Estrogen Receptor Antagonist Reverses the osteogenic effects of Simvastatin on Mouse Mesenchymal Stem Cell

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INTRODUCTION
Statins has been shown to stimulate osteogenesis in both in vivo and in vitro. Our previous studies showed that statin could prevent estrogen-deficient osteopenia and increase osteogenic protein in bones of ovariectomized (OVX) rats. Estrogen also stimulates the bone formation in osteoblasts. A recent study indicted the statin effect may through estrogen receptor α (ESR1), but the mechanism have not been clarified clearly. In addition, our microarray data in human bone marrow mesenchymal stem cells (hBMSCs) indicated that the statins induced osteogenesis was not only involved in the well-known BMP-2 pathway but also in enhancing ER-α gene expression. Therefore, we used estrogen receptor α antagonist ICI 182.780 (ICI) to confirm if the statins could enhance osteogenesis through estrogen receptor (ESR) signaling pathway.

METHODS
Mouse bone marrow mesenchymal stem cells (D1 cell) was used in this study. Simvastatin was used to stimulate D1 osteogenesis for 5 days and then changed into osteo-induction medium for 7 days. ESR antagonist ICI 180.782 (ICI) was used to block the function of ESR1. The cell viability was tested by MTT assay. Then, the alkaline phosphate activity (ALP) activity, ESR1 gene expression and the osteogenic marker genes such as BMP-2 and osteocalcin (OC) were used to test the osteogenesis. Mineralization was tested by Alizarin red 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
In our microarray bioinformatics and matacore analysis in hBMSCs, Estrogen receptor α (ESR1) had the higher expression after simvastatin treatment (Fig. 1). The optima simvastatin concentrations in D1 cells are 0.05-0.2 μM because of the cell viability and ALP activity (Fig. 2a and 2b). Simvastatin treatment on D1 cells showed the increased ALP activity in day 5 (Fig. 2b, p<0.05) and increased BMP-2 and OC gene expression after 1 and 2 days (p<0.05, Fig 2c-d). The ER-α gene expression also increased after 2 days (Fig. 2e, p<0.05). Moreover, after treated simvastatin, the ALP activity were blocked by ESR antagonist, ICI (Fig. 3). Besides, The D1 cells show the better mineralization after 5 days in osteo-induction medium by treated simvastatin (p<0.05) but inhibited by ICI (P<0.05) (Fig. 4).

DISCUSSION
According to our microarray data in hBMSCs indicated that the statins induced osteogenesis was not only involved in well-known BMP-2 pathway but also in ER-α gene expression. To clarify the signaling pathway of the statins and estrogen receptor involved osteogenesis, our in-vitro study results suggest that simvastatin-induced osteogenesis in D1 cells, at least in part, by induction of ESR1 because ICI can reverse the function of simvastatin enhanced the ALP activity and mineralization in D1 cells. Besides, simvastatin can promote the ESR1 gene expression. ESR1 is well-known to induce BMP-2 production that promotes osteogenesis. Therefore, these results provide a new insight in the mechanisms of simvastatin-induced bone formation in bone marrow stem cells. In the future, we will evaluate the ESR1 downstream gene and protein expression to confirm the ER-α signaling pathway.