

# Sigma-1 receptor attenuates osteoclastogenesis by promoting ER-associated degradation of SERCA2

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**INTRODUCTION:** There are two subtypes of Sigma receptor: Sigma-1 receptor (Sigmar1) and Sigma-2 receptor. Sigmar1 is widely expressed in various mammalian systems. In general, Sigmar1 is a transmembrane protein located in the mitochondria associated endoplasmic reticulum and is involved in a variety of physiological activities when stimulated or excited by external stimuli. Previous studies have revealed that Sigmar1 have protective effects on cells under various stress conditions. It is not clear whether Sigmar1 play a regulatory role in bone metabolic homeostasis.

**METHODS:** Firstly, bone marrow macrophages (BMMs) were extracted for osteoclast induction. We use western blot, qPCR and TRAP staining to investigate the roles of knockout of Sigmar1 and administration of dimemorfan in osteoclast formation. Next, using HEK-293T cells, we overexpressed Sigmar1 using plasmids and performed immunoprecipitation, mass spectrometry, molecular docking to explore Sigmar1's interaction proteins and potential regulatory mechanisms. Furthermore, we constructed global Sigmar1 knockout mice(gKO) and compared them with wild-type mice under steady conditions and by ovariectomy-induced osteoporosis model (OVX) to investigate whether Sigmar1 knockout can promote the progression of osteoporosis. Besides, we injected adeno-associated virus (AAV) to overexpressing Sigmar1 into wild-type OVX mouse model, to explore whether the overexpression of Sigmar1 has a protective effect. Besides, we performed bone marrow transfer experiment, to explore the effect of Sigmar1 gKO bone marrow on the pathological osteoporosis. Finally, we investigated the protective effects of intraperitoneal injection of dimemorfan in wild-type mouse models of acute osteolysis induced by subperiosteum injection of lipopolysaccharide (LPS), OVX-induced osteoporosis and collagen-induced arthritis (CIA).

**RESULTS SECTION:** Compared with WT littermates, knockout of Sigmar1 didn't affect bone mass, analysis of osteocytes, osteoblasts and osteoclasts showed identical results. After ovariectomy, Sigmar1 gKO mice exhibited severe osteoporosis comparing to WT mice. Whereas intramedullary injection of AAV overexpressing Sigmar1 showed protective effect with elevated bone mass compared with AAV vector injection. Besides, bone marrow transfer experiments demonstrated that WT mice received Sigmar1 gKO bone marrow transplantation developed less bone mass in comparison with those received WT bone marrow transplantation. In vitro osteoclast formation experiment, knock out of Sigmar1 promoted osteoclastogenesis, whereas dimemorfan inhibited osteoclast formation in a dose-dependent manner. Using co-immunoprecipitation-mass spectrometry, we determined that Sigmar1 regulates osteoclast differentiation by binding SERCA2. Confirmed by point mutations, protein truncations and co-immunoprecipitation experiments, we identified the Q615 site of SERCA2 as the key site of Sigmar1 interaction with SERCA2. Furthermore, we found that sigmar1 promote SERCA2 degradation, and this degradation process was related to ER-associated degradation pathways. By overexpressing or knockout components of the Hrd1/Sel1L complex, we discovered that Sigmar1 promotes endoplasmic reticulum-related degradation of Hrd1/Sel1L complex-dependent SERCA2. In mice with OVX, LPS and CIA induced bone loss models, intraperitoneal injection of dimemorfan effective reduced bone mass loss compared to vehicle groups.

**DISCUSSION:** We demonstrate that Sigmar1 regulate osteoclast differentiation by binding to SERCA2 and promoting SERCA2 through ER-associated degradation with the help of Hrd1/Sel1L complex, and that Sigmar1 knockout enhances OVX-induced bone loss. However, further human trials are needed to verify sigma-1 receptor function.

**SIGNIFICANCE/CLINICAL RELEVANCE:** (1-2 sentences): Sigmar1 agonists, especially dimemorfan, should be considered an anticatabolic agent in bone-related disease, in addition to its established role in cough relief.

**IMAGES:** Images and tables will appear at the end of the abstract and must be sized to fit within the abstract. Three images and/or tables are allowed per abstract.

