NUAK1 mediates osteoclast energy metabolism through the regulation of mitochondrial respiratory chain homeostasis

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INTRODUCTION: NUAK1 (NUAK family SNF1-like kinase 1) is a participant in important signaling pathways and is associated with various biological processes, including cell survival, metabolic regulation, and antioxidant responses, particularly playing a significant role in tumor development and metastasis, as well as fibrotic diseases. However, there is currently a lack of research on its regulatory role in bone metabolism. In this study, it was first observed that NUAK1 exhibited a notable increase during osteoclast differentiation. Subsequently, it was demonstrated that the NUAK1-specific inhibitor HTH-01-015 significantly suppressed osteoclast formation while having minimal effects on osteoblasts. The application of HTH-01-015 effectively improved bone volume, bone density, and osteoporosis caused by ovariectomy, as well as in a lipopolysaccharide-induced cranial defect mouse model. Furthermore, the research revealed that NUAK1 promotes osteoclast formation by binding to cytochrome C and regulating mitochondrial function within osteoclasts, which will influence bone metabolism and the occurrence of osteoporosis. Additionally, this study explored the potential of NUAK1 as a screening indicator for osteoporosis in humans, based on its expression levels in osteoporotic populations and ovariectomized mice, and provided new targets and strategies for the treatment or alleviation of osteoporosis.

METHODS: Samples of peripheral blood and cancellous bone were collected from osteoporosis patients to measure NUAK1 expression via quantitative PCR, Western blotting, and immunofluorescence. In vitro, mouse bone marrow macrophages were differentiated into osteoclasts using RANKL and treated with NUAK1-specific inhibitors (HTH-01-015 and WZ4003) and siRNA to study osteoclast formation, assessed by TRAP staining. Additionally, the effects on osteoblasts were evaluated in BMSC-induced differentiation. In vivo, NUAK1 inhibitors were applied to OVX-induced osteoporosis and LPS-induced inflammatory cranial bone resorption mouse models. Bone mass and osteoclast formation were analyzed using micro-CT and TRAP staining. Advanced techniques including transcriptome sequencing, co-immunoprecipitation, mass spectrometry, and docking studies were employed to investigate downstream pathways and interacting proteins of NUAK1, particularly in mitochondrial function, using JC-1, ROS, and calcium oscillation assays, alongside electron microscopy and ATP level measurements.

RESULTS SECTION: NUAK1 was significantly upregulated in osteoporosis patient's peripheral blood mononuclear cells and co-localized with osteoclast marker ACP5 in bone tissues. Its expression correlated with RANKL-induced osteoclast differentiation. Treatment with NUAK1-specific inhibitors and siRNA resulted in a substantial reduction in TRAP+ osteoclast formation and downregulation of osteoclast markers ACP5, NFATc1, and C-FOS, without affecting osteoblast differentiation. Micro-CT and histological analyses showed that NUAK1 inhibition mitigated bone loss and osteoclast activity in mouse models. Furthermore, transcriptome analysis indicated a significant downregulation in genes related to ATP production and TCA cycle, with impaired mitochondrial respiratory chain pathways. NUAK1's interaction with mitochondrial cytochrome C, which regulates mitochondrial function, was confirmed, highlighting its pivotal role in mitochondrial activation and energy metabolism during osteoclast differentiation.

DISCUSSION: This research confirms the critical role of NUAK1 in regulating mitochondrial function and energy metabolism during osteoclast differentiation, mediated through its interaction with cytochrome C. NUAK1's upregulation in osteoporosis and its impact on mitochondrial dynamics suggest its potential as a novel biomarker and therapeutic target for osteoporosis and related bone resorption conditions, offering possible new treatment avenues focused on mitochondrial regulation.

SIGNIFICANCE/CLINICAL RELEVANCE: This work has developed a novel NUAK kinase target towards osteoclast differentiation.

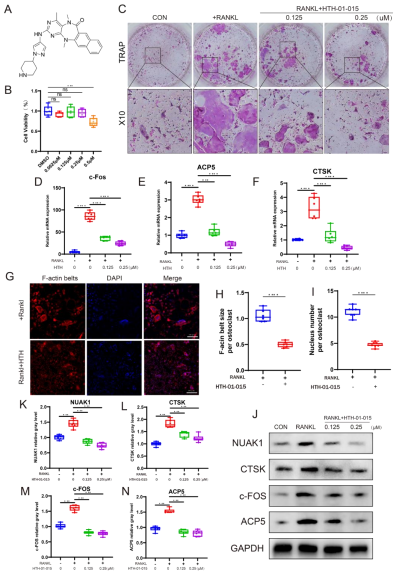

Figure 1. NUAK1-specific inhibitor HTH-01-015 blocks osteoclast differentiation. (A) Structural formula of the NUAK1-specific inhibitor HTH-01-015. (B) CCK-8 assay assessing the cytotoxicity of HTH-01-015 (n=6). (C) TRAP staining showing HTH-01-015's inhibition of osteoclast differentiation. TRAP staining under different concentrations of HTH-01-015 (0.125 μM, 0.25 μM), M-CSF, and RANKL. (D-F) qPCR analysis of HTH-01-015's inhibition of osteoclast differentiation. Quantitative analysis of c-FOS, ACP5, and CTSK mRNA levels (n=6). (G-I) F-actin fluorescence staining showing HTH-01-015's inhibition of osteoclast differentiation. Representative confocal images of F-actin (red) and DAPI (blue) in BMMs treated with or without HTH-01-015 (0.125 μM, 0.25 μM). (K-J) Western blot analysis of HTH-01-015's inhibition of osteoclast differentiation. Changes in protein levels under different concentrations of HTH-01-015 (0.125 μM, 0.25 μM) with or without RANKL stimulation. ns indicates no statistical significance. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Data are presented as mean ± SD.

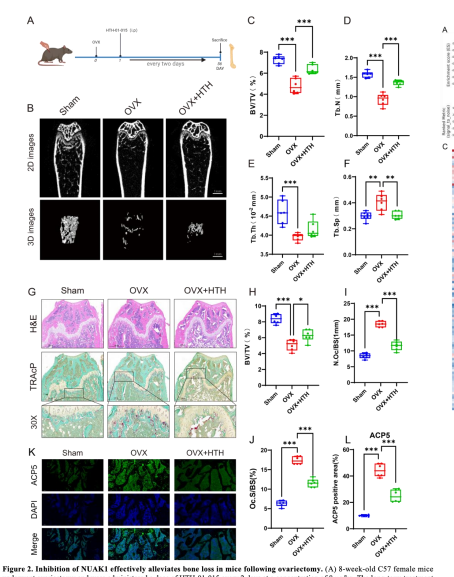

Figure 2. Inhibition of NUAK1 effectively alleviates bone loss in mice following ovariectomy. (A) 8-week-old C57 female mice underwent ovariectomy and were administered a dose of HTH-01-015 every 2 days at a concentration of 8 μg/kg. The long-term treatment group began treatment immediately after modeling. Mice were euthanized 8 weeks after modeling, and femurs were collected. (B) Representative cross-sectional and trabecular bone 3D reconstruction images. (C-F) Statistical analysis of Micro-CT parameters including trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and trabecular number (Tb.N) (n=2). (G) Femur tissue sections were subjected to H&E staining and TRAP staining. (H-I) Histomorphometric analysis of H&E and TRAP staining. (K-L) Immunofluorescence staining was performed to detect osteoclast-related markers. Green fluorescence indicates the presence of ACP5, while blue fluorescence represents DAPI staining. Fluorescence intensity analysis was conducted. Statistical analysis was determined using one-way ANOVA with Tukey's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Data are presented as mean ± SD.

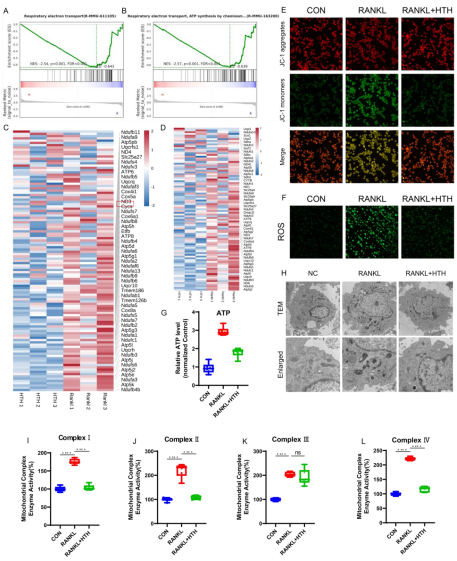

Figure 3. NUAK1 regulates mitochondrial quantity and energy metabolism and osteoclast formation. (A-B) GSEA analysis of transcriptome sequencing in osteoclasts with NUAK1 knockdown shows significant enrichment of genes related to oxidative respiratory chain and electron transport. (C-D) Heatmaps of oxidative respiratory chain and electron transport-related genes. (E) Mitochondrial membrane potential (JC-1) was measured in RANKL-treated BMMs cells with or without HTH-01-015 treatment. (F) ROS production was assessed in RANKL-treated BMMs cells under HTH-01-015 treatment conditions. (G) Intracellular ATP levels were determined using an ATP assay kit in RANKL-treated BMMs cells with or without HTH-01-015 influence. (H) Mitochondria were observed via transmission electron microscopy (n = 6). Scale bars: 2 μm or 1 μm. (I) Formation of oxidative respiratory chain complexes I, II, III, and IV was analyzed in RANKL-treated BMMs cells with or without HTH-01-015 treatment. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as mean ± SD.