Bone marrow adipogenic lineage precursors promote osteoclastogenesis in bone remodeling and pathologic bone loss

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Bone is maintained by coupled activities of bone-forming osteoblasts/osteocytes and bone-resorbing osteoclasts. Alterations in this relationship can lead to pathologic bone loss such as osteoporosis. It is well known that osteogenic cells support osteoclastogenesis via production of RANKL. Interestingly, our recently identified bone marrow mesenchymal cell population—marrow adipogenic lineage precursors (MALPs) that form a multidimensional cell network in bone—was computationally demonstrated to be the most interactive with monocyte-macrophage lineage cells through high and specific expression of several osteoclast regulatory factors, including RANKL. Using an adipocyte-specific Adipoq-Cre to label MALPs, we demonstrated that mice with RANKL deficiency in MALPs have a drastic increase in trabecular bone mass in long bones and vertebrae starting from 1 month of age, while their cortical bone appears normal. This phenotype was accompanied by diminished osteoclast number and attenuated bone formation at the trabecular bone surface. Reduced RANKL signaling in calvarial MALPs abolished osteolytic lesions after LPS injections. Furthermore, in ovariectomized mice, elevated bone resorption was partially attenuated by RANKL deficiency in MALPs. In summary, our studies identified MALPs as a critical player in controlling bone remodeling during normal bone metabolism and pathological bone loss in a RANKL-dependent fashion.

Introduction
Bone is a dynamic tissue, constantly undergoing remodeling through coupled activities of bone-resorbing osteoclasts and bone-forming osteoblasts/osteocytes. A shift in the balance of these 2 actions toward resorption leads to osteoporosis, an insidious disease characterized by excessive bone loss, microarchitectural deterioration, and increased risk of fracture. As a highly prevalent disorder, osteoporosis affects more than 75 million people in the United States, Europe, and Japan and is the underlying condition related to more than 8.9 million fractures annually worldwide (1).

Mature osteoclasts are large multinucleated cells derived from the monocyte-macrophage lineage of hematopoietic origin (2). They firmly attach to the bone surface and degrade bone matrix. Osteoclast differentiation predominantly depends on RANKL signaling (encoded by Tnfsf11 gene), a type II transmembrane protein of the TNF superfamily, and is modulated by other cytokines and growth factors (3). Tnfsf11−/− mice have no osteoclasts in bone and exhibit a severe osteopetrosis (high bone mass) phenotype (4, 5). Early studies indicated that osteoblasts and their progenitors are the major source of RANKL in bone to support osteoclastogenesis (6). Later, animal studies showed that osteoblast ablation does not affect osteoclast formation (7, 8). In growing mice, hypertrophic chondrocytes appear to be the main source of RANKL for bone resorption (9). In adult mice, osteocytes, the descendants of osteoblasts that are embedded in the bone matrix, have been demonstrated to be the major stimulator of osteoclastogenesis (9–11).

Osteoblasts and osteocytes are derived from bone marrow mesenchymal stem cells (MSCs), which also give rise to marrow adipocytes. Recently, we computationally delineated the hierarchy of mesenchymal lineage cells from MSCs to mature cells using large-scale, single-cell RNA-sequencing (scRNA-seq). Surprisingly, this study unveiled a new cell population, marrow adipogenic lineage precursors (MALPs), situating along the adipogenic differentiation route after mesenchymal progenitors and before classic lipid-laden adipocytes (LiLAs) (12). Labeled by mature adipocyte-specific Adipoq-Cre (13), MALPs are abundant, nonproliferative cells that express many adipocyte markers but have no lipid accumulation. Shaped as a central body with multiple cell processes, they exist as stromal cells and pericytes forming a 3D network.