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EMBO reports

# A critical relationship between bone and fat: the role of bone marrow adipose-derived RANKL in bone metabolism

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**Recent studies have unveiled unique functions of the bone marrow adipose tissue (BMAT), which represent over 10% of the total adipose tissue mass in healthy adults. Increasing evidence is emerging as to how BMAT deposition and osteoporosis are linked under normal and pathophysiological conditions, which is opening up novel treatment avenues. However, the means by which bone marrow adipocytes (BMAs) regulate bone remodeling and their involvement in osteoporosis remained unknown. A study in this issue of *EMBO Reports* (Hu *et al*, 2021) and a study in *Journal of Clinical Investigation* (Yu *et al*, 2021) reports independently that BMA-derived RANKL regulates osteoclastogenesis and bone remodeling, indicating that excessive RANKL generated by BMAs is an underlying cause for osteoporosis.**

**EMBO Reports (2021) 22: e52986**
See also: **Hu *et al*** (July 2021) and **Yu *et al*** (January 2021)

The activities of bone-forming (osteoblasts) and bone-resorbing (osteoclasts) cells are in a physiological equilibrium imperative to maintaining healthy bones. Breakdown of this equilibrium can lead to pathogenic bone disease conditions such as osteoporosis (brittle bone) and osteopetrosis (hardened bone). The receptor activator of NF-κB (RANK) and its ligand RANKL, a receptor–ligand pair of the TNF receptor superfamily, were identified as the critical regulators of osteoclast development and hence bone metabolism (Theill *et al,* 2002). RANKL/RANK regulates bone physiology by controlling the development, survival, and activity of osteoclasts. Osteoprotegerin (OPG) serves as a decoy receptor for RANKL and inhibits osteoclast development through competitive binding of RANKL (Fig 1) (Simonet *et al,* 1997).

In bone, RANKL is expressed by osteoblasts and osteocytes and binds to RANK on the membrane of osteoclast progenitors to promote osteoclastogenesis (Ono *et al,* 2020). Moreover, RANKL produced by B cells contributes to the induction of osteoclasts and excessive bone resorption induced by estrogen deficiency (Ono *et al,* 2020). Hypertrophic chondrocytes are an additional source of RANKL during juvenile development required for longitudinal bone growth. The RANKL/RANK pathway also controls many other physiological processes such as lymph node organogenesis, expansion of mammary gland progenitors, the formation of lactating mammary glands in pregnancy, or thymic rewiring in pregnancy.

Accounting for nearly 70% of bone marrow mass and over 10% of total adipose tissue mass in healthy adults, the BMAT was thought previously to play a "filler role" in the structural integrity of the skeletal system, which increases with age (Suchacki *et al,* 2020). Being distinctly different from brown, white, and beige adipocytes, bone marrow adipocytes have been identified as an independent class of adipocyte lineage. They are generated from distinct mesenchymal stem cell (MSC) progenitors (Horowitz *et al,* 2017) in the bone marrow that can differentiate into osteocytes, chondrocytes, and bone marrow adipocytes (Fig 1). Recent studies also revealed that the BMAT is involved in systematic energy regulation, by secreting adipokines such as adiponectin and leptin (Suchacki *et al,* 2020), as well as the formation of blood vessels in the bone marrow (Zhong *et al,* 2020). Complimentary to the increase in bone marrow adipocyte deposition seen with age, multiple studies have correlated increased BMAT concentration to reduced bone mass (i.e., osteoporotic presentation) (Horowitz *et al,* 2017). Importantly, bone marrow adipocytes (BMAs) also express RANKL (Horowitz *et al,* 2017). However, the mechanism by which BMAs regulate bone remodeling and how increased or dysregulation of fat tissue deposition in the bone results in bone loss remained largely unknown.

In the latest addition to this story, two independent studies published in this issue of *EMBO Reports* (Hu *et al,* 2021) and in *Journal of Clinical Investigation* (Yu *et al,* 2021) address the question how RANKL in BMAT

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**DOI** 10.15252/embr.202152986 | EMBO Reports (2021) 22: e52986 | Published online 13 June 2021

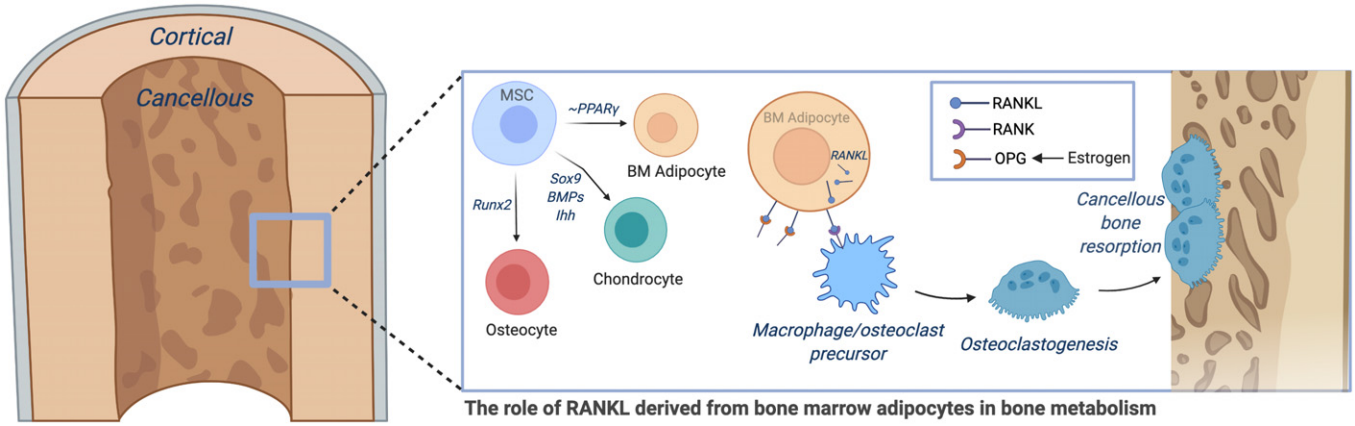


**Figure 1. The role of RANKL derived from bone marrow adipocytes in bone metabolism.**

The diagram illustrates the proposed role of bone marrow adipocyte (BMA)-derived RANKL in cancellous bone remodeling. Estrogen deficiency decreases OPG levels and increases BMA-derived surface RANKL binding to RANK on osteoclast progenitors resulting in subsequent osteoclast development and, under pathologic conditions, excessive bone resorption. The figure was created with BioRender.com.

regulates osteoclastogenesis and bone remodeling. Using single-cell RNAseq, the study by Yu *et al* (2021) demonstrates that adipose lineage cells in bone marrow are a source of RANKL. They crossed *rankl*-floxed mice with the adiponectin promoter-driven Cre (adipoq-Cre) line to generate *rankl*-deleted mice specifically in adipose lineage cells ($Adipoq^{cre}$; $rankl^{fl/fl}$). Under physiological conditions, $Adipoq^{cre}$; $rankl^{fl/fl}$ mice display impaired osteoclast formation and reduced bone resorption. The authors also show that loss of RANKL production had no effect on adipogenic or osteogenic differentiation from mesenchymal stromal progenitors (Yu *et al*, 2021).

Hu *et al* confirmed these findings by studying the impact of RANKL in diseased states. First, the authors removed estrogen via ovariectomy to induce osteoporosis. Using the same genetic approach to delete RANKL in adipose cells ($Adipoq^{cre}$; $rankl^{fl/fl}$), they observed no decrease in cancellous bone density, which was observed in ovariectomized control mice. Additionally, no alterations in cortical bone thickness or density were observed in these RANKL-ablated mice. As bone loss from estrogen deficiency was not reduced by blocking soluble RANKL, the authors concluded that membrane-bound RANKL expressed by BMAs has an important role in bone loss under estrogen-deficient conditions. To address the role of RANKL in pathologically increased BMAT, $Adipoq^{cre}$; $rankl^{fl/fl}$ mice were administered rosiglitazone. $Adipoq^{cre}$; $rankl^{fl/fl}$ mice showed impaired osteoclast formation and reduced bone loss, compared to control mice which suffered significant bone loss. Rosiglitazone treatment increased bone marrow adipogenesis in the control and *rankl* mutant mice. These results suggest excessive osteoclastogenesis and bone resorption are induced by RANKL expression from abundant BMAs observed in increased BMAT (Fig 1).

Thus, these two independent publications (Yu *et al*, 2021; Hu *et al*, 2021) found that BMAs play a key role in bone remodeling dynamics through RANKL/RANK-dependent regulation of osteoclastogenesis under physiological and pathological conditions. The authors of both studies propose that proper regulation of bone marrow adipogenesis and RANKL signaling induced by BMAs is crucial to avoid osteoporotic pathogenesis. Specific targeting of BMAs may thus serve as a novel therapeutic approach in osteoporosis and metabolic disease. However, it is paramount to further investigate the underlying mechanisms governing bone marrow adipogenesis to identify potential drawbacks and highly effective and safe methods of controlling BMA differentiation.

A word of caution is necessary: There are several studies questioning whether adipoq-Cre is specific for adipocytes. From the study from $Adipoq^{cre}$; $rankl^{fl/fl}$ mice (Yu *et al*, 2021) and (Hu *et al*, 2021), bone marrow adipocyte-derived RANKL seems to play a critical role in bone remodeling in cancellous bone. However in *Adipoq-Cre* reporter mice, a few osteocytes and osteoblasts are Cre-positive in aged mice (Yu *et al*, 2021); (Mukohira *et al*, 2019). Moreover, adiponectin expression was reported in primary human osteoblasts (Berner *et al*, 2004). Since adiponectin (Adipoq) is a marker of mature adipocytes as well as their progenitors, this non-specific expression of Cre could be explained by the fact that bone marrow adipocytes, osteoblasts, and osteocytes all originate from the same MSC progenitors.

Thus, although the extensive data of Yu *et al* (2021) and Hu *et al* (2021) reveal an important role of BMA-derived RANKL in osteoclastogenesis, bone remodeling, and osteoporosis, additional experiments using different constitutive or inducible Cre lines are warranted to come to a definitive conclusion. These two important studies will certainly stimulate new avenues for future research and therapeutic advancement.

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