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EXPERIMENTAL STUDY

Wnt/β-catenin signaling is involved in the Icariin induced proliferation of bone marrow mesenchymal stem cells

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Abstract

OBJECTIVE: To investigate the effect of icariin on proliferation of bone marrow mesenchymal stem cells (BMSCs) in Sprague-Dawley (SD) rats.

METHODS: BMSCs were obtained from SD rat bone marrow with differential time adherent method. Its characteristic was identified through differentiation cell surface antigens and the multi-lineage (osteo/adipo/chondo) differentiation potential. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and 5-Bromo-2-Deoxyuridine (BrdU) incorporation were applied to detect the effect of icariin on BMSCs proliferation. Flow cytometry was used to detect proliferation index of BMSCs. The mRNA level and the distribution of β-catenin were evaluated by Real-time Polymerase Chain Reaction (PCR) and Immunofluorescent staining respectively. Western blot was used to detect protein expression levels of β-catenin, glycogen synthase kinase-3 beta (GSK-3β), phospho-glycogen synthase kinase-3 beta (pGSK-3β) and cyclinD1.

RESULTS: Icariin promoted BMSCs proliferation at the concentration of 0.05-2.0 mg/L. The percentage of BrdU positive cells of BMSCs was increased from 40.98% to 70.42%, and the proliferation index value was increased from 8.9% to 17.5% with the treatment of 0.05 mg/L icariin, which significance values were both less than 0.05. Compared with the control group, total and nuclear β-catenin proteins, as well as β-catenin mRNA expression, were all increased with icariin treatment. Meanwhile, the phosphorylation level of GSK-3β and cyclinD1 protein expressions were also increased in BMSCs with icariin treatment.

CONCLUSION: The findings of the present study demonstrated that low dosage of icariin could promote BMSCs proliferation. The activation of Wnt/β-catenin pathways was involved in this process.

Key words: Icariin; Mesenchymal stromal cells; Wnt signaling pathway; Cell proliferation

INTRODUCTION

Bone marrow mesenchymal stem cells (BMSCs) are characterized by their self-renewal and multi-lineage

differentiation potential, which can differentiate into osteoblasts,[1] chondrocytes,[2,3] adipocytes,[4] endothelial cells and neuronal-like cells.[5] As the precursor cells of osteoblast, BMSCs not only regulate normal skeletal homeostasis, but also play an important role in fracture repair.[6] Although the role of BMSCs in the maintenance and repair of bone has been recognized, the influential factors and underlying mechanism has not been elucidated well yet.

The wingless-related mouse mammary tumor virus (MMTV) integration site (Wnt) family contains cysteine-rich secretory glycoproteins. Wnt proteins are involved in a variety of cellular processes including the growth, differentiation and survival of cells, through modulating target gene transcription by the canonical or non-canonical Wnt pathway. The canonical Wnt pathway is also known as the Wnt/β-catenin signaling pathway, which mainly activates the expression of target genes in the nucleus. In the absence of Wnt signaling, the destruction complex, which contains scaffolding protein, Axin and adenomatous polyposis coli protein (APC), mediates phosphorylation of beta-catenin by sequential recruitment of glycogen synthetase (GSK3Beta) and casein kinase 1 (CK). Phosphorylated β-catenin is recognized by the Skp1/Cul1/F-box (SCF) complex and ubiquitinated for degradation by the proteasome. Thus, cytoplasm β-catenin protein levels are kept low in the absence of Wnt signaling. When Wnt proteins such as Wnt3a bind to their receptor, low-density lipoprotein receptor-related protein 5/6 (LRP5/6) and Frizzled, a series of downstream cascade reactions are activated. The Disheveled protein (a compound of Wnt signaling pathway) and GBP [the glycogen synthase kinase-3 beta (GSK-3β) inhibition protein] are activated; the degradation of β-catenin is inhibited and the accumulation of intracellular β-catenin is increased by the phosphorylation of GSK-3β. Stabilized β-cateninthen translocate into nucleus to activate RNA transcription of downstream target genes by binding to lymphoid enhancer factor-1 (LEF1)/T-cell factor (TCF) and recruiting coactivators.[7,8] Additionally, some Wnt proteins such as Wnt5a and Wnt11 exert their biological functions by non-canonical Wnt signaling pathway, which would not induce the accumulation of β-catenin.[9,10] There have been a lot of studies indicating that MSCs expressed a number of Wnt ligands (Wnt2, Wnt 4, Wnt5a, Wnt11 and Wnt16) and Wnt receptors,[11] extensive researches also demonstrated that the activation of Wnt signaling result in a high bone mass phenotype in the human and murine, in which bone biopsies show increased bone volume and decreased fat within the marrow, and β-catenin is required for postnatal bone maintenance.[12-14] However, *in vitro* studies of the role of Wnt signaling in the osteoblast-differentiation of MSCs is inconclusive. On the one hand, studies showed that Wnt signaling promoted osteoblastogenesis of MSCs by increasing the transactivation ability of Runx2;[15,16] on the other hand, some researches indicated that Wnt signaling has an inhibitory effect on osteogenic and adipogenic differentiation in human MSCs, high levels of β-catenin signaling reduced osteogenic differentiation of stem cells.[17,18] Meanwhile, activation of Wnt signaling is found to be involved in the self-renewal and proliferation processes of some types of stem cells, such as haemopoietic stem cells,[19] neural stem cells[20] and BMSCs,[21,22] these effects are related to the up-regulation of cylcinD1 and decreases of cell apoptosis.[23] Suppression of β-catenin/TCF signaling leads to a decline of cyclin D1 and arrests BMSCs to re-enter into cell cycle.However, the overexpression of LRP5 can increase BMSC proliferation significantly.[12,24] All these results showed that Wnt signaling played a vital role in MSCs proliferation.

Icariin (the structure is shown in Figure 1) is a flavonoid isolated from Epimedium pubescens, which is a Chinese herb that is found to promote bone density and lumbar bone mineral density by increasing the mineral content.[25,26] As the active compound of pubescens, icariin was reported to have a definite antiosteoporotic effect on estrogen deficiency-induced bone fracture.[27] It also exert potent osteogenic effects through inducing expression of runt-related transcription factor 2 (Runx2) and activating bone morphogenetic protein (BMP) signaling in pre-osteoblastic mouse obsteoblastic MC3T3-E1 cells and mouse primary osteoblasts.[28-30] Our research group carried out a series of studies on Chinese herbs that are beneficial to bone metabolism. We found that the formula containing Epimedium pubescens and the single icariin promoted the proliferation of osteoblast and induced their differentiation by increasing the activity of alkaline phosphatase (ALP), collagen I and osteocalcin. These findings indicated that Icarrin might regulate the proliferation and osteogenic differentiation activity of BMSCs. However, the underlying mechanism remains unclear. In this study, the effect of icariin on rat BMSCs proliferation was investigated and its potential mechanism was addressed.

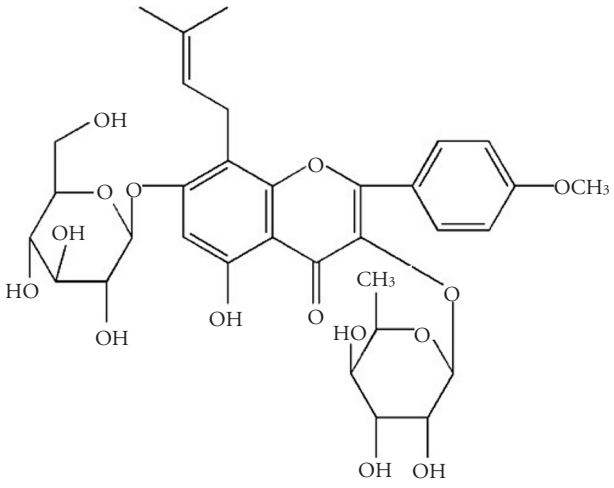

Figure 1 Chemical structure of icariin