Targeting senescent osteal macrophages ameliorate excessive bone loss in postmenopausal osteoporosis: a new approach for prevention of osteoporotic fractures

Yoshio Nishida¹, M Alaa Terkawi¹, Keita Kitahara¹, Junki Shiota¹, Taiki Tukuhiro¹, Tomohiro Shimizu¹
Hend Alhasan¹, Tsutomu Endo¹, Daisuke Takahashi¹, Norimasa Iwasaki¹
¹Department of Orthopedic Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan
Email of Presenting Author: nsd.yso@gmail.com

INTRODUCTION: Osteoporosis is the most common bone metabolic disease characterized by compromised bone volume and decreased mineral density resulting in an increased risk of fractures. It is estimated that over 50 million people worldwide were affected by osteoporosis in 2021, and 40% of women and 13% of men in ages of over than 50 years are at risk of experiencing one or more osteoporotic fractures in their lifetime [1]. Menopause is a major risk factor for skeletal bone homeostasis toward oxidative levels of bone resorption. Given the position of osteal macrophages in the bone microenvironment and their ability to secrete osteoclastogenic factors, understanding molecular and functional alterations in osteal macrophages in the post-menopausal state should provide a clue for discovery of therapeutics for prevention of osteoporosis. Our earlier study demonstrated osteal macrophages were heterogeneous subpopulations and had negative effects on bone homeostasis and inflammation toward the osteoporotic state in bone microenvironment [2]. These findings revealed that eliminating senescent osteal macrophages might be beneficial to ameliorate bone loss in the post-menopausal osteoporosis. Therefore, the aim of this study was to explore the pathological function of senescent osteal macrophages in osteoporosis and evaluate the therapeutic effects of targeting these cells in osteoporosis model.

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 (no. 22-0135). For in vitro oxidative stress model of macrophages, peritoneal macrophages that were isolated from SFF-BALB/c mice (CLEA, Tokyo, Japan) were seeded onto 24-well or 96-well plates and stimulated by treatment with hydrogen peroxide (Wako, Osaka, Japan) with or without 100nm β-estradiol (MedChemExpress, NJ, USA) and 20μM Glutaminase inhibitor compound (GIC) 968 (Cayman Chemical, MI, USA). Cells on 96-well plate were evaluated for the SA–Gal activity with a Senescence β-Galactosidase staining kit (Cell Signaling Technology, CST, USA). Cells on 24-well plate were collected to confirm the expression of target molecules using Western Blotting. For murine calvarial osteolysis model, SFF-BALB/c male mice were anesthetized then sagittal incision was made over the calvarial anterior site for implantation of oxidative stress-exposed macrophages (1.0 x 10⁶).

RESULTS: One-way analysis of variance (ANOVA) followed by Tukey’s multiple-comparison procedure was used for comparison the differences among groups (GraphPad Software, La Jolla, CA, USA). The results were considered statistically significant when p < 0.05.

DISCUSSION: Our findings suggest that decreased production of estrogen due to post-menopause state alters the osteal macrophages subsets, resulting in a shift toward cell senescence and inflammatory conditions in the bone microenvironment. These appear to be consistent with earlier findings highlighting that the accumulation of senescent myeloid cells in a bone microenvironment contributes to the development of inflammatory bone loss in the elderly [3]. Likewise, the increase in expression of senescence markers in macrophages skews these cells toward the inflammatory M1 phenotype that impairs bone metabolism and mediates skeletal fragility [4]. Importantly, the administration of GIC968 causes the elimination of senescent myeloid cells via inhibiting Gal activity with a Senescence β-Galactosidase staining kit (Cell Signaling Technology, CST, USA). Cells on 24-well plate were collected to confirm the expression of target molecules using Western Blotting. For murine calvarial osteolysis model, SFF-BALB/c male mice were anesthetized then sagittal incision was made over the calvarial anterior site for implantation of oxidative stress-exposed macrophages (1.0 x 10⁶).

CONCLUSION: Our findings suggest that targeting senescent osteal macrophages represents a promising therapeutic approach for the prevention of postmenopausal osteoporosis.