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REVIEW ARTICLE

Molecular Understanding of Osteoclast Differentiation and Physiology

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INTRODUCTION

Two types of cells, osteoblasts and osteoclasts, maintain bone homeostasis by balancing each other's function [1,2]. Osteoblasts, which build bone, are derived from a mesenchymal progenitor cell that can also differentiate into marrow stromal cells and adipocytes [3]. Osteoclasts, originating from hemopoietic progenitors of the monocyte/macrophage lineage, immigrate into bone via the blood stream and resorb mineralized tissues [1,2,4]. Bone through this continuous dynamic remodeling provides structural integrity, skeletal strength, and a reservoir for hematopoiesis. Elevated osteoclast numbers and activity cause osteoporosis, Paget's disease, tumor osteolysis, various arthritis, and periodontal disease. These diseases result in low bone mass and high fracture risk, which can also occur as a result of an osteoblast defect.

To become multinucleated mature osteoclasts, mononuclear precursor cells fuse together under two critical conditions, one of which is to have an 'optimal density' of precursor cells [5]. The other is an existence of two kinds of cytokines; macrophage-colony stimulating factor (M-CSF), a survival factor, and receptor activator of NF-κB ligand (RANKL), a differentiation factor [4]. Since spleen cells and stromal cells can secrete M-CSF and RANKL, the combined of bone marrow and these cells can have the same effect compared to their treatment [6].

Here I will provide brief description of osteoclast physiology and focus on the current understanding of the molecules affecting osteoclast differentiation. Enhanced understanding of bone biology will require reviewing the known molecular mechanisms involved in osteoclastogenesis.

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OSTEOCLAST PHYSIOLOGY

To solubilize the mineral component of bone, osteoclasts form a resorption space called the sealing zone and make it into an acidic micro-environment. To do that, cytoskeleton and the integrin of osteoclast are arranged in a ring for tight attachment to the substrate [1]. $\alpha_v\beta_3$ integrin, an adhesion receptor binding the Arg-Gly-Asp (RGD) motifs of matrix proteins, is essential for normal osteoclast function since mice lacking beta3 integrins fail to spread or form sealing zones, thus becoming osteosclerotic [7]. Similarly, mice deficient in src, a ubiquitously expressed non-receptor tyrosine kinase display osteopetrosis characterized by dysfunctional osteoclasts with abnormal sealing zones and $\alpha_v\beta_3$ localization [8,9].

Of note, $\alpha_v\beta_3$ integrin and c-Fms, the receptor of M-CSF, collaborate in the osteoclastogenic process by sharing the activation of the ERK/c-Fos, an immediate-early transcription factors in the osteoclastogenesis, signaling pathway [10]. In addition, M-CSF, the c-Fms ligand-deficient *op/op* mice develop severe osteopetrosis as a consequence of total absence of macrophages and osteoclasts [11,12].

The adherent cells are polarized and form ruffled border, which is the scaffold for large quantities of vacuolar H^+-ATPase, a proton pump implicated in the acidification process of osteoclasts. The vacuolar, electrogenic H^+-ATPase pumps protons across the ruffled border causing the ambient pH within the resorptive lacuna to fall and dissolving bone mineral, which is hydroxyapatite, a calcium-phosphate salt, containing hydroxyl ions [13,14]. The pH level at the ruffled border can be as low as 3 to 4.

Targeted disruption of Atp6i, a gene encoding a putative osteoclast-specific proton pump subunit (termed OC-116kD, a3, or TCIRG1), in mice results in severe osteopetrosis. Atp6i-/- osteoclast-like cells (OCLs) lose the function for extracellular acidification, but retain intracellular lysosomal proton pump activity [15,16]. This allows lysosomal enzymes, including cathepsin K, to cleave

collagen and release peptides. Cross-linked bone collagen and the peptides containing cross-links used to estimate the rate of bone resorption in serum or urine are not degraded by proteinases. A mutation in the Atp6i also causes an osteopetrosis in human [17, 18].

Several enzymes including carbonic anhydrase II supply protons for the proton pump [19]. In resorbing osteoclasts, carbonic anhydrase II is highly expressed, whereas in nonresorbing osteoclasts only low basal expression is maintained. Antisense RNA targeted against carbonic anhydrase inhibits bone resorption *in vitro* [20]. Similarly, the carbonic anhydrase inhibitor, acetozolamide can inhibit osteoclastic bone resorption [21]. Studies in patients with a congenital absence of this enzyme and osteopetrosis have shown the critical importance of carbonic anhydrase II in osteoclast [22].

Cathepsin K, a cysteine protease, was first cloned from rabbit and human osteoclasts [23-25]. Cathepsin K knockout mice develop osteopetrosis due to a deficit matrix degradation but not demineralization [26]. Similarly, mutations in the human cathepsin K gene have demonstrated an association with a rare skeletal dysplasia, pycnodysostosis [27,28]. Tartrate-resistant acid phosphatase (TRAP, encoded by *Acp5*), an osteoclast differentiation marker, as well as cathepsin K also affect the functional activity of osteoclast by regulating bone matrix resorption and collagen turnover [29].

The CLCN7 (*ClC-7*gene), a widely expressed chloride transporter in osteoclast membrane, acts as a chloride-proton antiporter rather than as a chloride channel in osteoclasts [30]. Deficiency of CLCN7 in osteoclast membrane causes osteopetrosis in mice as well as in human [31]. Therefore, osteoclasts need both functional CLCN7 and vacuolar H^+-ATPase in order to acidify the underlying resorption lacuna, a crucial part of the bone resorption process. Key mechanisms involved in bone resorption by osteoclasts differentiation are depicted in the Fig.1.

Molecular Control of Osteoclast Differentiation

Prior to discussing transcription factors, it should be mentioned the regulation mechanisms of osteoclast differentiation which consists of a RANKL/RANK/OPG system (Fig. 2). Receptor Activator of NF-κB (RANK), a type 1 transmembrane protein that belongs to the TNF receptor (TNFR) superfamily, is expressed primarily on

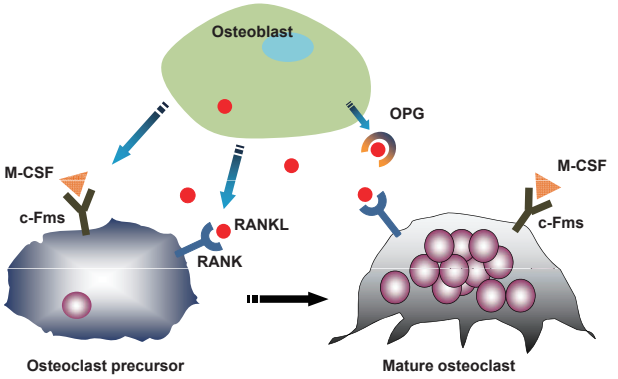

Fig. 2. RANKL/RANK/OPG system: the regulation of osteoclast differentiation by osteoblasts. RANKL from stromal/osteoblasts binds the RANK receptor on osteoclast precursors, thus inducing osteoclast formation whereas OPG inhibits osteoclastogenesis.

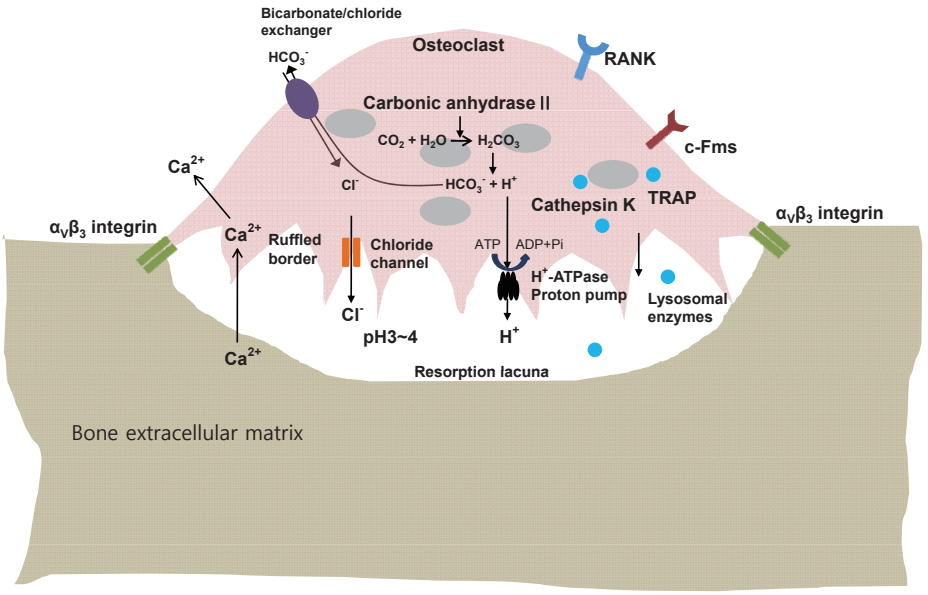

Fig. 1. The mechanisms of osteoclastic bone resorption. Several transport systems including the H^+-ATPase proton pump, Cl/HCO_3 exchanger and chloride channel are responsible for the acidification in the osteoclastic resorption lacunae. See text for further details.

monocytes/macrophages including osteoclastic precursors, activated T cells, dendritic cells, and mature osteoclasts [32,33]. RANKL (receptor activator of NF-κB ligand), also known as TRANCE (tumor necrosis factor-related activation-induced cytokine) is a type 2 membrane protein which belongs to the TNF superfamily and is synthesized by stromal cells/osteoblasts and activated T cells [34]. Osteoprotegerin (OPG), which is related to the TNF-receptor-superfamily, is a soluble decoy receptor for RANKL and is released from bone marrow stromal cells/osteoblasts as a soluble form [35].

By using RANK or RANKL-deficient mice showing similar osteopetrotic phenotypes, their association links their critical roles in bone remodeling [36,37]. On the contrary, OPG competitively binds to RANKL, thereby inhibiting osteoclast differentiation. OPG knock-out mice result in osteoporosis secondary to an excess of osteoclasts [35,38].

The binding of RANKL to RANK stimulates the differentiation of osteoclastic precursors into osteoclasts by inducing the expression of osteoclastogenesis-specific transcription factors or by activating them. The development of mouse genetics has contributed to better characterize osteoclast functions by identifying transcription factors regulating osteoclast differentiation.

Mouse mutants lacking transcription factors that function early in the lineage are osteopetrotic and lack either macrophages and osteoclasts or only osteoclasts. The PU.1, a myeloid- and B-cell specific transcription factor, regulates the initial stages of myeloid differentiation although it is expressed at all stages of osteoclast differentiation [39]. PU.1-/- mice exhibit osteopetrotic bone due to an arrested development of both osteoclasts and macrophages [39]. The PU.1-/- progenitor cells fail to express the RANK as well as c-Fms and reconstitution of PU.1 in these cells can induce RANK expression [39,40]. In addition, PU.1 and microphthalmia-associated transcription factor MITF, a basic helix-loop-helix-leucine zipper protein, collaborate to increase an expression of target genes like *cathepsin K (Ctsk)* and *acid phosphatase 5 (Acp5)* during osteoclast differentiation [41,42]. They recruit p38 mitogen activated protein kinase and Nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) to target genes during osteoclast differentiation [43].

Fos, a component of the dimeric transcription factor activator protein-1 (AP-1), is a key regulator of osteoclast-macrophage lineage determination [44]. The lack of Fos (encoding c-Fos) causes a lineage shift between osteoclasts and macrophages that results in increased numbers of bone marrow macrophages [44]. Fosl1 (encoding Fra-1) appears to be a transcriptional target of c-Fos since a transgene expressing Fra-1, a member of Fos proteins (c-Fos, FosB, Fra-1, Fra-2), rescues c-Fos–mutant mice from osteopetrosis *in vivo* [45]. Moreover, the rescue is both gene-dosage dependent and bone-development-specific [46].

The pleiotropic NF-κB transcription factor is critical for osteoclast formation [47,48]. NF-κB is a family consisting of five members: 'Rel' proteins including Rel A (p65), Rel B, and c-Rel (Rel) which synthesized as mature proteins and NF-κB1 (p105/p50) and NF-κB2 (p100/p52) which are synthesized as large precursors. These NF-κB proteins (p105 and p100) become shorter following post-translational processing into p50 and p52, respectively. They form dimers with Rel family members.

Both the generation of NF-κB1 null mice ($p50^{-/-}$) and NF-κB2 -deficient mice ($p52^{-/-}$) show an altered immune responses but without developmental defects [47]. Unlike single KO mice, the NF-κB p50/p52 double Knockout (dKO) mice are osteopetrotic and show growth retardation, craniofacial abnormalities with unerupted incisor teeth [47]. NF-κB p50/p52 dKO mice do not form osteoclasts and FACS analysis of the dKO mice showed a threefold increase in RANK-expressing splenocytes suggesting that NF-κB p50 and p52 are not required for RANK-expressing progenitor formation, but are necessary for RANKL-RANK-induced osteoclastogenesis [49]. In addition, RANKL or TNF failed to induce c-Fos in M-CSF-treated NF-κB p50/p52 double knockout splenocytes, whereas overexpression of c-Fos rescued the defect in osteoclast formation in osteoclast precursors in the absence of RANKL, indicating that c-Fos are downstream of NF-κB [45,47,50].

Nuclear factor of activated T-cells cytoplasmic 1 (NFATc1, NFAT2)-deficient embryonic stem cells fail to differentiate into osteoclasts. The overexpression of constitutively active NFATc1 in bone marrow monocytes/macrophages causes precursor cells to undergo efficient differentiation even in the absence of RANKL, suggesting that NFAT2 is not only indispensable but also sufficient for osteoclastogenesis [51].

NFAT, a calcineurin- and calcium-regulated transcription factor, is a family of transcription factors originally identified in T cells. The gene family has five members (NFATc1 through NFATc5). The release of Ca^{2+} activates the calmodulin-regulated phosphatase calcineurin, which binds the N-terminal domain of NFAT2 and dephosphorylates it [51,52]. NFAT2 undergoes nuclear translocation and regulates the expression of many osteoclast-specific genes, such as cathepsin K, TRAP, calcitonin receptor as well as NFAT2 it-

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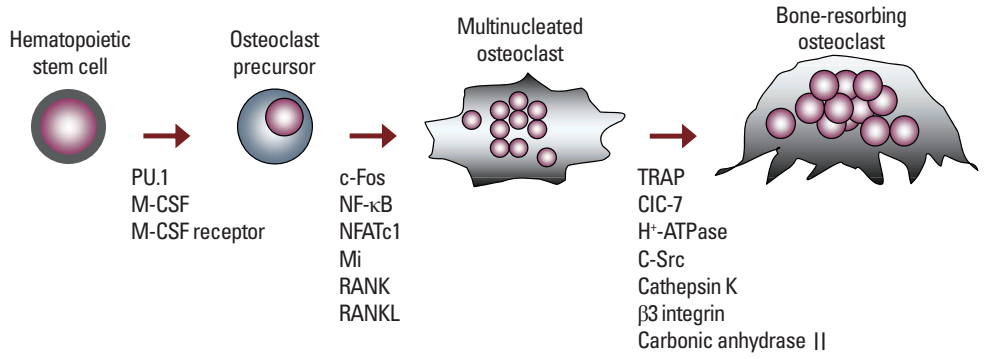

Fig. 3. The critical molecules affecting osteoclast differentiation and function. In the early stage, M-CSF/M-CSF receptor and PU.1 regulate the differentiation of hematopoietic stem cells into osteoclast precursors, which form multinucleated cells by cell-cell fusion. RANKL/RANK signaling stimulates the activation of downstream molecules, thus inducing the formation of mature osteoclast tat resorb bones.

self, in cooperation with other transcription factors, although the components of the transcriptional complex are not always the same [53-56]. The C-terminal domain of NFAT2 binds DNA sequence specifically and cooperates with AP-1 *in vitro* [52].

Activation of NFATc1 as well as c-Fos by RANKL signaling requires expression of NF-κB p50 and p52, indicating that c-Fos and NFATc1 are downstream of NF-κB [45,47,50]. In addition, NFATc1 seems to be activated by osteoblasts through a Ca^{2+} oscillation-independent signal pathway during osteoclastogenesis and this clearly differs from that seen during RANKL/M-CSF-induced osteoclastogenesis [57]. Recently, it has been found that NFATc1 functions in the osteoclast fusion process via up-regulation of the dendritic cell-specific transmembrane protein (DC-STAMP) and the d2 isoform of vacuolar ATPase V0 domain (Atp6v0d2) through co-activation with MEF2 and MITF, which would be followed [58,59].

Mice lacking genes that act later in the lineage are still osteopetrotic but have substantial numbers of osteoclasts which are not functional as a result of the absence of a normal ruffled membrane. Mice lacking Mi have morphologically normal osteoclast but fail to resorb bone since they are incapable of acidifying the resorptive microenvironment or cannot degrade the bone matrix. Stimulation of p38 MAP kinase results in the downstream activation of Mi/MITF, which controls the expression of genes encoding tartrate-resistant acid phosphatase (TRAP) and cathepsin K [60]. Moreover, interleukin-1 (IL-1) activates osteoclast-specific genes including TRAP and OSCAR, in part, via the MITF pathway [61]. *Mi* mutant osteoclasts are primarily mononuclear and express decreased levels of TRAP [41,62]. Mice heterozygous for both the mutant *Mi* allele and a *PU.1* null allele also develop osteopetrosis. The size and number of osteoclasts were not altered in the double heterozygous mutant mice, indicating that the defect lies in mature osteoclast function [41]. The critical molecules regulating osteoclast differentiation and function are summarized in Fig. 3.

CONCLUSIONS

Bone remodeling occurring throughout life is an important biological and medical issue. The discovery of RANKL/RANK and the production of several mouse models produced considerable advancements in osteoclast biology. The importance of several molecules including transcription factors that regulate osteoclast differentiation and function has been highlighted by mouse studies. Nonetheless, we still require increasing-knowledge about bone physiology given that our current knowledge is at a particularly primitive state. Increasing detailed molecular understanding while integrating current information of osteoclast differentiation is required. More studies are needed to help develop novel therapeutic drugs that target the formation and bone resorption activity of osteoclast.

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