

# Identification of osteal macrophage Neuropeptide Like Protein as an inhibitor of osteoclast differentiation and bone loss for therapeutic applications in osteoporosis

Taiki Tokuhira<sup>1</sup>, M. Alaa Terkawi<sup>1</sup>, Yuki Ogawa<sup>1</sup>, Takuya Ogawa<sup>1</sup>, Junki Shiota<sup>1</sup>, Keita Kitahara<sup>1</sup>, Yoshio Nishida<sup>1</sup>, Hend Alhasan<sup>1</sup>, Tomohiro Shimizu<sup>1</sup>, Daisuke Takahashi<sup>1</sup>, Norimasa Iwasaki<sup>1</sup>

<sup>1</sup>Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University  
Email of Presenting Author: tokuhira.taiki.y9@elms.hokudai.ac.jp

**Disclosures:** Taiki Tokuhira: None. Mohamad Alaa Terkawi: None. Yuki Ogawa: None. Takuya Ogawa: None. Junki Shiota: None. Keita Kitahara: None. Yoshio Nishida: None. Hend Alhasan: None. Tomohiro Shimizu: None. Daisuke Takahashi: None. Norimasa Iwasaki: None.

**INTRODUCTION:** The aging of the population is rapidly progressing all over the world and a healthy life expectancy has emerged as an important issue for public health services. Osteoporotic fragility fractures are one of the main factors tackling healthy life expectancy. Osteoporosis is a metabolic bone disease characterized by decreased bone mineral density and bone mass due to impairment of bone remodeling process. This process requires tight coordination between cells in the bone, including osteoblasts and osteocytes of mesenchymal origin, and osteoclasts and osteal macrophages of hematopoietic origin. Osteoporosis occurs when the function of bone-resorbing osteoclasts increases over the function of bone-building osteoblasts. Current therapies for osteoporosis mainly target osteoclast and osteoblast functions, but their effectiveness in increasing bone density and preventing bone fractures is limited, and the number of fragility fractures caused by osteoporosis remains high and increasing globally. Therefore, a new approach is needed to regulate bone metabolism and restore bone homeostasis. Emerging evidence highlights that osteal macrophages play essential roles in maintenance of bone homeostasis. Thus, we speculate that exploring regulatory function of osteal macrophages may provide a clue for discovery of innovative therapeutic agents for prevention of osteoporosis [1]. In our earlier study, single cell RNA sequencing of osteal macrophages revealed that they were classified into six subsets, whereas subsets 1 and 6 which are expressing gene signatures of M2 macrophages and efferocytotic macrophages, were decreased during osteoporosis. Importantly, we identified a gene of Gm1673 (Neuropeptide-like protein C4orf48 homolog) that specifically expressed in subsets 1 and 6. The molecule was named as Osteal macrophage Neuropeptide-Like Protein (OmNLP). Therefore, the objective of the current study is to evaluate the beneficial effects of OmNLP in the treatment of osteoporosis.

**METHODS:** The procedures for the animal experiments were approved by the Institute of Animal Care and Use Committee of the Hokkaido University Graduate School of Medicine. *in vitro*, qPCR in osteoblasts and osteocytes culture models stimulated by OmNLP, bone formation evaluation using human fetal osteoblasts, and bone resorption evaluation and gene expression analysis by RNA-seq using osteoclasts. *in vivo*; We used two osteoporosis models. (1) A local RANKL administration model was created to induce osteoclasts by local injection of RANKL on the calvaria bone of mice. OmNLP was administered once daily and the calvaria bone was harvested on the fifth postoperative day for  $\mu$ CT and histological evaluation. (2) The OVX model of osteoporosis was created by excision of bilateral ovaries in female mice, OmNLP was administered three times a week for four weeks, and the distal femur was harvested 28 days postoperatively for  $\mu$ CT and histological evaluation. Statistical analysis was performed using unpaired t test (GraphPad Software Inc.). The significant level was set at  $p < 0.05$ .

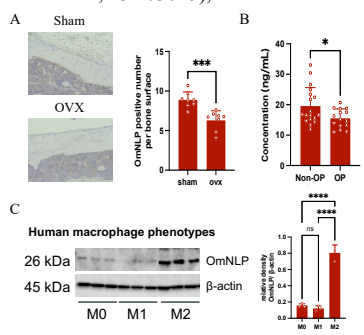
**RESULTS:** To confirm our scRNA-seq data, we first examined the expression of OmNLP in bone tissues. Importantly, expression of OmNLP in lining bone cells was significantly decreased in femoral bone tissue of osteoporotic mice (Fig. 1A). Likewise, protein level of OmNLP was decreased in serum samples collected from clinically diagnosed patients with osteoporosis (Fig. 1B). The expression of OmNLP was significantly increased in *in vitro* differentiated M2 macrophages as compared M0 and M1 (Fig. 1C). Furthermore, we evaluated the effects of this molecule on cells in bone microenvironment. Importantly, there was a little increase in the expression of anabolic factors of osteoblasts after stimulation with OmNLP. This stimulation seemed to inhibit osteoclast differentiation and bone resorption in a dose-dependent manner (Fig. 2A). Bulk RNA-seq analysis revealed that OmNLP stimulation resulted in a decrease in transcription factors of osteoclasts and an increase expression of factors involved in interferon signaling, such as IRF1 and STAT4 (Fig. 2B). In consistent with the *in vitro* results, OmNLP administration reduced local bone resorption area induced by RANKL injection on the calvarial bone and alleviated bone loss in post-menopausal osteoporosis mouse model (Fig. 3). These results demonstrated that OmNLP has regulatory function in bone metabolism and highlighted it as a novel therapeutic agent for osteoporosis.

**DISCUSSION:** OmNLP inhibited osteoclast differentiation and suppressed bone loss *in vitro* and *in vivo* using two osteoporosis models. OmNLP inhibited osteoclastogenesis and bone resorption through repressing the NF- $\kappa$ B signaling pathway and problems activating IFN-signaling. It was suggested that molecules that can trigger IFN-signaling pathway might open a new window for development of new therapeutic interventions in osteoporosis [2]. These findings are consistent with previous reports documenting that the mice lacking IFN signaling components had reduced bone density compared to wild-type mice. In fact, IFN- $\beta$  produced by mature osteoclasts is known to be a negative regulator of osteoclastogenesis and has been traditionally used for treatment of osteoporosis [3]. These collective findings point to a novel immunoregulatory function of OmNLP in bone homeostasis and highlight it as a potential therapeutic agent for the treatment of osteoporosis.

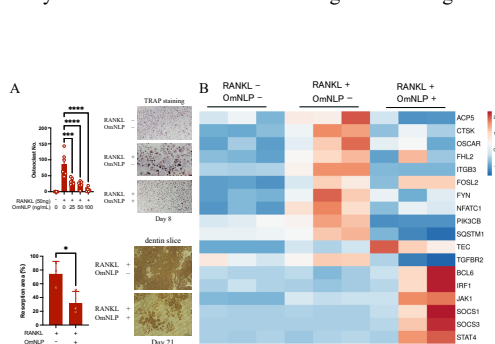
**SIGNIFICANCE/CLINICAL RELEVANCE:** OmNLP derived from osteal macrophage, is a novel immunoregulator of bone metabolism and can be a new therapeutic agent for treatment of osteoporosis.

**REFERENCES:** [1] Terkawi, et al. J Mol Sci. 2022. [2] Yokota, et al. Bone. 2021. [3] Zhao, et al. Arthritis Res Ther. 2011.

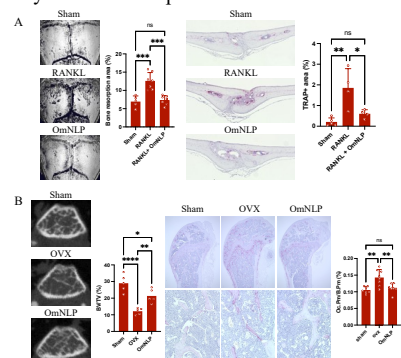
**ACKNOWLEDGEMENTS:** This work was supported by research grants from Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research B; 23H03025), Hirose Foundation, Kobayashi Foundation and AMED Program for Drug Discovery Seeds Development.



**Figure 1:** A) Expression of OmNLP in bone lining cells was significantly decreased in femoral bone tissue of osteoporotic mice. B) Protein level of OmNLP was decreased in serum samples collected from clinical samples. C) Expression of OmNLP was significantly increased in M2 macrophages.



**Figure 2:** A) OmNLP stimulation seemed to inhibit osteoclast differentiation and bone resorption in a dose-dependent manner. B) Bulk RNA-seq analysis revealed that OmNLP stimulation resulted in a decrease in transcription factors of osteoclasts and an increase expression of factors involved in interferon signaling.



**Figure 3:** OmNLP administration reduced local bone resorption area induced by RANKL injection on the calvarial bone and alleviated bone loss in post-menopausal osteoporosis mouse model