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BASIC RESEARCH

Effects of Angelica Sinensis Extract on Differentiation of Rat Bone Marrow Mesenchymal Stem Cells

Shimao Yang
Jinan Stomatology Hospital

Abstract

The present study evaluated the effect of Angelica sinensis extract (AS extract) on differentiation of rat bone marrow mesenchymal stem cells (BMSCs). The results revealed that AS extract promoted the alkaline phosphatase (ALP) activity and calcium content of rat BMSCs in a dose-dependent manner in the range 20-40mg/L. This result was further confirmed by the upregulation of osteogenic marker genes, runt-related transcription factor 2 (Runx2) and osteocalcin (OCN). The decreased expression of peroxisome proliferator activated receptor γ2 （PPARγ2） and lipoprotein lipase (LPL) suggested that AS extract also inhibited adipogenic differentiation of rat BMSCs. Further study demonstrated that the transcriptional levels of the phosphorylation of GSK3β in the Wnt/β-catenin signaling pathway increased significantly during AS extract induced osteogenesis. Dickkopf 1 (DKK1), a well known inhibitor of Wnt/β-catenin signaling pathway, suppressed AS extract-mediated regulation of rat BMSCs differentiation, suggesting that AS extract promoted osteogenesis via activating the Wnt/β-catenin signaling pathway. These results indicated that the effects of AS extract on upregulate the osteogenic differentiation of BMSCs might provide an attractive and promising treatment for osteoporosis, especially the osseointegration around implants under osteoporosis.

Background and Aim

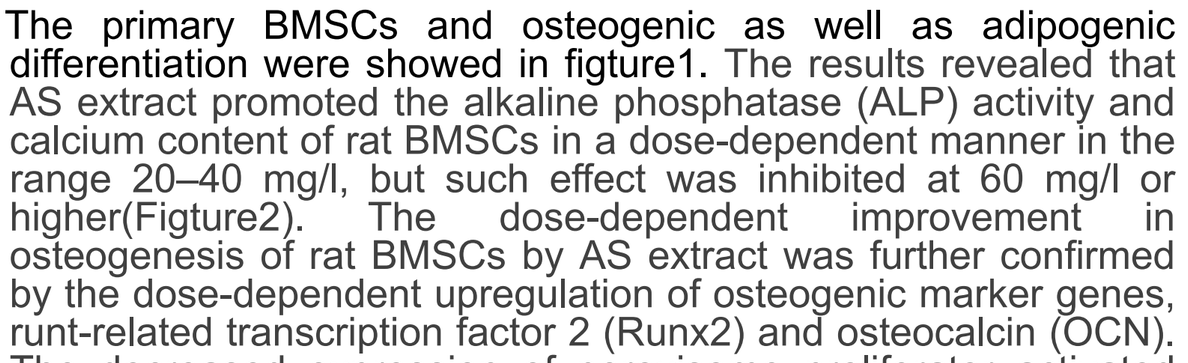
Postmenopausal osteoporosis is a common skeletal disorder disease because of estrogen deficiency, characterized by increased bone turnover and reductions in bone mineral density. Simultaneously, this disorder is a potential risk factor for dental implant surgery because it could increase the risk of oral infectious disease and decrease osseointegration around implants. Angelica sinensis root is one of the herbs most commonly used in China, and its extract has been proven to have anti-osteoporotic effects on ovariectomized rats. However, reports about the effect of AS on osteoblastic and adipocytic differentiation from bone marrow derived mesenchymal stem cells (BMSCs) are limited. The purpose of this study is to evaluate whether AS extract affects the proliferation and the osteogenic or adipogenic differentiation of rat BMSCs.

Methods and Materials

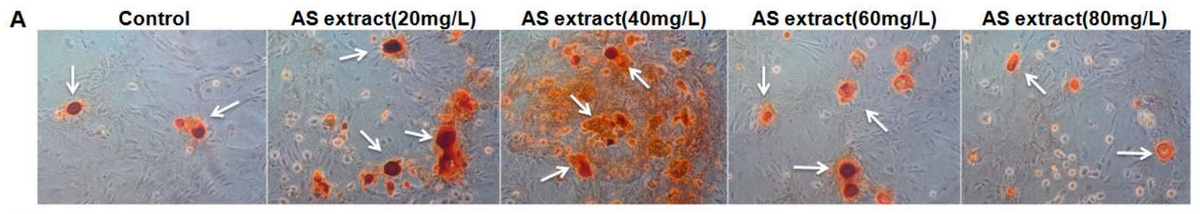
Rat BMSCs were were isolated and identified by osteogenic as well as adipogenic differentiation. The effect of AS extract with different concentrations on the differentiation of rat BMSCs was investigated in vitro. The alkaline phosphatase (ALP) activity and alizarin red staining were performed after 3 weeks. Then, RT-PCR was carried out to detect the expression of osteogenic and adipogenic markers. Furthermore, the expression of CyclinD1 and β-catenin was also evaluated to confirm the effect of Wnt/β-catenin signaling pathway.

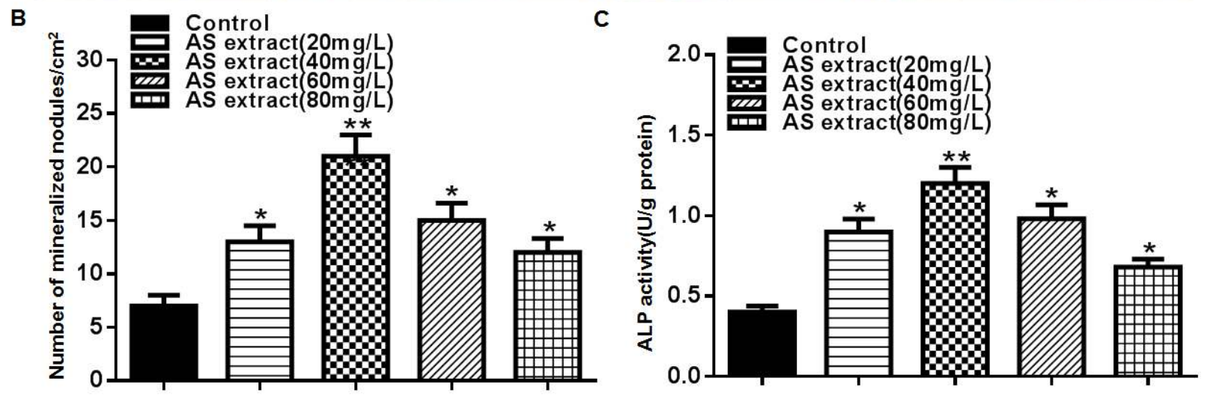
Results

The primary BMSCs and osteogenic as well as adipogenic differentiation were showed in figture1. The results revealed that AS extract promoted the alkaline phosphatase (ALP) activity and calcium content of rat BMSCs in a dose-dependent manner in the range 20–40 mg/l, but such effect was inhibited at 60 mg/l or higher(Figture2). The dose-dependent improvement in osteogenesis of rat BMSCs by AS extract was further confirmed by the dose-dependent upregulation of osteogenic marker genes, runt-related transcription factor 2 (Runx2) and osteocalcin (OCN). The decreased expression of peroxisome proliferator activated receptor γ2（PPARγ2）and lipoprotein lipase (LPL) suggested that AS extract also inhibited adipogenic differentiation of rat BMSCs. Further mechanistic study demonstrated that the transcriptional levels of CyclinD1 and β-catenin increased significantly during AS extract induced osteogenesis(Figture3). Dickkopf 1 (DKK1), a well known inhibitor of Wnt/β-catenin signaling pathway, suppressed AS extract-mediated regulation of rat BMSCs differentiation, suggesting that AS extract promoted osteogenesis via activating the Wnt/β-catenin signaling pathway(Figture4 and 5).

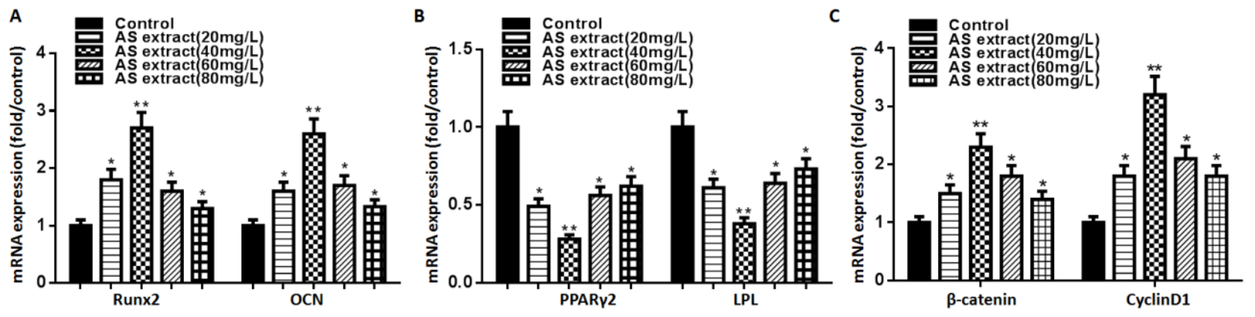


Figture 1. **A**. Primary BMSCs **B**. Alizarin red staining **C**. Oil red O staining

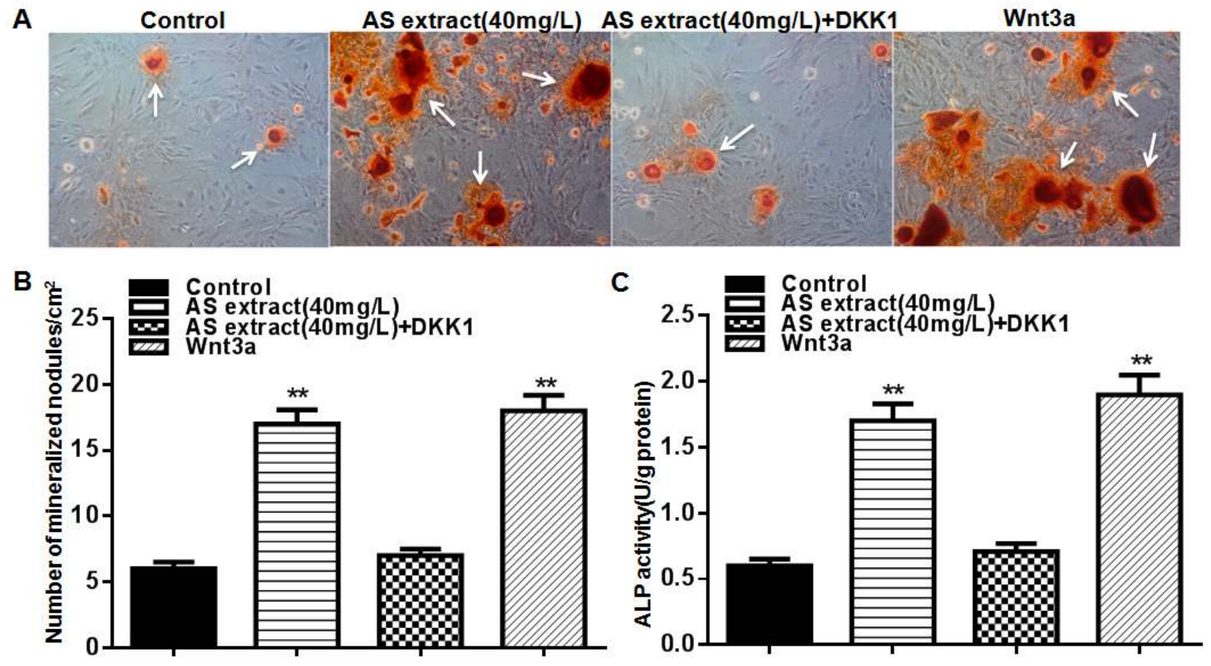




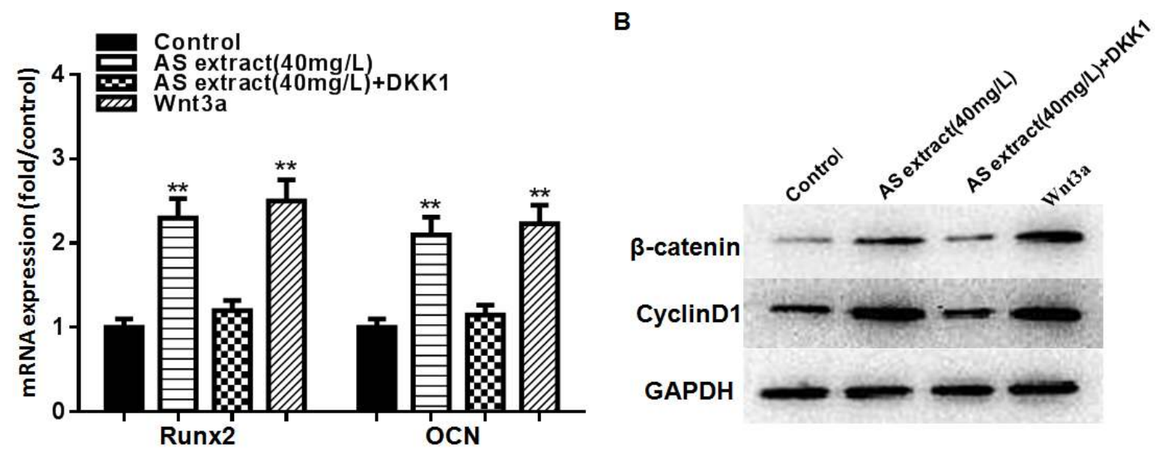
Figture2. **A.** Representative alizarin red staining images in different groups.(White arrows indicate the mineralized nodules) **B.** The number of mineralized nodules in different groups. **C.** The ALP activity in different groups. Data were presented as mean ± SEM. *p < 0.05 and **p < 0.01 vs control.



Figture3. **A.** Runx2 and OCN mRNA expression normalized to GAPDH by RT-PCR analysis. **B.** PPARγ2 and LPL mRNA expression normalized to GAPDH by RT-PCR analysis. **C.** β-catenin and CyclinD1 mRNA expression normalized to GAPDH by RT-PCR analysis. Data were presented as mean ± SEM.*p < 0.05 and**p < 0.01 vs control.



Figture4. **A.** Representative alizarin red staining images in different groups.(White arrows indicate the mineralized nodules) **B.** The number of mineralized nodules in different groups. **C.** The ALP activity in different groups. Data were presented as mean ± SEM. **p < 0.01 vs control.



Figture5. **A.** Runx2 and OCN mRNA expression normalized to GAPDH by RT-PCR analysis. **B.** β-catenin and CyclinD1 protein expression normalized to GAPDH by Western blot analysis. Data were presented as mean ± SEM.**p < 0.01 vs control.

Conclusions

These results indicated that the effects of AS extract on upregulate the osteogenic differentiation of BMSCs might provide an attractive and promising treatment for osteoporosis, especially the osseointegration around implants under osteoporosis.

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