under osteogenic and adipogenic conditions to differentiate into osteoblasts and adipocytes, respectively. Then, they were co-cultured for 3 days, and osteoblasts were cultured for another 24 hours in serum-free medium to produce CM. New osteoblasts were cultured for 3 days in this CM. Osteoporosis was induced by orchiectomy (ORX) and osteoblasts differentiated from bone marrow MSCs of ORX and Sham rats were compared. The inhibitory effect of CM on osteoblast differentiation was similar to that induced by osteoporosis (Fig. 1A-D) as well as decreased histone H3 acetylated (AcH3) protein expression (Fig. 1E-F). Trichostatin A (TSA), an inhibitor of histone deacetylase, was used to increase AcH3, which reverted the deleterious effect of CM and osteoporosis on osteoblast differentiation (Fig. 2A-F). In conclusion, adipocytes recapitulate the inhibitory effect of osteoporosis on osteoblast differentiation by downregulating histone acetylation.

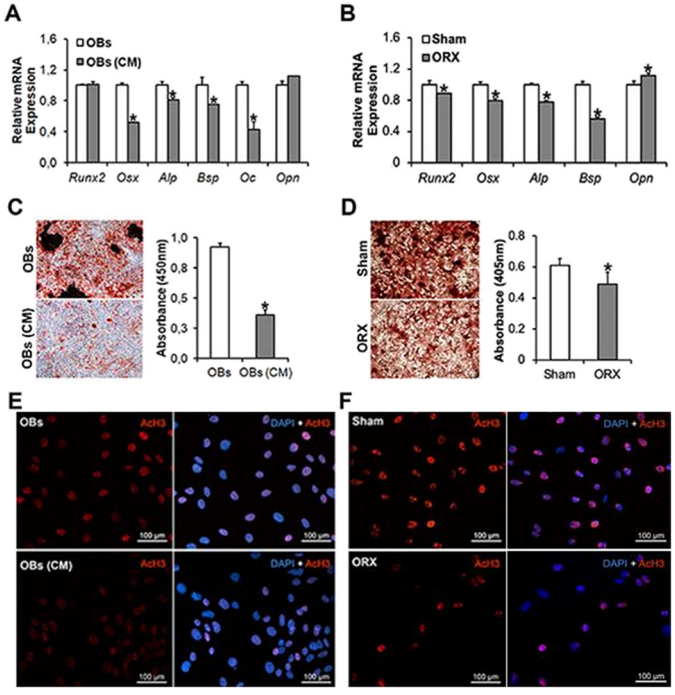

Fig. 1. Effect of CM and ORX on osteoblast differentiation. *Student's t-test, n=3, p≤0.05).

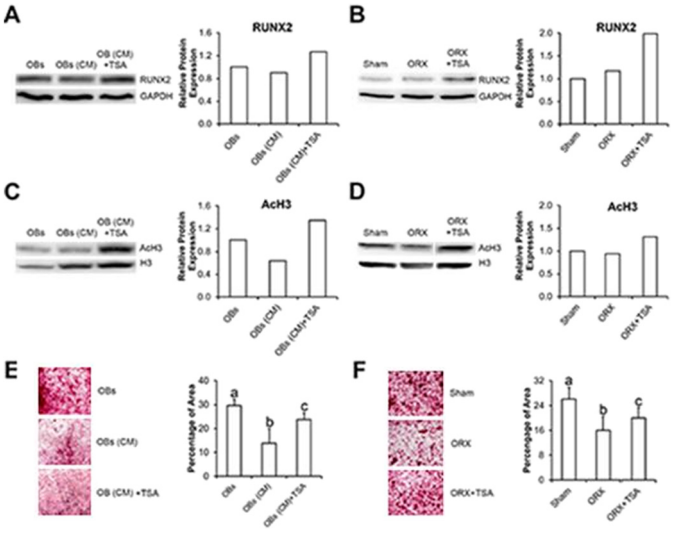

Fig. 2. Effect of CM and ORX on osteoblast differentiation involves AcH3. ANOVA, n=3, p≤0.05.

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P089

Positive effects of mesenchymal stem cells from healthy rats on the impaired osteoblast differentiation of mesenchymal stem cells from osteoporotic and diabetic rats

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Osteoporosis and diabetes mellitus are systemic diseases that impaired the osteoblast differentiation of mesenchymal stem cells (MSCs). Considering cell therapy applications to treat bone defects under osteoporotic and diabetic conditions, we hypothesized that MSCs from healthy rats (HE-MSCs) have positive effects on the ability of MSCs from osteoporotic (ORX-MSCs) and diabetic (DM-MSCs) rats to differentiate into osteoblasts. Thus, the aim of this study was to evaluate the influence of HE-MSCs on the osteoblast differentiation of both ORX-MSCs and DM-MSCs, using an indirect co-culture model. All animal procedures were approved by Ethics Committee in Animal Research. Osteoporosis and diabetes mellitus were induced by orchiectomy surgery and streptozotocin injection, respectively. Then, MSCs were isolated from bone marrow of healthy, osteoporotic and diabetic rats, co-cultured under osteogenic condition and *Runx2* gene expression (n=3) and alkaline phosphatase (ALP) activity (n=5) were evaluated on day 10 and extracellular matrix mineralization (n=5), on day 14. Co-cultures of cells at the same condition (healthy, osteoporotic or diabetic) were used as controls. The data were compared by ANOVA (p≤0.05) and indicate that MSCs derived from healthy rats partially recovered the osteogenic potential of MSCs from rats with osteoporosis and diabetes mellitus (Fig. 1). These findings suggest that the use of MSCs from healthy donors may be an interesting strategy in cell therapy approaches to repair bone tissue under osteoporotic and diabetic conditions.

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P090

Revealing the localization of Annexin A6 in matrix vesicles during physiological mineralization

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Annexin A6 (AnxA6, ~68 kDa) is the largest member of the annexin family of proteins present in matrix vesicles (MVs). MVs serve as nucleation sites for crystal deposition during physiological mineralization.