MALPs Promote Osteoclastogenesis in Bone Remodeling and Pathologic Bone Loss

Wei Yu, MD/PhD[1]
Leilei Zhong, PhD[1]
Lutian Yao, MD/PhD[1]
Yulong Wei, MD[1]
Tao Gui[1]
Ziqing Li, MD[2]
Hyunsoo Kim, PhD[3]
Nicholas Holdreith[4]
Xi Jiang, MS/MD[1]
Wei Tong, PhD[4]
Nathaniel Dyment, PhD[1]
Xiaowei S. Liu, PhD[1]
Shuying Yang[2]
Yongwon Choi, PhD[3]
Jaimo Ahn, MD/PhD[1]
Ling Qin, PhD[1]

[1]Department of Orthopaedic Surgery
Perelman School of Medicine,
University of Pennsylvania

[2]Department of Basic & Translational Sciences
Penn Dental Medicine,
University of Pennsylvania

[3]Department of Pathology and Laboratory Medicine
Perelman School of Medicine,
University of Pennsylvania

[4]Department of Pediatrics,
University of Pennsylvania
Perelman School of Medicine, University of Pennsylvania

Disclosures

None

Introduction

Bone is maintained by coupled activities of bone-forming osteoblasts/osteocytes and bone-resorbing osteoclasts. Osteoclast differentiation predominantly depends on the signal from RANKL (encoded by *Tnfsf11* gene) and is modulated by other cytokines and growth factors. It is well known that osteogenic cells, particularly osteocytes, support osteoclastogenesis. Osteogenic cells are derived from mesenchymal stem cells (MSCs), which also give rise to marrow adipocytes. Using single cell RNA-sequencing (scRNA-seq), we recently discovered a new bone marrow cell population, marrow adipogenic lineage precursors (MALPs), which functions to maintain vessel structure and inhibit bone formation[1]. Our scRNA-seq datasets also contained many hematopoietic cells, including osteoclasts. To our surprise, our in-silico data indicated that MALPs, but not osteoblasts or osteocytes, are the most supportive cells for osteoclast formation. To validate this finding, we constructed adipocyte-specific *Tnfsf11 CKO* mice to investigate the role of MALP-derived RANKL in regulating bone remodeling under physiological and pathological conditions.

Methods

Animals

All procedures were approved by our institution's Animal Care and Use Committee. *Col2-Cre Tomato* (*Col2/Td*) mice, *Adipoq-Cre Rosa-tdTomato 2.3kbCol1-GFP* (*Adipoq/Td/Col1-GFP*) mice, *Adipoq-Cre Tnfsf11*$^{flox/flox}$ (*RANKL CKO*^{Adipoq}) mice and their *WT* siblings (*Tnfsf11*$^{flox/flox}$) were generated. Mice received a PBS or Lipopolysaccharides (LPS, 25mg/kg) injection above calvariae at 1.5 month of age and were harvested 7 days later. Female mice at 3 months of age received ovariectomy surgery and vertebrates were harvested 1.5 month later.

ScRNA-seq analysis

Td^+ cells sorted from bone marrow of 1-month-old (2 batches, n = 5) and 3-month-old (1 batch, n = 3) male *Col2/Td* mice were subjected to 10X Genomics library construction and sequencing. Unsupervised clustering was conducted by Seurat and trajectory analysis was conducted by Monocle.

μCT

Bones were scanned by vivaCT 35 (Scanco Medical AG) at a resolution of 6 and 15 μm for trabecular/cortical bone and calvaria analysis, respectively.

Histology and bone histomorphometry

Bones were processed for paraffin and frozen sections for immunostaining, TRAP staining, and dynamic histomorphometry.

Statistics

Data are expressed as means±SD and analyzed by Student's t-test or two-way ANOVA.

Results

We obtained a total of 9952 mesenchymal cells and 6977 hematopoietic cells, clustered in 18 groups, in our scRNA-seq dataset (Fig. 1A). Computational analysis of monocyte-macrophage lineage cells generated one monocyte cluster (Mono) at one end of the pseudotime trajectory, one macrophage cluster (Mϕα) at the branch point, one macrophage cluster (Mϕβ) at the 2nd end, one early osteoclast cluster (OC) and one late osteoclast cluster at the 3rd end (Fig. 1B), suggesting that monocytes undergo bi-lineage differentiation into mature Mϕβ cells and osteoclasts via Mϕα as the intermediate cell type. Ligand-receptor pair analysis suggested that MALPs, but not osteoblasts nor osteocytes, have the most signal interactions with macrophage lineage cells (Fig. 1C) due to their high expression of osteoclast regulatory factors, including RANKL (Fig. 1D). *Adipoq-Cre* has been used to label mature adipocytes in peripheral fat tissues. In bone marrow, Td^+ cells in 3-monthold *Adipoq/Td/Col1-GFP* mice labeled stromal cells, pericytes, lipid-laden adipocytes (LiLAs, which are hundred times less than MALPs[1]) but not osteoblasts, or osteocytes (Fig. 1E a-d), indicating that *Adipoq-Cre* is specific for MALPs in adult mouse bone.

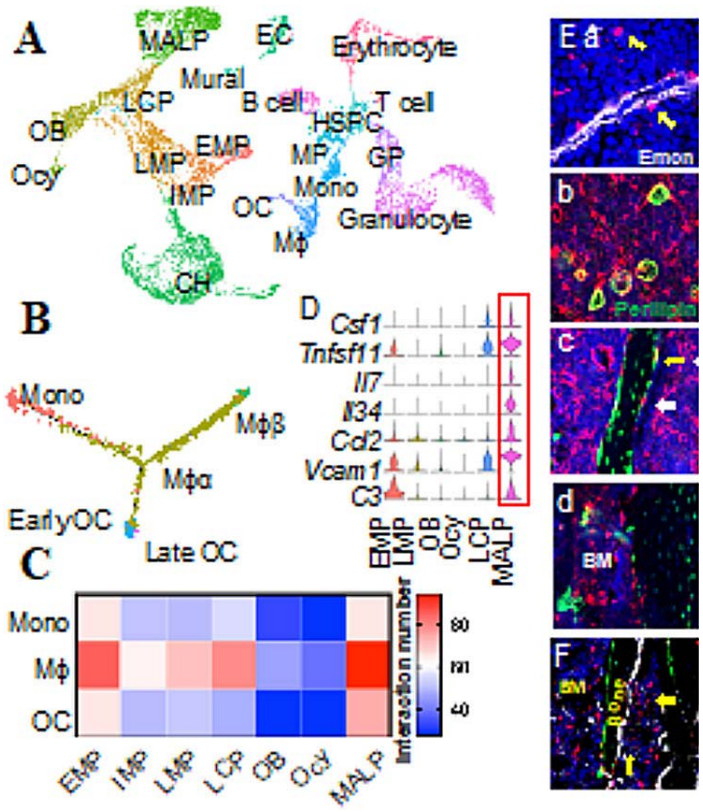

Figure 1. MALPs are the major source of RANKL that regulate osteoclastogenesis in vivo. (A) The UMAP plot of cells isolated from bone marrow of 1-3-month-old Col2/Td mice. EMP: early mesenchymal progenitor; LMP: late mesenchymal progenitor; OB: osteoblast; Ocy: osteocyte; LCP: lineage committed progenitor; CH: chondrocyte; EC: endothelial cells; HSPC: hematopoietic stem and progenitor cells; MP: monocyte progenitor; GP: granulocyte progenitor **(B)** Monocle trajectory plot of monocyte-macrophage lineage cells. **(C)** Ligand-receptor pair analysis of mesenchymal subpopulations with monocytes, macrophages, and osteoclasts. **(D)** Violin plots of osteoclast regulatory factors in mesenchymal subpopulations. **(E)** Representative fluorescent images of 3-month-old Adipoq/Td/Col1-GFP mouse bone reveal that Td labels stromal cells, pericytes (a), and Perilipin$^+$ adipocytes (b), but not osteoblasts or osteocytes (c, d). c: a yellow arrow points to a Td$^+$GFP- cell on bone surface and a white arrow points to a Td$^+$GFP$^+$ cell, which only accounts for 4% of GFP$^+$ osteoblasts/osteocytes. **(F)** Representative fluorescent TRAP staining image reveals that Td$^+$ MALPs touch nearby osteoclasts (white).

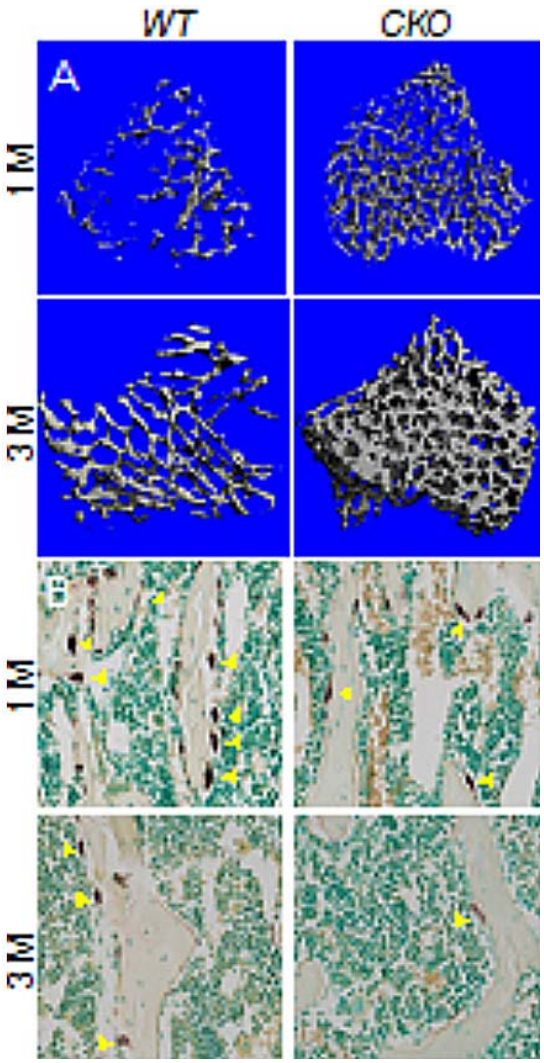

Figure 2. *RANKL CKO^{Adipoq}* mice have high trabecular bone mass. (A) 3D μCT images of tibial trabecular bone from *WT* and *RANKL CKO^{Adipoq}* mice at 1 and 3 months of age. **(B)** TRAP staining shows that osteoclasts are reduced at secondary spongiosa area in *RANKL CKO^{Adipoq}* mice.

Interestingly, we observed that bone attaching osteoclasts are often contacted by cell processes of neighboring Td$^+$ MALPs but rarely by osteoblasts (Fig. 1F). Strikingly, both male and female *RANKL CKO^{Adipoq}* mice (n = 5-6/group) displayed a drastic increase of trabecular bone mass (BV/TV) in long bones (1.6- and 2.9-fold, at 1 and 3 months of age, respectively, Fig. 2A) and vertebrates, due to a remarkable decrease of osteoclast number and activity (65% and 60%, Fig. 2B). Osteoblast number and activity was also reduced, implying a crosstalk between osteoclasts and osteoblasts. Growth plate and cortical bone were not affected. MALPs also exist in calvarial bone marrow. Osteolytic lesion in calvaria induced by LPS injections was completely abolished in *RANKL CKO^{Adipoq}* mice (n = 6/group) due to reduced osteoclast number (Fig. 3A, C). Furthermore, in ovariectomized mice (n = 5-6/group), elevated bone resorption in vertebrates was partially attenuated by RANKL deficiency in MALPs (Fig. 3B, D).

Discussion

In our study, we delineate the in vivo differentiation routes of osteoclasts and macrophages from bone marrow monocytes. MALPs, which form a multi-dimensional cell network in bone, are the most interactive mesenchymal subpopulation with monocyte-macrophage lineage cells in the bone marrow. We propose that osteoclast formation is controlled by a variety of mesenchymal cells in skeletal site-specific and disease-dependent manners. While osteocytes play a major role in promoting cortical bone resorption, MALPs predominately contribute to trabecular bone osteoclast formation, especially in young mice, because mice with osteocyte-specific deficiency of RANKL showed no or minor bone changes at 1 month of age[2].

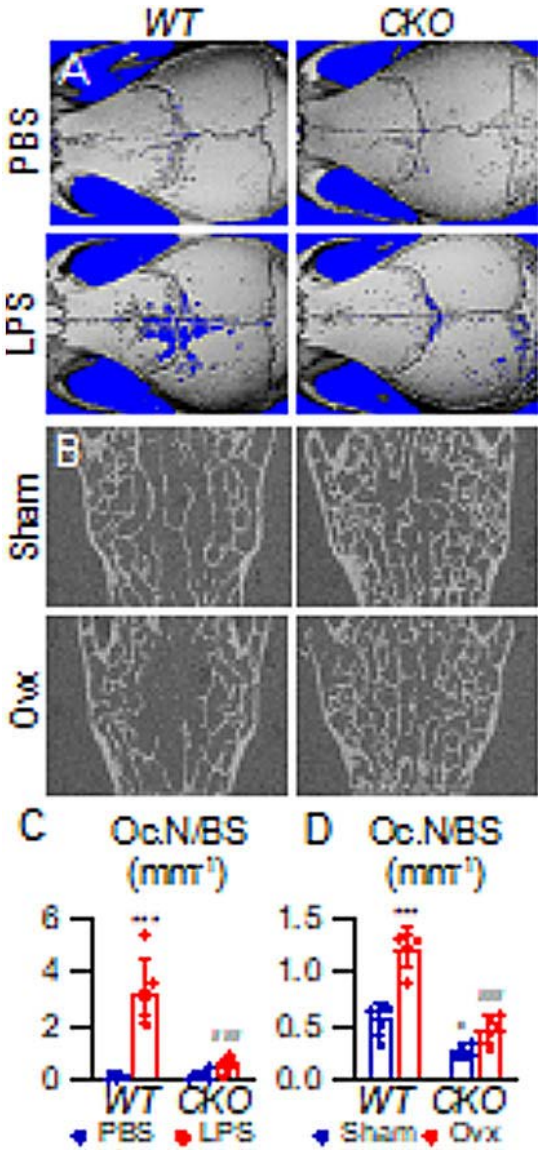

Figure 3. *RANKL CKO*^{Adipoq} mice are protected from pathologic bone loss. (A) Representative 3D μCT reconstruction of mouse calvaria at 1 week after vehicle (PBS) or LPS injections. **(B)** 2D μCT reconstruction of *WT* and *RANKL CKO*^{Adipoq} mouse vertebrate at 1.5 months after sham or ovx surgery. **(C)** Quantification of osteoclast number (Oc.N) in calvaria after injection. **(D)** Quantification of osteoclast number (Oc.N) in vertebrate after surgery.

Significance

MALPs are a critical player in controlling bone remodeling during normal bone metabolism and pathological bone loss in a RANKL-dependent fashion.

References

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2. Xiong J, Onal M, Jilka RL, *et al.* Matrix-embedded cells control osteoclast formation. *Nat Med* 2011; 17(10):1235-41.