

Osteoclast biology in bone resorption: a review

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ABSTRACT

What we know about bone resorption has changed a lot in the last few decades. The osteoclast is the only cell to nibble and break down the bone, and in the formation and resorption of bone tissue, osteoclasts play an important role. Once the balance of bone formation and bone loss is out of control, diseases like osteopetrosis and osteoporosis occur. Bone resorption is a unique function of osteoblasts, which are multinucleated cells formed by the fusion of mononuclear progenitor cells of the monocyte/macrophage family. In the formation of osteoclasts, there are two main factors affecting this process, macrophage colony-stimulating factor (M-CSF) and ligand-activated receptor (RANKL) of nuclear factor kappa B (NF- κ B). The identification of RANK-RANKL signaling and other classic signaling pathways such as Wnt and Notch, as the major signaling regulation in osteoclast differentiation, was a significant breakthrough in the field of osteoclastogenesis. In this review, we briefly describe the latest knowledge of osteoclast-induced bone resorption and cellular factors that regulate the activity of osteoclasts and cell fusion, for the purpose of understanding osteoclastogenesis and the development of drugs that enhance bone resorption to improve pathological bone diseases.

Keywords: Osteoclast · Bone resorption · M-CSF · RANKL pathway · Cell fusion

Introduction

Bone is the hardest organ in the body that makes up the internal bones of vertebrates. Its function is moving, supporting and protecting the body and storing minerals. One of the components of the bone is mineralized skeletal tissue with a solid honeycomb-like 3D structure inside, and there are tissues include bone marrow, periosteum, nerves, blood vessels and cartilage in the bone.

Osteoclasts are described as the only cells to be capable of destroying the bone tissue. They express markers that are thought to be specific to skeletal tissues, such as α v β 3 integrin, cathepsin K (CTSK), calcitonin receptor, and tartrate-resistant acid phosphatase (TRAP) (1). The osteoclast is essential for the two basic processes of bone biology, the first is bone modeling, which forms bone elements and ensures the correct shape and density of the bone. The second process is bone remodeling, and the mechanism of bone remodeling ensures bone tissue

renewal and adapting to the environment. The balance of bone remodeling and resorption is essential for health. When the functions of osteoclasts and osteoblasts are disordered, it will cause bone diseases. The exacerbated bone resorption is associated with osteoporosis, rheumatoid arthritis and periodontal disease, while decreased bone resorption leads to osteopetrosis, a rare genetic disease. On the other hand, except for the function of bone resorption, osteoclasts are able to regulate cells in the bone marrow (2). We give this concise summary of previous work to better understand the osteoclast differentiation and identification of potential therapeutic targets (3).

The differentiation of osteoclasts

There are two basic groups in the blood cells which are differentiated by hematopoietic stem cells (HSCs): lymphoid lineage and myeloid lineage, such as macrophages. Osteoclasts are derived from the hematopoietic system (4). In the bone marrow, HSCs experience several differentiation and self-renewal, each subtype of the cell has a specific surface marker. There are four stages in the osteoclast differentiation: 1. HSCs differentiate into multipotential progenitor cells (c-Fms-

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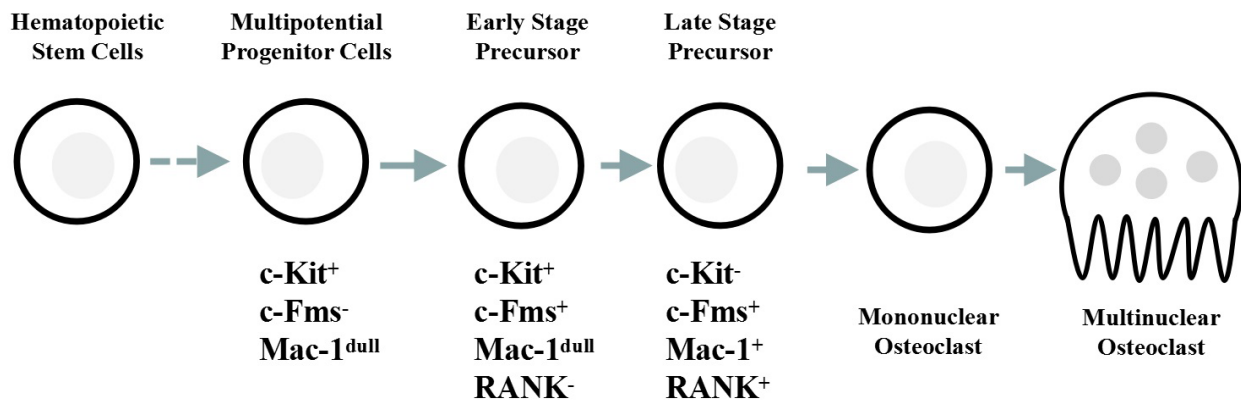


Figure 1. Stages in osteoclast differentiation. Phenotypic changes during the osteoclast differentiation and regulatory cytokines in the maturation and differentiation of osteoclasts.

c-Kit⁺, Mac-1^{dull}); 2. multipotential progenitor cells differentiate into early-stage precursor (c-Fms⁺, c-Kit⁺, Mac-1^{dull}, RANK⁻) and late-stage precursor (c-Fms⁺, c-Kit⁺, Mac-1⁺, RANK⁺); 3. precursors differentiate into mononuclear osteoclasts; 4. mononuclear osteoclasts differentiate into multinuclear osteoclasts (**Figure 1**) (5). D. G. Walker found that the capacity of resorbing bone was restored in osteopetrosis mice with intravenous administration of normal spleen and bone marrow cells (6). Interestingly, the dendritic cells are undergoing trans-differentiation into functional osteoclasts by stimulated with the microbe and the presence of receptor activator of nuclear factor kappa B (NF- κ B) ligand (RANKL). Furthermore, The activation of dendritic cell-derived osteoclasts can be inhibited by aspirin which downregulates the expression of NFATc1 via the NF- κ B pathway (7).

The differentiation of osteoclasts is mainly regulated by two critical cytokines, RANKL and macrophage-colony stimulating factor (M-CSF). PU.1 is a domain transcription factor of myeloid, B-lymphoid cells also regulate the transcription of c-fms and CD11b/CD18 which controls the osteoclast phenotype (8).

Mature osteoclasts are large whose size is up to 100 μ m, multinucleated and polarized, firmly adhering to the surface of the bone. In the process of bone resorption, there are four different cell structures of osteoclast: 1. sealing zone, isolates the resorptive area from the extracellular environment; 2. ruffled board, facing the bone matrix, is composed of the plasma membrane to absorb the bone matrix; 3. basolateral membrane, facing the vascular compartment, is participating in bone resorption, which contributes to transporting the bone degradation products; 4. functional secreted domain.

Factors and pathways which regulate the formation of osteoclasts

M-CSF is critical for osteoclastogenesis (5). It is well known that the differentiation of osteoclasts needs two key molecules: NF- κ B ligand RANKL and M-CSF (9). Osteoclasts are derived from the monocyte/macrophage cell line, and PU.1 controls the switch of activation of

stem cell precursor. PU.1 drives a positive regulation of the M-CSF receptor, called c-fms. In the next step, the precursor becomes an osteoclast lineage by M-CSF and RANKL. On the other hand, macrophage or B lymphocyte can also trans-differentiate into an osteoclast. C-fms supports the progenitors to survive and give rise to osteoclastogenesis by inducing the expression of RANK. Malt1^{-/-} mice induced M-CSF production which played an important role in osteoclastogenesis and decreased osteoprotegerin (OPG) production while in the presence of inflammatory stimuli (10). GM-CSF can induce bone marrow-derived macrophages to differentiate to dendritic cells (11). M-CSF knockout mice (op/op mice) causes osteopetrosis by inducing the numbers of osteoclasts (12). RANKL is one member of the tumor necrosis factor superfamily and belongs to type II transmembrane protein. It is crucial for bone metabolism. It is a membrane protein (also known as CD254) on the osteoblast membrane that can activate the osteoclast and accelerate the formation of osteoclast and bone loss, which is important for bone regeneration. When RANKL on the osteoblast membrane activates the RANK protein on the osteoblast membrane, osteogenesis will begin (13). RANK is the critical receptor that mediates the function of osteoclasts on bone resorption and remodeling (14). Study shows that, when RAW264.7 type CRL-2278 cell line was cultured in the hydroxyapatite surface, the hydroxyapatite induces autocrine of RANKL and RANK by monocyte/macrophage cells to differentiate itself into osteoclasts (15). NF- κ B is an essential factor for the differentiation of osteoclasts (16). The overexpression of OPG results in osteopetrosis by decreasing the differentiation of osteoclasts (**Figure 2**) (17).

Several kinds of research have shown that the RANK/RANKL/OPG pathway is a key signal pathway in bone metabolism and bone diseases resulting in imbalances in bone formation and resorption. RANK^{-/-} mice (18), RANKL^{-/-} mice (14), and the overexpression of OPG in rat (19) have shown symptoms of osteopetrosis. On the other hand, adolescent and adult OPG^{-/-} mice developed osteoporosis and arterial calcification (20). The RANK/RANKL/OPG pathway tightly and precisely controls the balance between bone formation and resorption. Some

bone diseases in human patients such as familial expansile osteolysis (FEO) and the familial form of early-onset Paget's disease of bone (PDB2) occur when this process is out of control. In 2010, an investigation has found an extremely rare bone disease called dysosteosclerosis in a 3-year-old girl, the "Osteoclast-Poor" symptoms are similar to osteopetrosis caused by the deficiency of osteoclasts, but there are no mutations in the genes that encode RANK, RANKL, OPG or M-CSF (21). How the lack of bone resorption occurs in this disease is still unclear.

Recent research has found that the salt-inducible kinase (SIK) signaling pathway takes part in the checkpoints of controlling the osteoclast formation. SIK inhibitors may denote a potential new treatment for preventing bone erosion (22). The SIKs are a serine/threonine kinase subfamily which belongs to the AMP-activated protein kinase (AMPK) family (23). SIK inhibitors can reduce the expression of osteoclast differentiation markers, such as TRAP and CTSK. Furthermore, the levels of c-Fos and NFATc1 proteins have an extreme downregulation when SIK inhibitors are present (22). Pkn3 bound to c-Src is critical for the activity of bone resorption by osteoclasts, while in the Wnt5a-Ror2 signal pathway, Pkn3 bound to c-Src can enhance the activity of osteoclasts (24). The Wnt signaling pathway has been extensively reported in osteoblasts lineage, but less known in osteoclasts. Recent studies found that early Wnt3a treatment inhibited the activation of NFATc1, which was also activated during macrophage differentiation into osteoclasts (25). Moreover, injection of microRNA-410 or downregulated Wnt-11 inhibited osteoclast function in osteonecrosis of the femoral head (26). On the other hand, the Notch signaling pathway plays a different role in osteoclastogenesis: in the osteoclast precursor cells, the stimulation of the Notch signal leads to large osteoclast with numerous nuclei and the activity of resorption but depresses the small osteoclast resorptive activity (27).

Osteoclastic bone resorption

The cytoskeleton of osteoclasts is unique because the polarization forms different sections to comply with different functions. The ruffled border is the isolated structure that shapes an actin-ring or sealing zone to segregate the absorption microenvironment from the outer space of the cell. This process needs the presence of $\alpha\beta 3$ integrin. $\alpha\beta 3$ integrin combined with the M-CSF receptor c-Fms to activate a signaling pathway constituted with Vav3, Syk, Dap12, c-Src, Slp76 and Rac which switches on the formation of actin-ring (28). $\beta 3$ gene knockout mice show that $\beta 3^{-/-}$ cells are still multinucleated and express TRAP but are not able to form normal actin-ring, indicating osteoclast cytoskeleton dysfunction (29). In the contacting area between osteoclasts and bone surface, matured osteoclasts release enzymes like CTSK and TRAP to destroy the minerals. In healthy physical condition, there is high expression of TRAP by osteoclasts, activated macrophages and neurons (30). Under some pathological circumstances, TRAP expression is increased. These

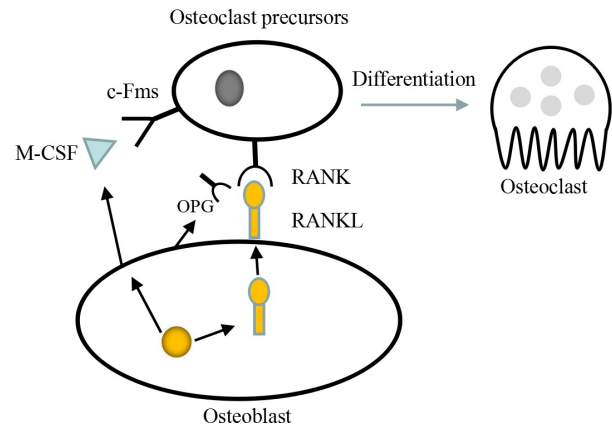


Figure 2. Regulation of osteoclast differentiation by osteoblasts through M-CSF and RANKL. RANKL and M-CSF expressed by osteoblasts are essential for osteoclast differentiation and fusion into multinucleated cells. On the other hand, OPG inhibits osteoclast differentiation by blocking the combination of RANKL and RANK.

diseases include osteoclastoma and osteoporosis, as well as metabolic bone disease. CTSK is expressed mainly in osteoclasts, which is a protease that performs the function of bone resorption by catabolizing elastin, collagen and gelatin to break down bone tissues (Figure 3) (31).

Recently, some evidence has shown that the inhibitors of CTSK like 2-(3-(2-fluoro-4-methoxyphenyl)-6-oxo-1(6H)-pyridazinyl)-N-1H-indol-5-ylacetamide is a potential therapeutic drug for osteoporosis (32). Kent et al using the specific marker and time-lapse found that the resorption model of osteoclasts was not only the formation of a round pit, more importantly and more aggressively, there were long trenches that osteoclasts resorbed while moving (33).

To develop the capability of resorbing bone matrix, polarization is required. Through an area named sealing zone, osteoclasts can communicate with the bone and form an isolated zone with the bone matrix. This process requires the participation of molecules like c-Src, vitronectin receptor, carbonic anhydrase and CTSK (34). The osteoclasts secrete protons through the ruffled border membrane to acidify the bone surface. In this process, carbonic anhydrase enzyme transports protons by V-ATPase. Thus, the creation of pH 4.5 in isolated area decomposes bone mineral components and exposes the organic material such as type I collagen, which can be decreased by CTSK (35).

Cellular fusion in osteoclastogenesis

The fusion of plasma membranes is a common phenomenon in almost every cell, such as the fusion of intracellular membranes which makes hormone secretion and neurotransmission possible. On the other hand, less is known about the mechanisms by which intercellular fusion occurs during osteoclastogenesis. Cell-cell fusion is a fundamental process in the generation of osteoclasts and multinucleated giant cells (36). The progenitor cells of osteoclast move to some specific location of hard tissues through the blood vessel, then these cells gradually form a

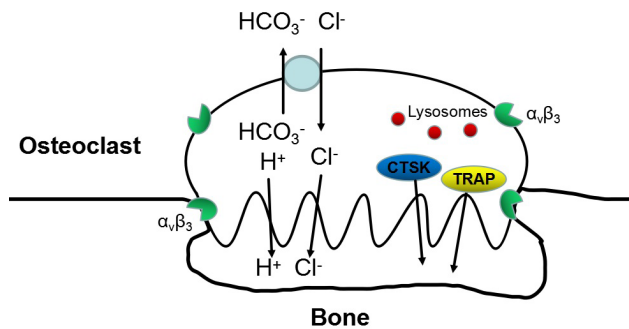


Figure 3. Mechanism of bone resorption. Osteoclasts release acid to the bone surface. Under acidic conditions, the inorganic minerals of bone are dissolved in the area of absorbing microenvironment. Osteoclasts degrade collagen by secreting several lysosomal enzymes, particularly CTSK and TRAP.

cluster and fuse with each other, and in the end become a mature osteoclast (37). Masaru put forward that cell fusion was the key process of osteoclast formation and regulating this process would provide potential therapeutic targets in bone diseases (38).

The cell fusion process in osteoclastogenesis involves many molecules, including dendritic cell-specific transmembrane protein (DC-STAMP) (39, 40), dendritic cell-specific protein, integrin, src family kinases, and integrin and metalloproteinase family proteins (41). Fusion regulatory protein FRP-1 is also in charge of cell fusion. The antibody of FRP-1 enhanced the generation of multinucleated giant cells and induced the generation of functional osteoclasts (42).

The process of cell fusion depends on DC-STAMP including the presence of phosphatidylserine. At the surface of the fusion area, molecules like Anx A5 and Syn-1 play an important role in forming protein scaffold structure to link the phosphatidylserine with S100A4 in cell surface to regulate the fusion process (43). On the other hand, the activation of G protein-coupled receptor 119 can downregulate DC-STAMP to suppress preosteoclast fusion (44). Researchers find that actin binding LIM 1 (abLIM1) plays the role of negative control in cell fusion. Overexpression of abLIM1 suppresses osteoclast differentiation and formation. But when the expression of abLIM1 was downregulated by small interfering RNA, the formation and the marker genes of osteoclasts increased (45).

Conclusion

In summary, as a key cell in the bone metabolism system, studies on the mechanisms of osteoclast formation and activation have further deepened our understanding of osteoclasts. Research in bone immunology suggests that osteoclasts are involved in the regulation of the bone immune system, and the discovery of new signaling pathways, such as Semaphorin, may provide a new perspective on the mechanism of bone metabolic balance (46). With further research, especially in bone immunology (47, 48) and the RANK/RANKL pathway, it is expected to provide new ideas for the biological

mechanism of osteoclasts and new directions for drug development for bone diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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