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.

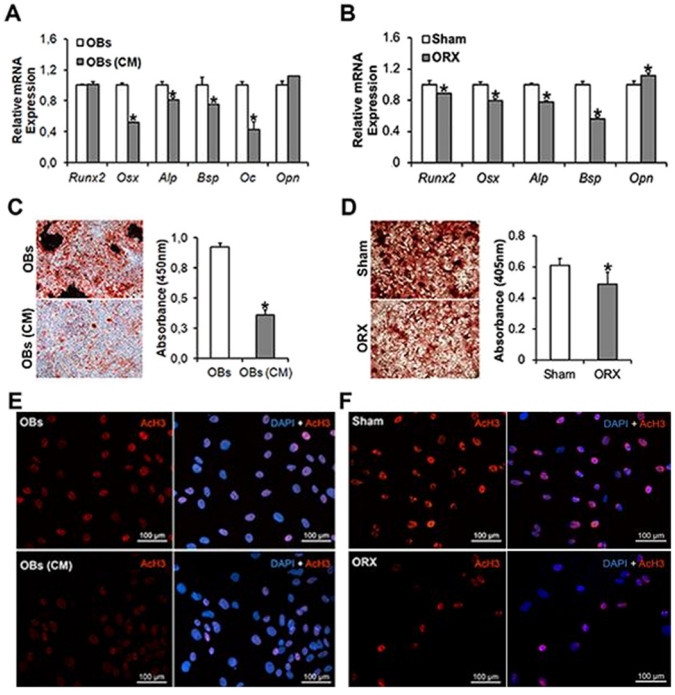

Fig. 1. Effect of CM and ORX on osteoblast differentiation. *Student's t-test, n=3, p≤0.05).

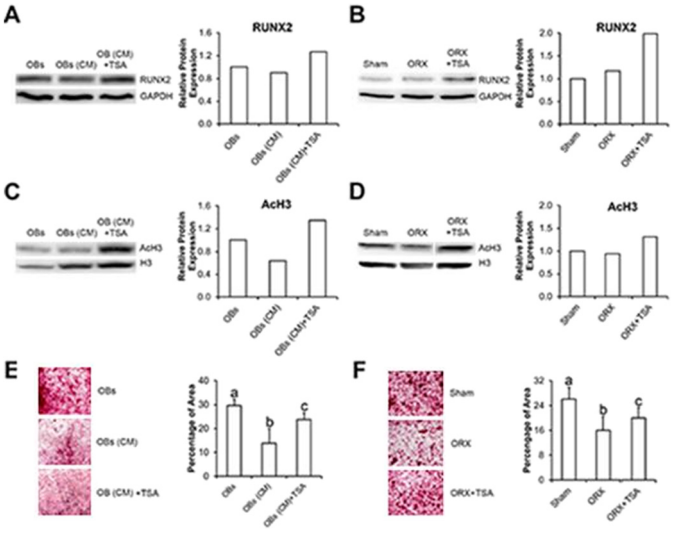

Fig. 2. Effect of CM and ORX on osteoblast differentiation involves AcH3. ANOVA, n=3, p≤0.05.

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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.

Here, we assess the localization of AnxA6 in the MV membrane using native MVs and MVs biomimetics. Biochemical analyses revealed that AnxA6 is present in three distinct regions of the MV membrane. The first, corresponds to Ca^{2+}-bound AnxA6 interacting with the inner leaflet of MV membrane, the second, is AnxA6 localized on the surface of the outer leaflet of MV membranes, and the third, is AnxA6 inserted in the membrane's hydrophobic bilayer and co-localized with cholesterol. Using monolayers and proteoliposomes composed of either dipalmitoylphosphatidylcholine (DPPC) to mimic the outer leaflet of the MV bilayer or a 9:1 DPPC:dipalmitoylphosphatidylserine (DPPS) mixture to mimic the inner leaflet, we confirmed that AnxA6 interacts differently with MV membranes in agreement with the biochemical data. Thermodynamic analysis based on the measurement of the surface pressure exclusion, enthalpy and phase transition cooperativity ($\Delta t_{1/2}$) showed that AnxA6 interacts with both the lipid models and that this interaction increases in the presence of cholesterol. The selective recruitment of AnxA6 by cholesterol molecules was observed in MVs as probed by the addition of methyl-β-cyclodextrin (MβCD). AnxA6-lipid interaction was Ca^{2+}-dependent as evidenced by the greater increase in surface pressure in negatively charged 9:1 DPPC:DPPS monolayers and a larger decrease in enthalpy in 9:1 DPPC:DPPS proteoliposomes caused by the addition of AnxA6 in presence of Ca^{2+} compared to zwitterionic bilayers composed of DPPC. We conclude that the different localizations and ways of interaction of AnxA6 with the lipid membrane suggest distinct functions in MV during biomineralization.

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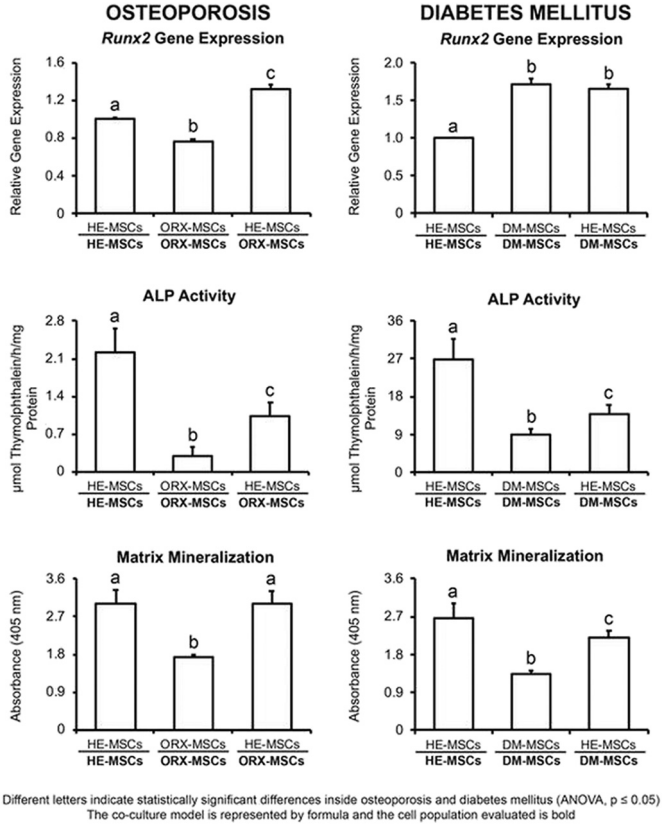

Fig. 1. Runx2 gene expression (n=3), ALP activity (n=5), and matrix mineralization (n=5).

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P091

The type 1 lysophosphatidic acid receptor is involved in osteoblastogenesis up to osteocytogenesis

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Multiple factors, systemic and local, participate in the regulation of bone cell activity. One of those factors is lysophosphatidic acid (LPA). LPA is a natural bioactive lipid exhibiting growth factor-like activities on a large range of normal and neoplastic cells. LPA activates at least six different G-coupled receptors (LPA1-6). LPA_1 is ubiquitous Gi coupled GPCR and is the most interesting for bone homeostasis. Indeed, our team have shown that global deletion of *Lpar1* (the LPA_1 gene) alters the growth of mice as a consequence of a bone formation defects. The aim of this study was to better understand the specific role of LPA via its receptor LPA_1 in the accrual of bone mass during puberty. Thus, we generated *Lpar*1flox/flox;Osx:GFP-Cre/+ mouse lines, in which the *Lpar*1 was specifically invalidated from preosteoblats to osteocytes. Our results show that LPA_1 is essential for bone mineralization. We also found that the absence of LPA_1 slowdown osteocytogenesis and disturbs osteocyte maturation. Osteocytes are mechanosensor cells that control bone formation. Our results suggest a new role for LPA in bone mass control through osteocyte activity.

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P093

Effects of ginsenoside Rb2 on osteogenic differentiation of C2C12 cells

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The most current therapies for osteoporosis have focused on inhibiting bone resorption by osteoclasts. Although the conventional drugs have therapeutic benefits, they also have disadvantages such as breast cancer and osteonecrosis of jaw. The purpose of this study is to develop the new anabolic agents for treatment of osteoporosis that have fewer risks compared to conventional therapies. *Panax Ginseng* is one of the most commonly used herbal medicines. Most of the biological activities of ginseng are derived from main components, ginseng saponins (ginsenosides). To determine the effect of ginsenosides on bone formation, we examined the effect of ginsenosides Rb2(G-Rb2) on C2C12 cell proliferation and osteogenic differentiation.