**Introduction:** Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can differentiate into a variety of other cell types, such as osteoblasts, adipocytes, and chondrocytes [1]. Stimulating the osteogenic differentiation property is considered as one of the strategies for bone tissue engineering applications. Retinoid signaling plays very important roles in skeletal development. CRBP1 (cellular retinol binding protein 1), a key component of retinoid signaling pathway, is known to take part in vitamin A metabolism and intracellular transporting of retinoid [2]. However, little is known about the roles of CRBP1 in MSCs. We aim to study the effect of CRBP1 on multi-differentiation potential and migration ability of MSCs.

**Methods:** Cultures of bone marrow-derived MSCs were established from 6-8 weeks SD rats. The gene encoding rat CRBP1 (GenBank number: NM_012733) was amplified and cloned into pLenti-MCS-dsRed vector using in vitro recombination. The gene encoding rat β-catenin (GenBank number: NM_053357) and RXRa (GenBank number: NM_012805) was cloned into pCMV-Flag plasmid separately. Two different shRNAs were chosen from rat CRBP1 mRNA sequence, which target nucleotides 420-439 and 620-639 respectively, and one nonspecific shRNA was designed as control. Pseudo-lentivirus was produced by transient transfection of 293FT cells. MSCs were then transduced with lentivirus carrying CRBP1, dsRed, or shRNAs. The transduction efficiency was checked by immunofluorescence; CRBP1 gene expression was checked by quantitative real-time polymerase chain reaction (RT-PCR) and western blot. Osteogenic and adipogenic differentiation were performed according to the published protocols. Osteoblasts or adipocytes were stained with Alizarin Red S or Oil red O. Genes associated with osteogenesis and adipogenesis were assayed by quantitative RT-PCR. Bone matrix protein (OPN), β-catenin and Erk signaling pathways which were involved in osteogenesis were checked by western blot. Finally, the effect of CRBP1 on osteogenesis of MSCs was evaluated by ectopic bone formation carried out in nude mice.

**Results:** CRBP1 was successfully transduced into MSCs with more than 80% of cells being positive for CRBP1 reporter gene dsRed. CRBP1 overexpression could enhance osteogenic differentiation of MSCs (Fig.1A-C), while inhibit their adipogenic differentiation (Fig.2A-C), as demonstrated by quantitative RT-PCR (ALP, Runx2, OCN, OPN, ColIα2), western blot (OPN), Alizarin Red S or Oil red O staining and ectopic bone formation. When endogenous CRBP1 was knocked down in MSCs using shRNA, the effect of CRBP1 on osteogenesis and adipogenesis was reversed. Furthermore, we showed that CRBP1 may have promoted osteogenic differentiation of MSCs through inhibiting RXRa-induced β-catenin degradation, maintaining β-catenin and pErk1/2 at higher levels (Fig. 3). CRBP1 overexpression had no significant effect on migration of BM-MSCs. Finally, the effect of CRBP1 on osteogenesis and adipogenesis of BM-MSCs was confirmed in vivo by ectopic bone formation carried out in nude mice.

**Discussion:** We showed that over-expression of CRBP1 promoted osteogenic differentiation and inhibited adipogenic differentiation of BM-MSCs in vitro and in vivo. Furthermore, we demonstrated that CRBP1 promotes osteogenic differentiation of MSCs through inhibiting RXRa-induced β-catenin degradation, maintaining β-catenin and pErk1/2 at higher levels. Taken these data together, our study shows that CRBP1, apart from its known role in vitamin A homeostasis, CRBP1 inhibits RXRa-induced β-catenin degradation, leading to enhanced osteogenesis of BM-MSCs.

**Significance:** New function of CRBP1 was elucidated. Apart from its known role in vitamin A homeostasis, CRBP1 inhibits RXRa-induced β-catenin degradation, leading to enhanced osteogenesis of BM-MSCs.

**References:**