Estrogen Inhibits Calcification of VSMCs via Promotion of Autophagy

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Background: Medial artery calcification is an active cell-regulated process that involves the differentiation of vascular smooth muscle cells (VSMCs) to an osteoblast-like phenotype. Autophagy is a highly regulated homoeostatic process involved in the lysosomal degradation of damaged cell organelles and proteins, which considered an important pro-survival mechanism under diverse stress conditions. Arterial calcification and osteoporosis are both associated in postmenopausal women. Estrogen is a key therapeutic method for postmenopausal osteoporosis and is contribute to decrease the coronary-artery calcium and can promote the autophagy of endothelial cell and VSMC via ER–ERK–mTOR signaling pathway. But autophagy, as well as the contribution of estrogen to vascular calcification, is poorly understood.

Objective: To clarify the effect and the mechanism involved in estrogen on autophagy and calcification of VSMCs.

Methods: Primary mouse VSMCs were treated with β glycerophosphate to induce calcification. Estrogen was incubated with VSMCs during osteoblastic differentiation. Inhibitors to the following estrogen receptors were used: estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), combined ERα and ERβ (ERαβ). 3MA or knock down ATG5 were selected to block autophagy, rapamycin (pmTOR) was used to promote autophagy, GFP-LC3 redistribution, expression of LC3I/II and ATG5 detected by western blot were employed to determine the presence of autophagy analysis in VSMCs.

Results: 1. Estrogen reduced the expression of RUNX2 of VSMCs mainly through ERα but not ERβ in the osteogenic medium; 2. Estrogen enhanced the autophagy of VSMCs during the process of osteogenic differentiation: Estrogen increased the level of the lipid-conjugate form of the autophagosome marker light-chain 3-II (LC3II) and ATG5 in VSMCs. Treatment with rapamycin, an inducer of autophagy, Estrogen further increased the LC3II and ATG5 level. Estrogen also increased the number of LC3 puncta tagged with green fluorescent protein (GFP); Electron microscopy of typical autophagic structures were increased in VSMCs treated with estrogen. 3. Estrogen inhibited the calcification of VSMC via enhancing autophagy. Inhibition of autophagy with 3MA or knocking down ATG5, the expression of RUNX2 was increased compared with those only treated with estrogen. Furthermore, the expression of runx2 was further decreased in the VSMCs treated with estrogen and rapamycin together.

Conclusion: Estrogen inhibits calcification of VSMC via promotion of autophagy through the ERα–mTOR signaling pathway.

Antiosteoporotic Activity of Dihydrophaseic Acid 3'-O-b-D-Gluco-pyranoside Derivatives from Lycii Radicis Cortex

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Objective: Our previous study demonstrated that ethanol extract of Lycii Radicis Cortex (LRC) prevented the ovariectomy-induced bone mineral density loss in mice via promoting the differentiation of osteoblast lineage cells. A single compound, D3G, was isolated from the LRC extract as a candidate component enhancing bone formation. The aim of this study was to investigate the antiosteoporotic effects of dihydrophaseic acid 3'-O-b-D-glucopyranoside (D3G).

Methods: The 70% ethanol extract of LRC was evaporated, suspended in H2O, and then partitioned successively with CH2Cl2, EtOAc, n-BuOH, and aqueous fractions. Fractionation of the LRC extract was performed to identify the bioactive compound(s) responsible for the bone formation-enhancing effect of LRC extract. The structure of the isolated single compound was elucidated by 1H-NMR, 13C-NMR, and mass spectrometry analyses. To confirm the bioactivity of the isolated single compound, three different concentrations (1, 5, and 10 µg/ml) of the compound were treated in the osteoblastic cell lines C3H10T1/2 and MC3T3-E1, and alterations in the ALP activity of the cells were examined

Results: The highest alkaline phosphatase activity was observed with 5 µg/ml of D3G in both cell lines: C3H10T1/2 and MC3T3-E1. The D3G treatment for 21 days increased the mineralized nodule formation in the MC3T3-E1 cell line. The expression of osteoblastic markers, Alpl, Runx2, and Bglap, was significantly increased in the