

Osteoclast Differentiation and Bone Disease: The RANKL Angle

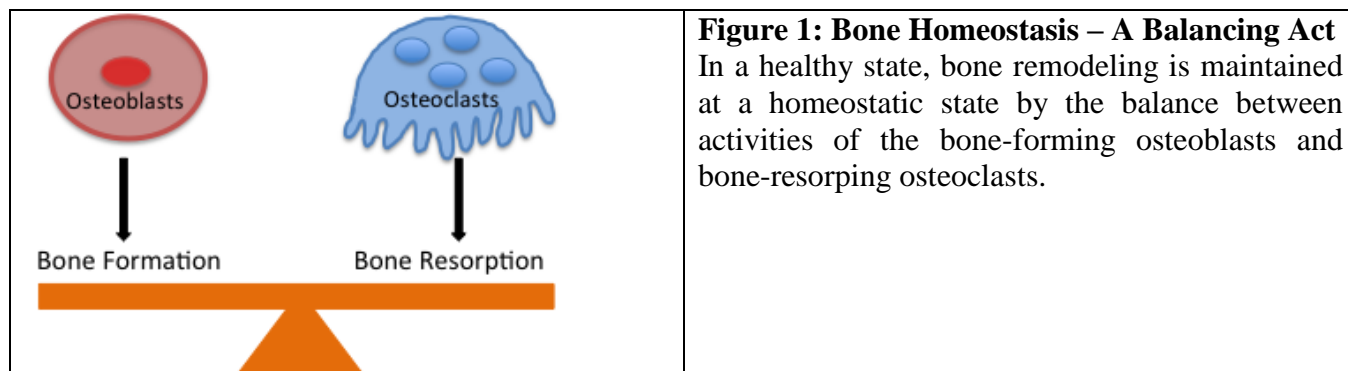
Bone Remodeling and Osteoclasts:

Bones are complex, living organs that distinguish vertebrates from other animals. Bones serve multiple purposes in the human body, including assisting in mobility, serving as warehouses for the production of blood cells, and functioning as reservoirs for storage and release of essential minerals like calcium. While the bone might appear rigid and inert, it is in fact a dynamic organ that undergoes constant remodeling (Crockett et al., 2011). Estimates show that approximately 10% of the total human bone mass is remodeled every year (Jones et al., 2002). Bone remodeling, also termed bone metabolism, is a life-long process required for many skeletal functions including bone growth, fracture healing, tooth eruption, and maintenance of steady state calcium levels in the blood. Constituent skeletal cell types involved in the bone remodeling cycle are the bone-forming osteoblasts and the bone-resorping/degrading osteoclasts (Crockett et al., 2011; Roodman, 1999) (Figure 1). In young adults, the integrity of the skeletal system is maintained by a delicate equilibrium between osteoblast and osteoclast numbers and functions. With increasing age, and in certain forms of disease, the balance is tipped in favor of osteoclasts, leading to perturbation of bone remodeling. As a result, an increase in bone resorption occurs, sometimes leading to brittle bones and fractures. Conversely, certain pathological bone diseases are associated with reduced bone resorption thereby leading to conditions like osteopetrosis, which is characterized by abnormally dense bones (Rodan and Martin, 2000).

Osteoclasts are highly specialized, large, multinucleated cells, with the unique ability to dissolve components of the bone during bone development and remodeling. Derived from the hematopoietic precursors of the monocyte-macrophage lineage, their path to differentiation is common to that of dendritic cells and macrophages. A pluripotent hematopoietic stem cell gives rise to a pro-myeloid precursor, which upon exposure to the right stimuli, further differentiates and fuses to form the characteristic multi-nucleated, mature osteoclast (Boyce et al., 2009; Boyle et al., 2003). Given that deregulated osteoclast-mediated bone resorption is implicated in a number of skeletal diseases, there is a great deal of interest in unraveling the molecular mechanisms of osteoclastogenesis (development of osteoclasts), with an emphasis on generating pharmacological agents that inhibit either the formation or activity of osteoclasts (Rodan and Martin, 2000).

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RANKL/RANK/OPG Signaling Pathway in Osteoclastogenesis:

Identification of the receptor activator of nuclear factor- κ B ligand (RANKL)/ receptor activator of nuclear factor- κ B (RANK)/osteoprotegerin (OPG) signaling axis as a key regulator of osteoclastogenesis served as a big breakthrough in the field of bone biology (Boyce and Xing, 2007). Experimental evidence had previously shown that proximity of bone marrow to stromal cells is essential for osteoclast formation, suggesting a critical role for a stromal-derived factor in osteoclastogenesis (Takahashi et al., 1988). Subsequent research revealed that interactions between stromal and bone marrow cells aided in osteoclastogenesis through the secretion of various molecules including the TNF family-related cytokine, RANKL (Lacey et al., 1998) (Nakagawa et al., 1998) (Figure 2).

RANKL, a type II membrane protein is expressed by stromal cells/osteoblasts and can either be membrane bound or secreted. The expression of RANKL is triggered by factors like calcitropic hormones and pro-resorptive cytokines that mediate osteoclast differentiation (Khosla, 2001). RANKL-induced osteoclastogenesis is initiated when RANKL binds to its cognate receptor RANK, which is expressed by pre-osteoclasts. The binding of RANKL to RANK triggers a cascade of signaling events resulting in activation and translocation of nuclear factor- κ B (NF- κ B) to the nucleus, and culminates in the expression of differentiation-inducing osteoclast-specific genes. These events cumulatively lead to the differentiation and activation of mature osteoclasts. The activity of RANKL is counter-balanced by OPG, a naturally occurring decoy receptor secreted by stromal cells and osteoblasts. OPG is a soluble, secreted glycoprotein and was the first molecule to be discovered in the TNF family member of proteins. Thus, the rate of bone turnover is regulated by a balance between expression levels of RANKL and OPG. Although best known for its role in osteoclastogenesis, RANKL signaling also plays important roles in development of lactating mammary gland during pregnancy, and in the regulation of T cell-dependent immune responses (Anderson et al., 1997; Fata et al., 2000).

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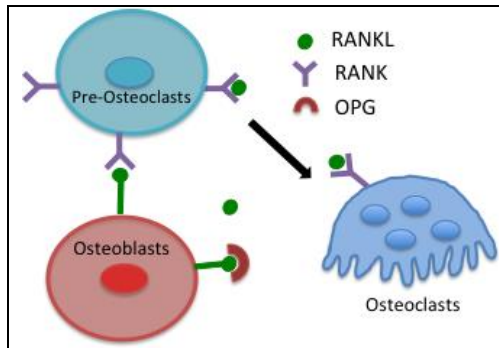


Figure 2: RANKL Signaling in Osteoclast Differentiation

RANKL, expressed by osteoblasts, interacts with its cognate receptor RANK on the surface of pre-osteoclasts and mature osteoclasts. The interaction leads to osteoclast differentiation, maturation, and survival, thereby promoting bone resorption.

Animal Models and Bone Diseases:

Aberrant regulation of osteoclast number and/or activity contributes to the pathogenesis of several skeletal diseases (Figure 3). Since signaling through the RANKL/RANK pathway is critical for osteoclastogenesis, it is not surprising that modulation of the pathway has been explored in different bone pathologies (Boyce and Xing, 2007). Our understanding of bone pathologies has been greatly advanced by the availability of animal models, both large and small. While rodent models offer the advantages of easy genetic manipulation and high breeding capacity, larger animal models like sheep, canines, and porcine are more akin to humans in their bone properties, bone micro and macrostructures, and in the process of bone remodeling.

Loss-of-function mutations of RANK/RANKL or a block in the RANKL signaling pathway are associated with defects in osteoclast activity and bone resorption. RANKL^{-/-} mice display severe osteopetrosis, a condition characterized by abnormally high bone density (Kong et al., 1999). This phenotype can be attributed to an intrinsic defect in the ability of osteoblasts from RANKL^{-/-} mice to support osteoclastogenesis, leading to a deficit in bone resorbing-osteoclasts. Exogenous administration of recombinant RANKL restores osteoclast formation and activity *in vivo*. RANK^{-/-} mice phenocopy the RANKL^{-/-} mice, further highlighting the importance of the RANKL-RANK pathway in maintaining bone homeostasis (Li et al., 2000). These mouse models elucidated a physiological role for the RANKL pathway in regulating osteoclastogenesis. The RANKL^{-/-} mouse model has been used to gain insight into human bone pathologies such as autosomal recessive osteopetrosis (ARO) a rare disorder in humans, caused by a defect in bone resorption. RANKL^{-/-} mice closely resemble the human condition and studies have shown that administration of soluble RANKL to the RANKL^{-/-} mice significantly reduces the bone defects associated with the disease. These studies thereby pave the way for further exploring the role of soluble RANKL as a therapeutic tool in humans with ARO (Lo Iacono et al., 2012).

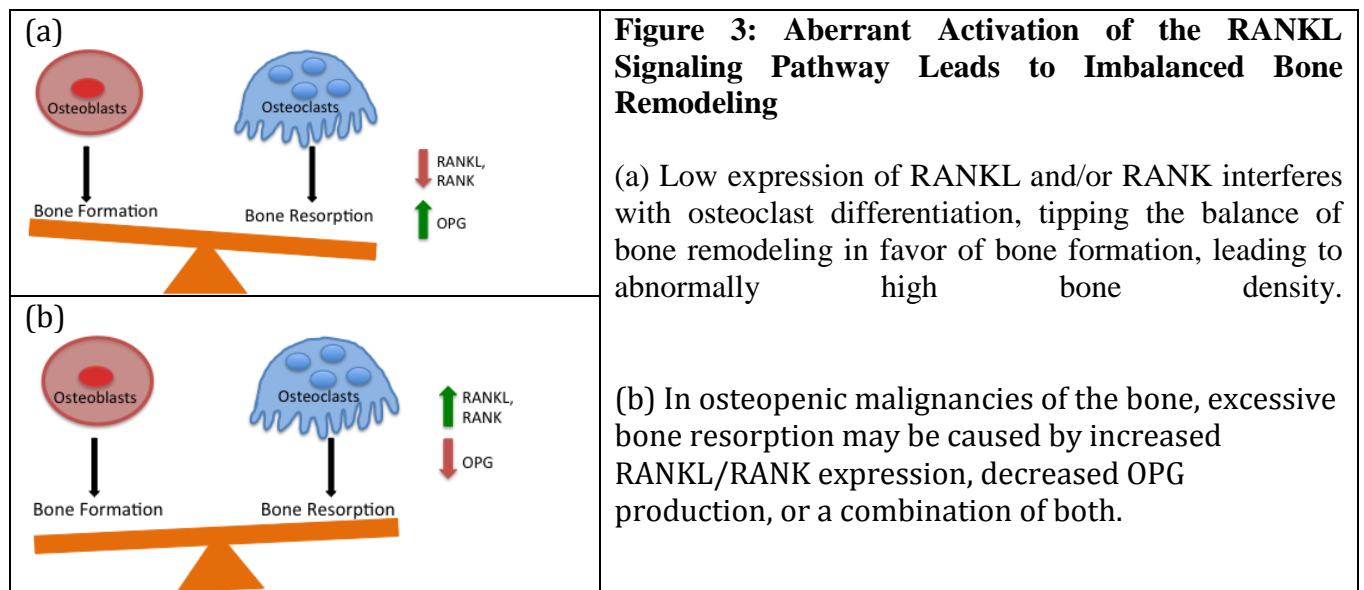
As mentioned earlier, hyperactivation of the RANKL signaling pathway, which is often associated with high expression of RANK /RANKL, is observed in a slew of osteopenic conditions including osteoporosis, arthritis, and other bone malignancies. An increase in the number of RANKL- and RANK-positive cells have been observed in the elbow joints of arthritic canines compared to healthy

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canine bone tissues, and this is proposed to contribute to the pathologic bone resorption (Spahni et al., 2009). Expression of RANKL is also observed in bone neoplasms of dogs and cats (Barger et al., 2007). Osteosarcoma is far more prevalent in animals compared to humans, providing a strong rationale to further investigate RANKL pathway inhibition as a therapeutic strategy for veterinary bone malignancies. Blocking the RANKL signaling pathway has already proven successful in many disease models. For instance, in some rodent models of arthritis, blocking the activity of RANKL by expressing its natural decoy receptor, OPG, prevents bone loss. Of great clinical relevance is the development of denosumab, a human monoclonal antibody that serves as a targeted therapy available for the treatment of certain bone diseases in humans (Jones et al., 2002). Denosumab binds to and inhibits RANKL, thereby preventing the maturation of osteoclasts, effectively serving as a brake on uncontrolled bone resorption (Kostenuik et al., 2009).

In conclusion, unraveling the molecular underpinnings of the RANKL signaling pathway has enabled a giant leap in the understanding of osteoclastogenesis, and has opened several avenues for therapeutic intervention in bone diseases. While the focus of this review is the RANKL system in the bone, as discussed before, the RANKL signaling axis also plays roles in other functions beyond bone remodeling. Future research efforts are needed to illuminate the roles of RANKL/RANK/OPG pathway in these alternate organ systems in health and disease. Also, by no means is RANKL the only regulator of bone homeostasis and it is vital to consider the contributions of other signaling cascades in bone maintenance. A holistic understanding may help elucidate promising combinatorial approaches in the battle against bone diseases, and help alleviate pain and discomfort in humans and their companion animals. The development of reagents to enable the study of relevant animal models is therefore critical to ensure success in these endeavors.



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