TNF (Fig. 1F).

The expression of mRNAs for the BMPs in response to naringin was analyzed by RT-PCR. Treatment of pOB cells with naringin induced the mRNA expression of BMP-2 but not other BMPs (Fig. 2A). The RT-PCR findings therefore support the findings from the cDNA microarray analysis. The induction of BMP-2 expression in response to naringin in pOB cells was also observed using quantitative real-time RT-PCR analysis (Fig. 2B). On the other hand, treatment with naringin also increased BMP-2 protein expression in a time- and dose-dependent manner as determined by Western blotting and BMP-2 ELISA assay, respectively (Fig. 2C&D). On the other hand, naringin also decreased the mRNA expression of BMP-3 by using quantitative real-time RT-PCR (Fig. 2E). To confirm the hypothesis that induction of BMP-2 is required for naringin-induced osteogenic function, BMP-2 signaling in osteoblasts was blocked with a BMP-2-specific neutralizing antibody or with the antagonist, noggin. Treatment with BMP-2-specific neutralizing antibody, but not IgG control antibody, decreased naringin-induced ALP activity in MC3T3-E1 cells as well as in pOB cells (Fig. 2F). In addition, treatment of osteoblast cells with noggin also antagonized the naringin-induced ALP activity (Fig. 2F). On the other hand, treatment with either BMP-2-specific neutralizing antibody or noggin abolished naringin-mediated anti-apoptotic activity in osteoblastic cells (Fig. 2G).

DISCUSSION:

The present study demonstrated that naringin induce osteoblast proliferation, differentiation and maturation in cultured osteoblasts. Naringin also increased BMP-2 expression via PI3K, Akt, c-Fos/c-Jun and AP-1-dependent pathways. Naringin prevented the bone loss inducing by ovariectomized. Therefore, naringin may be beneficial in stimulating expression of BMP-2 and inducing the bone formation in osteoporosis diseases.