INTRODUCTION
Genistein, one of the primary isoflavones in soybeans, exhibits a multitude of biological effects that influence cell growth and regulation. It has attracted much attention not only because of its potential estrogen-related effects, but also because several of its key enzymes are thought to be involved in cell proliferation. Recently, Anderson et al. reported that isoflavones in soybeans might also directly inhibit bone resorption. This was followed by a report from Ishida et al. on the preventive effects of the genistein on bone loss in ovariectomized rats fed a calcium-deficient diet. Reports on the health benefits of soy isoflavones in prevention of osteoporosis have also been made by Banes et al. and Brandi et al. However, the direct effects of isoflavones on osteoblastic cells have not been clarified. In this study, we examined the effects of genistein on cell proliferation, the cell cycle, and cell differentiation in MC3T3-E1 osteoblastic cells.

MATERIALS and METHODS
A mouse calvaria-derived osteoblast cell line, MC3T3-E1, was grown at 37°C in Dulbecco’s modified Eagle medium (DMEM, Gibco, Grand Island, NY, U.S.A.) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, U.S.A.), 100 U/ml penicillin and 100 mg/ml streptomycin under a humidified 7% CO2 atmosphere.

Genistein (FUNAKOSHI Co., Tokyo, Japan), an isoflavone, was dissolved in dimethyl sulfoxide (DMSO) and included at various concentrations in the medium. The medium with or without genistein was changed every 3 days.

Cells were plated at a concentration of 5 x 104 cells /4 ml medium in 60-mm diameter wells. Samples were harvested from the cultures on days 1, 2, 4, and 8, and assayed.

1) DNA synthesis was measured by [3H]-thymidine incorporation.
2) Cell cycles were analyzed with FACSscan (Becton Dickinson Immunocytometry Systems, Mountain View, CA, U.S.A.).
3) ALP activity was measured by modified Lawry methods.
4) Total RNA was isolated from the cultures on days 1, 2, 4, and 8 with the aid of an RNA extraction kit (ISOGEN, Nippon Gene, Tokyo, Japan). Ten-microgram aliquots of total RNA from cultures obtained at various time-points were used for Northern blot analysis.

Values are expressed as the mean±standard deviation (S.D.) for three culture dishes. Significant differences between samples were assessed by analysis of variance (ANOVA). P values of less than 0.05 were considered to be statistically significant.

RESULTS
Effects of Genistein on DNA synthesis
MC3T3-E1 cells treated with 40 µM genistein dramatically decreased to 1.9% of the control culture. This inhibition of DNA synthesis was observed throughout the culture period. The measurement at 48 hours indicated that DNA synthesis was inhibited by genistein in a dose-dependent.

Effect of genistein on cell cycle progression
Genistein predominantly increased the G2/M phase population in a dose-dependent manner while it decreased the percentage of G1 cells. In the time course of alterations in the cell cycle, the percentage of G2/M cells began increasing at 24 hours and then were observed to increase rapidly until the end of the measurement period at 192 hours.

Effects of Genistein on ALP activity
ALP activity corrected for protein content increased in the presence of genistein as compared to that of the control culture in the absence of genistein.

Effects of Genistein on the expression of osteocalcin and estrogen receptor genes
Expression of the osteocalcin gene was low in untreated cells, however in treated cells gene expression increased dose-dependently (Figure 1) and time-dependently up to 4.5-fold (genistein 40 µM) (Figure 2). In the same way, estrogen receptor gene expression showed a gradual increase in a dose-dependent (Figure 1) and time-dependent manner up to 5.5-fold (genistein 40 µM) (Figure 2).

DISCUSSION
Our results indicate that genistein not only induces osteoblastic differentiation following G2/M arrest, but also activates expression of the estrogen receptor gene in osteoblast-like MC3T3-E1 cells. Genistein may have important roles in cell cycle regulation and cellular differentiation through the activation of estrogen receptor expression. Moreover, genistein may become a key therapeutic agent that prevents chronic disease including heart disease, renal disease, cancer, and osteoporosis. Additional studies will be required to elucidate the nature of this flavonoid’s biological targets and mechanisms of action that leads to cellular differentiation following G2/M arrest and apoptosis in osteoblastic cells.

REFERENCES

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