Introduction: Ethanol and glucocorticoid are risk factors associated with osteonecrosis (ON). Previous reports suggest ethanol and glucocorticoid induces adipogenesis, decreases osteogenesis in bone marrow stromal cells, and produces intracellular lipid deposits resulting in death of osteocytes. Wnt/β-catenin signaling pathway is involved in the regulation of homeostasis of bone. In this study, we propose that ethanol and glucocorticoid may induce ON in human via a similar mechanism related to Wnt/β-catenin signaling pathway. We have three hypotheses. Firstly, HMCs from human ilium retains the characters of mesenchymal stem cells. Second, ethanol, like glucocorticoid, decreases osteogenesis and increases adipogenesis through the regulation of Wnt/β-catenin signaling pathway on HMCs. Thirdly, ethanol decreases intranuclear translocation of β-catenin. The novel observations of this study provide a possible mechanism of ethanol-induced ON through Wnt/β-catenin signaling pathway.

Materials and Methods: Bone marrow fluid was aspirated from iliac crest of the patients undergoing hip surgery. 5 ml of bone marrow fluid were used for Percoll separation and the nucleated stroma cells were isolated for culture. Pooled bone marrow stroma cells were cultured in Dulbecco's Modified Eagle's Medium, check stem cell characteristic by cell surface markers. Cells from the third passage of the cultures were used for experiments. The cells were then treated with ethanol, 10 and 30 μmol/L for 3 days. The mRNA expression of adipogenic genes (PPAR γ, AP2, adipsin) and osteogenic genes (BMP2 and osteocalcin) were evaluated by real-time RT-PCR. The protein expressions of β-catenin and PPAR gamma were obtained by western blotting. Immunohistochemistry was used to investigate the nuclear translocation of β-catenin.

Results: The HMCs showed extensive mineralization in the plate after 2-week osteogenic induction, sulfated proteoglycans using alcian blue staining after 2-week chondrogenic induction and multiple lipid vacuoles by Oil red O staining after adipogenic induction for 2 weeks (Fig. 1). The surface protein expression of HMCs were characterized, the positive staining was defined as fluorescent intensity greater than 99% of that obtained with the isotype-matched control antibody. The surface markers were compatible with those of mesenchymal stem cells (Fig. 2). Ethanol decresed the mRNA expression of osteogenic genes, including BMP2, Runx2, and osteocalcin. On the contrary, the mRNA expression of PPARγ and adipsin was up-regulated after the treatment of ethanol, besides, ethanol suppressed β-catenin expression and increased PPARγ expression in a dose-dependent manner similar to mRNA expression with Western blot analysis (Table 1). The ethanol suppressed β-catenin expression and increased PPARγ expression in a dose-dependent manner similar to mRNA expression by Western blot analysis (Fig. 3). In addition, immunofluorescence studies showed reduced levels of nuclear β-catenin after ethanol treatment (Fig. 4).

Discussion: We propose that ethanol and glucocorticoid may induce ON in human via a similar mechanism, Gaur et al. reported canonic Wnt signaling promoted osteogenesis by directly stimulating Runx2 gene expression. We found ethanol activated the mRNA expression of adipogenic genes, including adipin and PPARγ. Our observation indicated positive relationship between ethanol-induced adipogenic differentiation in HMCs and the inhibition of BMP2, Runx2, and osteocalcin that could partly attenuate osteogenic differentiation. Thus, ethanol causes HMCs toward adipogenic differentiation rather osteogenic differentiation. This may be the novel possible mechanisms of ethanol-induced ON. Wang et al. reported death of osteocytes in alcohol-induced ON[1]. Calder et al. reported steroid-induced and alcohol-induced ON was accompanied by widespread apoptosis of osteoblasts and osteocytes[2]. Previous report also indicated that β-catenin modulate cell proliferation and survival. In this study, we found ethanol decreased the quantity of β-catenin and hampered the intra-nuclear translocation of β-catenin in HMCs. It is worthwhile to delineate the relationship between β-catenin and alcohol-induced apoptosis. HMCs have the ability to differentiate into osteoblasts, chondrocytes and adipocytes thru proper induction. Both dexamethasone and ethanol decreased the osteogenic gene and protein expression and increased adipogenesis via Wnt signaling related genes. Ethanol also hampered intranuclear translocation of β-catenin as evidence by immunofluorescence analysis. The results have provided a novel possible mechanism of ethanol-induced ON through Wnt/β-catenin signaling pathway.