FATP2 Promotes Osteoclastogenesis by Regulating Lipid Metabolism and ROS production

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INTRODUCTION: The maintenance of bone homeostasis is dependent on a delicate balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Disruption of this equilibrium can result in various bone-related disorders, including pathological fractures, osteosclerosis and osteoporosis. Obese patients and HFD mice both exhibited decreased bone mass and density, as well as increased numbers and activity of osteoclasts, indicating that excessive lipid intake can enhance osteoclastic activity. Current clinical treatments for osteoporosis primarily focus on inhibiting OCs to reduce bone loss through the use of bisphosphonates; however, effective therapeutic strategies specifically targeting lipid accumulation-induced osteoporosis are currently lacking. FATP2, which is encoded by the Slc27a2 gene, belongs to the family of fatty acid transport proteins. It is primarily located on the endoplasmic reticulum membrane, cell membrane and peroxisome membrane where it functions as a transporter for long-chain and very-long-chain fatty acids while also serving as a long-chain and very-long-chain fatty acyl-CoA synthetase. However, the precise role of FATP2 in osteoclastogenesis remains unclear. The objective of this investigation is to explore the correlation between FATP2 and lipid metabolism in osteoclasts, with the aim of devising effective clinical strategies for preventing and treating osteoporosis.

METHODS: Murine bone marrow-derived macrophages (BMMs) were isolated for osteoclastogenesis, followed by protein and gene analysis as well as TRAP staining to evaluate osteoclast differentiation. To mimic the phenotype of obesity-induced osteoporosis, mice were fed a high-fat diet for 12 weeks. Lipopolysaccharide (LPS) and ovariectomy (OVX) were employed to establish models of osteoporosis, with intervention performed via intraperitoneal injection of the FATP2 inhibitor Lipofermata. Micro-CT was utilized for bone mass measurement, while immunofluorescence staining was employed to detect protein expression.

RESULTS SECTION: Lipid metabolism plays a crucial role in maintaining bone homeostasis, particularly in osteoclasts (OCs) formation. Here, we found the expression level of FATP2, a transporter for long-chain and very-long-chain fatty acids, was significantly upregulated during OC differentiation and in the bone marrow of mice fed a high-fat diet (HFD). Notably, the use of FATP2 siRNA or a specific inhibitor (Lipofermata) resulted in significant inhibition of OC differentiation while only slightly affecting osteoblasts (OBs). In pathological models of bone loss induced by LPS or OVX, in vivo treatment with Lipofermata was able to rescue the loss of bone mass by inhibiting OC differentiation. RNA sequencing (RNA-seq) revealed that Lipofermata reduced fatty acid β-oxidation and inhibited energy metabolism, while regulating reactive oxygen species (ROS) metabolism to decrease ROS production, ultimately inhibiting OC differentiation. Treatment with Lipofermata, either in vivo or in vitro, effectively rescued the overactivation of OCs, indicating that FATP2 regulated OC differentiation by modulating fatty acid uptake and energy metabolism. These findings suggested that targeting FATP2 may represent a promising therapeutic approach for pathological osteoporosis.

DISCUSSION: The inhibition of osteoclastogenesis by Lipofermata, a FATP2 inhibitor, was achieved through the reprogramming of energy metabolism and regulation of ROS levels. In both pathological bone loss and HFD-induced osteoporosis models, the expression levels of FATP2 were significantly upregulated and Lipofermata demonstrated potential therapeutic effects in the pathological bone loss model.

SIGNIFICANCE/CLINICAL RELEVANCE: FATP2 may be a novel therapeutic target for osteogenesis and osteoporosis treatment.

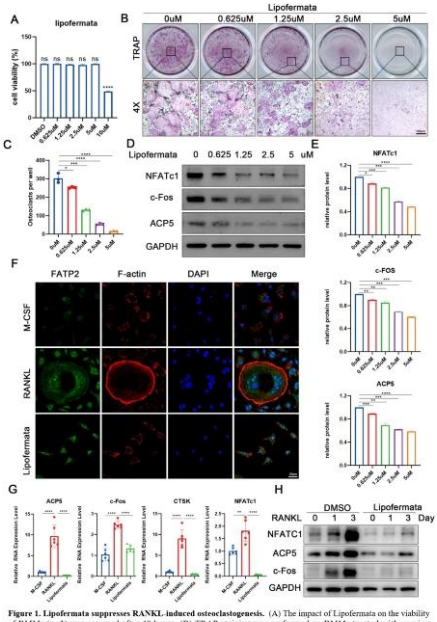

Figure 1. Lipofermata suppresses RANKL-induced osteoclastogenesis. (A) The impact of Lipofermata on the viability of BMMs (n=3) was assessed after 48 hours. (B) TRAP staining was performed on BMMs treated with varying concentrations of Lipofermata and stimulated with M-CSF and RANKL for 5 days. The images above depict low magnification, while the images below show high magnification. (C) Quantitative analysis was performed to determine the number of TRAP-positive multinuclear cells (n=3). (D) The protein levels of OC markers were assessed by Western blotting following treatment with varying concentrations of Lipofermata. (E) Quantitative statistical analysis was performed on the protein levels (n=3). (F) Representative confocal images depicting FATP2 (green), F-actin (red), and DAPI (blue) in BMMs treated with or without 2.5μM Lipofermata were obtained. (G) mRNA levels of ACP5, c-Fos, CTSK, and NFATc1 were quantified (n=6). (H) Different time points by RANKL stimulation, with or without treatment of Lipofermata (2.5μM), result in alterations in protein levels. Data are presented as mean ± SD and were statistically analyzed using a one-way ANOVA test (A, C, E, G). ns indicates no statistical difference. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

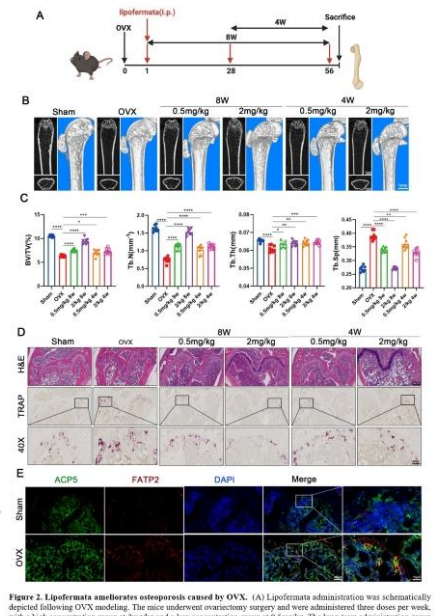

Figure 2. Lipofermata ameliorates osteoporosis caused by OVX. (A) Lipofermata administration was schematically depicted following OVX modeling. The mice underwent ovariectomy surgery and were administered three doses per week, with a high concentration group at 2mg/kg and a low concentration group at 0.5mg/kg. The long-term administration group commenced treatment immediately after modeling, while the short-term administration group received treatment after 4 weeks of modeling. Mice were sacrificed and femurs were collected 8 weeks post-modeling. (B) Representative images of cross-sectional views and three-dimensional reconstructions were presented, with the image in the top left corner depicting the coronal plane and the one in the bottom left corner representing the horizontal plane. (C) Statistical analysis was conducted on micro-CT parameters including BV/TV, Tb.N, Tb.Th, and Tb.Sp. (D) Femoral tissue sections were subjected to H&E staining and TRAP staining. (E) Representative images of ACP5 and FATP2 immunofluorescence staining in femoral tissue sections were obtained from the Sham group and OVX group. ACP5 (green), FATP2 (red), and DAPI (blue). The far-right image displays a magnified view, with white arrows indicating cells that exhibit positive expression for both ACP5 and FATP2.

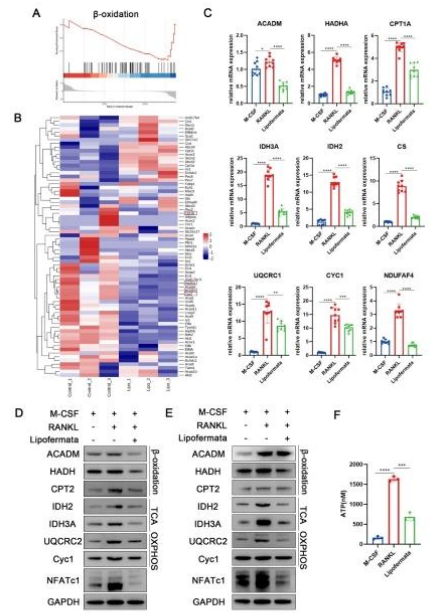

Figure 3. Lipofermata inhibits OC differentiation by reprogramming energy metabolism. (A) The GSEA analysis revealed a significant enrichment of genes associated with the process of β-oxidation. NES=-1.35. (NES, normalized enrichment score). (B) Heatmap of β-oxidation related genes, with or without Lipofermata administration (2.5μM). (C) Expression levels of representative genes for β-oxidation, TCA cycle, and OXPHOS (n=9). M (M-CSF); R (M-CSF + RANKL); Lipofermata (M-CSF + RANKL + Lipofermata). Western blot analysis was conducted to assess the expression levels of key markers involved in β-oxidation, TCA cycle, and OXPHOS upon treatment with Lipofermata (D) or FATP2 siRNA (E). (F) The ATP levels were measured by means of an ATP detection assay kit, both with and without Lipofermata treatment (n=3). Statistical analyses were determined by one-way ANOVA (C, F). *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. Data are presented as mean±SD.