Estrogen Receptor contributed to simvastatin-stimulated osteogenic effects on osteogenic lineage cells

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INTRODUCTION
Simvastatin, a hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is known to inhibit cholesterol biosynthesis and recent studies have reported that simvastatin stimulated bone formation in vitro and in vivo. Simvastatin act on the mevalonate pathway in osteogenic lineage cells and enhance the expression of the bone morphogenetic protein-2 (BMP-2), which is an important growth factor for osteoblast differentiation. Estrogen also stimulates the bone formation in osteoblasts and bone marrow mesenchymal stem cells (hBMSCs). A Recent study indicted the statin effect may through estrogen receptor α (ESR1), but the mechanism have not been clarified clearly. In our previous study, we clarified the simvastatin-stimulated osteogenesis was related to ESR1, but the detail mechanism were not investigated clearly. Besides, how simvastatin acts on ESR1 doesn’t understand. Therefore, the purposes of this study were (1) to know if simvastatin can act through ESR1 directly; (2) to know how simvastatin effects on osteogenic gene expression and osteogenesis. Besides, we also detected the effect of simvastatin combined with E2. We used estrogen receptor α antagonist ICI 182.780 (ICI) and luciferase reporter gene assay to confirm if the simvastatins could act through ERS1 to enhance osteogenesis.

METHODS
Mouse bone marrow mesenchymal stem cells (D1 cell), MG63 and C2C12 cells were used in this study. In lucificase reporter assay, human estrogen receptor vector (pHE0) and estrogen response element reporter vector (pERE-Luc) were co-transfected to MG63 and C2C12 to detect if the simvastatin can react on estrogen receptor directly. Simvastatin and E2 was used to stimulate D1 osteogenesis for 5 days and then changed into osteo-induction medium for 5 days. ESR1 antagonist ICI 180.782 (ICI) was used to block the function of ESR1. The osteogenic marker genes such as BMP-2, osteocalcin (OC) and ALP were measures by real-time PCR. The alkaline phosphate activity (ALP) was measured by luciferase reporter gene expression through estrogen receptor pathway. These results showed that simvastatin can activate luciferase gene in C2C12 and MG63 when co-transfect pHE0 and pERE-Luc vector, but the reaction was reversed by using ICI. It meant that simvastatin can bind to ESR1 directly or be a co-activator in ESR1 signaling pathway. On the other hand, 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. Besides, simvastatin can promote the osteogenic gene (BMP-2, OC, ALP and OPG) expression. It also can be reversed by treating ICI. Therefore, simvastatin also influenced the gene expression through estrogen receptor pathway. These results showed that simvastatin-induced osteogenesis in D1 cell occurs via an ESR1-dependent pathway. It provided 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.

RESULTS
In lucificase reporter gene assay, pERE-Luc vector express was increased after treated simvastatin for 24 hr in MG63 and C2C12 cells, but the ICI inhibited the pERE-Luc vector expression. Besides, simvastatin combined with E2 can promote the pERE-Luc vector expression than simvastatin alone and it was also inhibited by ICI (Fig. 1). After treated simvastatin, the ALP activity stain and mineralization in D1 cell were increased and had dosage dependent effect (*, p<0.05). On the other hand, ICI inhibited the ALP activity stain and mineralization (#, p<0.05 compared to the same simvastatin concentration) (Fig. 2). Besides, simvastatin combined with E2 can promote osteogenic differentiation than simvastatin alone. It was also inhibited by ICI (Fig. 3). The expression of BMP-2, osteocalcin, ALP and Osteoprotegerin (OPG) were increased after treated simvastatin (*, p<0.05, Fig 4a-d). However, when simvastatin treated with ICI, the gene expressions were not significant change.

Discussion
According to our previous study, it indicted the statin effect may through estrogen receptor α (ESR1), but the detail mechanism were not investigated clearly. Besides, how simvastatin acts on ESR1 doesn’t understand. According to our results, simvastatin can activate lucificase gene in C2C12 and MG63 when co-transfect pHE0 and pERE-Luc vector, but the reaction was reversed by using ICI. It meant that simvastatin can bind to ESR1 directly or be a co-activator in ESR1 signaling pathway. On the other hand, 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. Besides, simvastatin can promote the osteogenic gene (BMP-2, OC, ALP and OPG) expression. It also can be reversed by treating ICI. Therefore, simvastatin also influenced the gene expression through estrogen receptor pathway. These results showed that simvastatin-induced osteogenesis in D1 cell occurs via an ESR1-dependent pathway. It provided 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.

Reference