Estrogen receptor and GRP30 contribute to simvastatin-stimulated osteogenic effects on murine bone marrow mesenchymal stem cells

INTRODUCTION
Estrogen plays important physiological processes in bone formation. Besides of the classical nuclear estrogen receptor α (ERα), the G protein-couple receptor (GPR30) activates cellular signaling pathways in response to estrogen. In our previous study, we clarified the simvastatin-stimulated osteogenesis was related to estrogen receptor by using antagonist ICI 182.780 (ICI). However, the ICI is both the antagonist of ERα and agonist of GPR30. Therefore, in this study, we want to clarify the roles of ERα and GPR30 on osteogenesis. The ERα specific antagonist MPP, the GPR30 specific agonist and antagonist, G1 and G15, were used respectively. Murine bone marrow mesenchymal stem cells are used to examine the function of COX-2 on osteogenic-differentiation.

METHODS
Murine bone marrow mesenchymal stem cells (D1 cell) were used in this study. Simvastatin was used to stimulate D1 osteogenesis for 5 days and then changed into osteo-induction medium for 5 days. The classical and specific ERα antagonist, ICI 182.780 (ICI) and MPP, and the specific GPR30 agonist and antagonist, G1 and G15, were used to affect the estrogen receptors after simvastatin treatment. The ERα and GPR30 genes expression were measures by real-time PCR. Mineralization was tested by Alizarin red S staining. Significant differences were tested by using ANOVA. The mean of different treatment groups was tested using Duncan’s new multiple-range test. A p value < 0.05 was taken as significant.

RESULTS
After treated with simvastatin, the mineralization in D1 cell was increased and had dosage dependent effect. On the other hand, ICI inhibited the mineralization (6, p<0.05 compared to the same simvastatin concentration) (Fig.1). The ERα gene expression was not significantly changed, but the GPR30 was decreased in osteo-induction medium (p<0.05) (Fig.2). After MPP treatment, the simvastatin-stimulated mineralization of D1 cells was attenuated by using MPP 5uM (p<0.05) (Fig.3). The simvastatin-stimulated mineralization was not affected after G15 treatment (Fig.4), but the effect of simvastatin-stimulated mineralization of D1 cells was suppressed when treated with G1 (Fig. 5).

DISCUSSION
According to our previous study, we clarified the simvastatin-stimulated osteogenesis was related to estrogen receptor by ICI treatment. However, the ICI is both the antagonist of ERα and the agonist of GPR30. Therefore, it is necessary to further clarify the role of ERα and GPR30 on osteogenesis. According to our data, the simvastatin-stimulated mineralization was decreased by MPP treatment, but not affected by G15 treatment. It meant that ERα, but not GPR30, might contribute simvastatin-stimulated osteogenesis. However, the contribution might be mediated through the ERα function, not the gene expression, because the gene expression of ERα was not affected.

On the other hand, the gene expressions of GPR30 were decreased in D1 cell during osteogenesis induction, and the enhancement of GPR30 by G1 treatment suppressed the osteogenesis of D1 cells. Therefore, GPR30 might also play some roles in regulating the osteogenic differentiation of mesenchymal stem cells in coordinating with ERα. In the future, the GPR30 downstream gene and protein expression were needed to evaluate to confirm the function of GPR30 on osteogenesis.

Significance
The results showed the roles of estrogen receptor, ERα and GPR30, on simvastatin-stimulated osteogenesis on murine bone marrow mesenchymal stem cell osteo-differentiation. It provides new information and basic medicine science in bone physiology.