Siglec-15 Is A Potential Therapeutic Target For Postmenopausal Osteoporosis

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Disclosures:

Introduction:
Sialic acid binding Ig-like lectin (Siglec)-15 is a novel type of immunoreceptor that regulates osteoclast development in association with an immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptor DAP12. Recent studies demonstrated that Siglec-15 deficient mice exhibit no growth retardation but mild osteopetrosis due to impaired development of osteoclasts, suggesting that Siglec-15 plays an important role in physiological bone remodeling [1]. The role of Siglec-15 in pathological bone loss; however, remains to be elucidated. Especially, it is of great interest whether Siglec-15 is implicated in postmenopausal osteoporosis. Although Siglec-15 was shown to regulate osteoclast development by modulating RANKL signaling, it is unknown whether Siglec-15 is associated with TNFα signaling, which is one of the key molecules for postmenopausal osteoporosis [2]. Therefore, we aimed to investigate whether Siglec-15 deficient mice are resistant to ovariectomy in terms of bone loss and to examine the involvement of Siglec-15 in TNFα induced osteoclastogenesis.

Methods:
Mice: Both WT and Siglec-15−/− mice were on a C57BL/6 background. Female mice were ovariectomized (OVX) or sham operated at 14 wk of age. Mice were sacrificed either at 4 weeks after ovariectomy for histology or at 8 weeks after ovariectomy for micro-computed tomography (micro-CT) analysis.
Micro-CT analysis: Left tibiae and 5th lumbar vertebral bodies were scanned individually by micro-CT (CT, R_mCT2; Rigaku, Tokyo, Japan) at a 10-µm isotropic resolution. Both were quantitatively analyzed using a TRI/3D-BON (Ratoc System Engineering Co. Tokyo, Japan).
Histological evaluation: Proximal tibiae of either OVX or sham operated mice were fixed in paraformaldehyde (PFA), decalcified in EDTA, and embedded in paraffin. Sections were stained for tartrate-resistant acid phosphatase (TRAP) to observe osteoclasts.
In vitro osteoclastogenesis: Bone marrow macrophages (BMMs) were cultured with 30 ng/ml M-CSF and 100 ng/ml TNFα for 5 d at 37°C in a 5% humidified CO2 incubator to generate osteoclasts.
Immunocytochemistry: Cells were cultured on plastic wells or on bovine bone slices, and fixed for 5 min with 4% PFA and then treated with 0.1% Triton X-100. The cells were treated with polyclonal anti-Siglec-15 antibody followed by staining with Alexa Fluor-labeled secondary antibody (Molecular Probes, Carlsbad, CA). The cytoskeletal actin was stained using Alexa Fluor 633 phalloidin (Molecular Probes). The nuclei were visualized using 4′,6-diamidino-2-phenylindole reagent (Dojindo Laboratories, Japan). Immunoblot analysis: Cell lysates were extracted using the PhosphoSafe Extraction Reagent (Novagen, Madison, WI). Cell lysates were subjected to immunoblot analyses using the appropriate antibodies.
Statistical analysis: Data of two-group comparisons were analyzed using a two-tailed Student’s t test. A P value of less than 0.05 was considered statistically significant. The data are represented as mean ± SD.

Results:
Trabecular bone loss after ovariectomy was observed in both WT and Siglec-15−/− mice, but the extent of bone loss in Siglec-15−/− mice was much smaller than that in WT. The percent difference between Sham and OVX groups in proximal tibiae was 50.7% in WT and 20.2% in Siglec-15−/−, and those in vertebral bodies were 27.4% in WT and 12.1% in Siglec-15−/− mice, respectively (Fig. 1).
Histologically, the number of TRAP positive osteoclasts in OVX mice was much higher than that in sham mice in both WT and Siglec-15−/− mice. Osteoclasts in Siglec-15−/− mice were small in size and did not spread on the bone surface and the number of multinuclear osteoclasts in Siglec-15−/− mice were significantly smaller than that in WT mice (Fig. 2). In vitro experiment showed that Siglec-15 is required for the TNFα-induced development of functional osteoclasts. Under the existence M-CSF, TNFα induced differentiation of TRAP-positive multinuclear cells from WT cells, while it induced predominantly TRAP-positive mononuclear cells from Siglec-15−/− cells. When cultured on bovine bone slices, Siglec-15−/− osteoclasts showed impaired bone resorptive activity and could not form an actin ring (Fig. 3). On the contrary to our expectation; however, TNFα-induced intracellular signaling including ERK, Akt, PI3K, IkB, JNK and p38 was not altered in Siglec-15 deficient cells (data not shown).

Discussion:
This study shows that Siglec-15 is a potential therapeutic target for postmenopausal osteoporosis. Although Siglec-15 deficiency did not completely block estrogen-deficiency induced bone loss, it reduced the estrogen-deficiency induced bone loss to less than half of WT mice.

Early studies have shown that upregulated production of TNFα by activated T cells is a primary mechanism for estrogen deficiency-induced bone loss [2]. Although Siglec-15 deficiency does not affect known intracellular signaling downstream of TNFR1, Siglec-15 deficient osteoclasts were morphologically abnormal and showed impaired bone resorptive function, suggesting that Siglec-15 is involved in TNFα induced osteoclastogenesis. Our data suggests that blocking of Siglec-15 reduces bone resorptive activity of osteoclasts in acute estrogen deficiency and that Siglec-15 will be a potential therapeutic target for postmenopausal osteoporosis.

**Significance:**

This is the first study to show the relationship of Siglec-15 in postmenopausal osteoporosis. Although further studies are required to confirm our speculation, Siglec-15 may be a target molecule for a novel and effective strategy for the treatment of postmenopausal osteoporosis.

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**References:**

Figure 2. Impaired osteoclast development and bone resorption after ovariectomy in Siglec-15⁻/⁻ mice. A. Micrographs of the proximal tibia of WT and Siglec-15⁻/⁻ mice stained with TRAP and methyl green. B. Histomorphometric parameters at the secondary spongiosa.
Figure 3. Siglec-15 deficiency results in abnormal TNFα-induced osteoclast development in vitro. A. Differentiation efficiency of bone marrow macrophages into TRAP-positive multinuclear cells is suppressed in Siglec-15−/− cells compared to WT cells. B. BMMs were cultured in the presence of M-CSF and RANKL on bovine bone slices for 10 d. The percentage of substrate resorption (brown areas) was quantified. C. Osteoclasts generated on bovine bone slices were stained with anti-Siglec-15 antibody, Alexa Fluor 633 phalloidin (red), and DAPI.