

Molecular and cellular basis of bone resorption

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Summary Osteoclast research has an exciting history and a challenging future. More than 3 decades ago, it became evident that bone-resorbing osteoclasts are of hematopoietic origin and are ultimately linked to the “basic multicellular unit,” where they team up with the other cell types, including bone-forming osteoblasts. Since 2 decades, we have learned about the signaling pathways controlling genes relevant for osteoclastogenesis and bone resorption. It took another decade until the hypothesized “osteoclast differentiation factor” was discovered and was translated into an approved pharmacologic strategy. Here, the focus is on another molecular target, cathepsin K, a cysteine protease being released by the osteoclast into the resorption compartment. Genetic deletion and pharmacological blocking of cathepsin K reduces bone resorption but with ongoing bone formation. This observation not only holds great promise to become a new pharmacologic strategy, but it also provides new insights into the coordinated work of cells in the “basic multicellular unit” and thus, bridges the history and future of osteoclast research. This article is a short primer on osteoclast biology for readers of the special issue on odanacatib, a cathepsin K inhibitor.

Keywords Osteoclast · Bone remodeling, signaling · Resorption · Sealing zone · Ruffled boarder

Molekulare und zelluläre Grundlagen der Knochenresorption

Zusammenfassung Die Erforschung der Osteoklasten hat eine interessante Historie und eine herausfordernde Zukunft. Vor mehr als drei Jahrzehnten wurde deutlich, dass die Osteoklasten hämatopoetischen Ursprungs sind und sich gemeinsam mit den anderen Zelltypen, einschließlich der knochenbildenden Osteoblasten, in der „Basic Multicellular Unit“, befinden. Seit zwei Jahrzehnten verdichtet sich das Wissen über die Signalwege zur Kontrolle der Osteoklastogenese und der Knochenresorption. Es dauerte ein weiteres Jahrzehnt bis der hypothetische „Osteoklasten-Differenzierungsfaktor“ entdeckt wurde der, in Folge, die Grundlage für eine zugelassene pharmakologische Strategie bildete. Ein anderes molekulares Ziel ist Cathepsin K, einem Cystein-Protease die vom Osteoklasten während der Resorption freigesetzt wird. Genetische Deletion und pharmakologische Blockierung von Cathepsin K reduzieren die Knochenresorption, ohne Unterbrechung der Knochenneubildung. Dieses Prinzip hat das Potential eine neue pharmakologische Strategie zu werden und bietet neue Einblicke in die koordinierte Arbeit der Zellen in der „Basic Multicellular Unit“. Dieses Prinzip verbindet auch die Historie und Zukunft der Erforschung der Osteoklasten. Dieser Artikel ist ein kurzer Abriss der Osteoklastenbiologie für die Leser der Sonderausgabe zum Thema Odanacatib, einem Cathepsin-K-Inhibitor.

Schlüsselwörter Cathepsin K · Osteoclast · Resorption · Signaling · Review · Remodeling

Anatomy and biochemistry of the osteoclast

The molecular and cellular basis of bone resorption should not be reviewed without referring to the “basic

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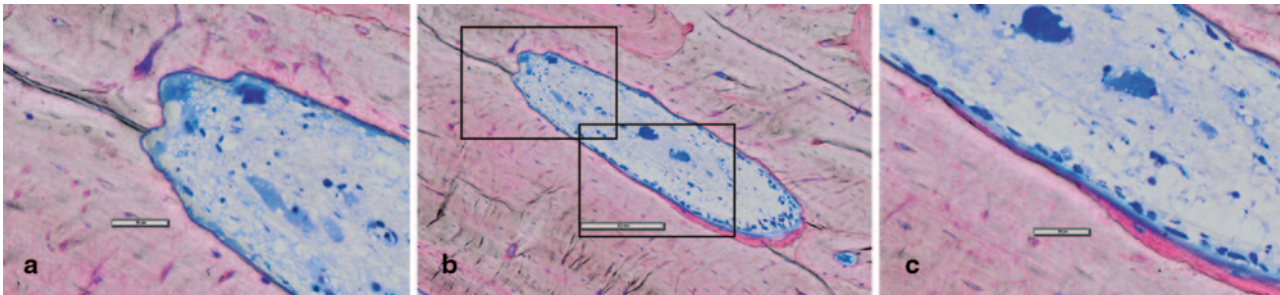


Fig. 1 The “Basic multicellular unit.” Bone-resorbing osteoclasts remove the bone and prepare the site for the following bone-forming osteoblasts. The blood capillaries are a sign that osteoclast and osteoblasts develop out of their respective hematopoietic and mesenchymal progenitors. Thus, the BMU holds the microenvironment that initiates osteoclastogenesis and the osteoblastogenesis, and as the two processes are functionally coupled, also the underlying molecular mechanisms are beginning to be discovered. Among the key regulators of this process are the osteocytes that can RANKL and sclerostin, thereby pushing osteoclastogenesis while suppressing osteoblastogenesis, respectively. Also, osteoclasto-

genes and growth factors released by the osteoclasts likely control osteoblastogenesis. Moreover, the osteoclasts leave an osteoblastophilic surface. The birth and death of bone cells and the complex interplay between the two key effector cells hold the key to understand the biology and also the pathophysiology of bone remodeling and reveal potential clinical targets, for example, cathepsin K to which this review series is dedicated. **a** Osteoclasts at the resorption front; **b** overview on a active osteon; **c** osteoblast at the formation front. Note the blood vessels in the center on the osteon. The histology was taken from the porcine mandible.

multicellular unit” (BMU) in the cortical bone (Fig. 1) or the “Howship’s lacunae” in the trabecular compartment. In both anatomical locations, the bone-resorbing osteoclast and the bone-forming osteoblasts coordinately work on the remodeling of bone, as proposed early on by Frost [1], refined by Parfitt [2], and recently updated by Sims and Martin [3] and Delaisse [4]. Modeling is the structural adaptation of bone during growth and to functional loading where osteoblast and osteoclast work independently [1]. Remodeling and modeling depend on the recruitment of the respective progenitors, which are hematopoietic cells for osteoclasts and mesenchymal cells for osteoblasts. Considering further that cells have a limited life span and require constantly renewal, understanding bone remodeling is challenging—in physiologic situation, but, even more, in pathologic conditions such as osteoporosis [5].

Bone resorption is accomplished by osteoclasts, highly specialized multinucleated cells in intimate contact with the bone matrix. Mature osteoclasts have two characteristic morphological features: the sealing zone and the ruffled border. The sealing zone attaches the osteoclast via $\alpha_v\beta_3$ integrins to the extracellular bone matrix, particularly to osteopontin and bone sialoprotein [6]. The intracellular domains of the integrins are connected to podosomes, which are F-actin cores associated with other structural proteins (vinculin, paxillin, and talin) and kinases (small GTPases, c-Src, and Pyk2) [7]. The sealing zone tightens the osteoclasts toward the resorptive compartment, allowing the cell to generate a secondary lysosome with low pH and proteases. The ruffled border is formed by the fusion of intracellular vesicles containing the enzymes and transmembrane proteins driving bone resorption.

The vacuolar H^+ -ATPase (V-ATPase) and the chloride channel type 7, pump hydrochloride acid into the resorp-

tion lacunae. The carbonic anhydrase type II provides the protons. SLC4A2 exchanges Cl^- for HCO_3^- at the basolateral site. Together, these enzymes drive the acidification of the resorption lacunae and thus the lysis of the mineralized matrix [8]. The remaining collagen-rich matrix is removed by proteases, mainly cathepsin K [9]. Calcium, phosphate, matrix degradation products, and also growth factors stored in the bone matrix are transported through the osteoclast and released on the basolateral site, where blood vessels provide the connection to the circulation. Thus, urine and serum are used to monitor collagen degradation products [10] (Fig. 2).

Pioneer work on osteoclastogenesis

More than 3 decades ago, it became evident that osteoclasts are of hematopoietic origin [11, 12]. Multinucleated osteoclast-like cells expressing tartrate-resistant acid phosphatase and capable of resorbing dentine were originally generated from murine bone marrow [13]. This process was induced by the same hormones and cytokines that are responsible for bone resorption in vivo: 1,25(OH)₂ vitamin D₃, prostaglandin E₂, PTH, and inflammatory cytokines of the IL-6 family [14]. The presence of mesenchymal cells was found to be essential allowing the hormones and cytokines to induce osteoclastogenesis [15]. It was therefore proposed that mesenchymal cells produce a membrane-bound factor termed “stromal osteoclast-forming activity” (SOFA) or “osteoclast differentiation factor” (ODF) [11, 14]. In 1997, this osteoclastogenic factor, now termed RANKL, was discovered by Snow Brand [16–18] and Amgen [19, 20], but was first described to link T cells and dendritic cells [21, 22].

RANKL competes with its decoy receptor OPG for activating RANK on osteoclasts and the respective progeni-

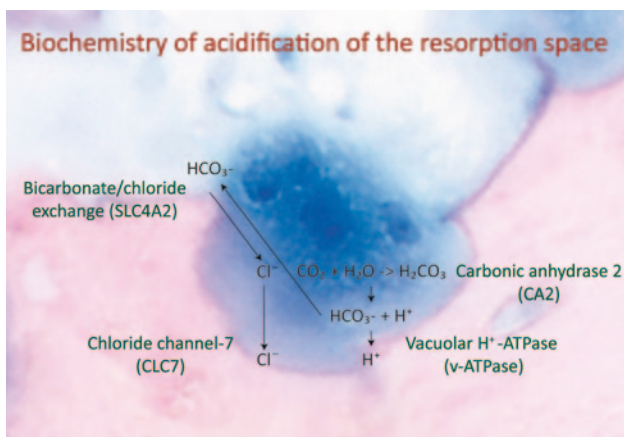


Fig. 2 The biochemistry of acidification of the resorption lacunae. Once the osteoclasts have sealed off the space between their apical cell membrane and the underneath bone matrix, protons and proteolytic enzymes are pumped into this secluded space, which later becomes the “cutting cone” (BMU) or the Howship’s lacuna. The sealing zone is rich in α_3 integrins that connect to SIBLINGs (for Small Integrin-Binding Ligand, N-linked Glycoproteins; e.g., osteopontin and bone sialoprotein) on the bone matrix to the podosomes in the cytoplasm. Acidification is accomplished by the interplay of enzymes: the carbonic anhydrase 2 (CA2) providing the unstable carbonic acid that has equilibrium with protons and bicarbonate. The proton is pumped into the resorption site by the vacuolar H^+ -ATPase, and the bicarbonate is exchanged for chloride by the respective transporter (SLC4A2). The chloride is also pumped into the resorption site by means of the chloride channel 7 (CLC7). The pH in the resorption sites drops to approximately 3–4, which releases minerals from the matrix and is optimal for the proteolytic activity of cathepsin K

tors [19, 20]. With the recognition that RANKL produced by T-cell that induce osteoclastogenesis, the term “oste-immunology” was coined [23]. RANKL, by far, exceeds aspects of bone biology [24]. Nevertheless, pharmacologic strategies were developed, first with recombinant OPG [25] and then with a RANKL-neutralizing antibody [26]. Denosumab is now approved in osteoporosis therapy [27] and applied in bone metastasis [28]. Thus, it took 1 decade from discovering the molecular triad to the approval of a global pharmacological product.

Signaling mechanisms controlling osteoclast differentiation, activation, and survival

RANK is a receptor with an intracellular domain linked to TRAF6, which is characteristic for members of the TNF-receptor family. Downstream are c-fos [29] and NF κ B [30], which support the expression of nuclear factor of activated T cell cytoplasmic 1 (NFATc1) [31, 32], together all key transcription factors regulate osteoclastogenesis. However, NFATc1 is not exclusively controlled by RANK.

Signaling via immunoreceptor tyrosine-based activation motif (ITAM), as part of Fc receptor common gamma chain (FcR γ) and DNAX-activating protein, 12 kDa

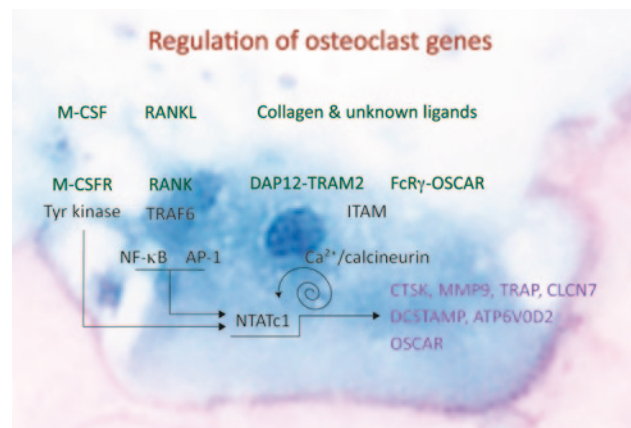


Fig. 3 The regulation of osteoclastic genes. Three main signals of osteoclast signaling are required for osteoclastogenesis, activity, and survival of the cells. In a simplified model, M-CSF activates the receptor tyrosine kinase, RANK signals via TRAF6 and activates NF κ B and AP-1 transcription factors and the ITAM-motif containing receptors DAP12, and FcR γ stimulate Ca^{2+} -dependent calcineurin signaling—all together cumulate in the increased expression of NFATc1 that is further pushed by feed-forward regulation. Thus, NFATc1 accumulates and, together with other transcription factors, stimulates the expression of the characteristic osteoclast genes responsible for bone resorption, fusion into multinucleated cells, and activation by ligands. Not shown is the signaling involved in formation of the cytoskeleton

(DAP12), is also essential for osteoclastogenesis [33]. The respective costimulatory receptors are osteoclast-associated receptor (OSCAR), triggering receptor expressed in myeloid cells-2 (TREM-2), signal-regulatory protein β 1 (SIRP β 1), and paired immunoglobulin-like receptor-A (PIR-A). For example, OSCAR serves as a collagen receptor that stimulates osteoclastogenesis [34].

ITAM- and also RANKL-dependent signaling activates phospholipase C γ and thus Ca^{2+} release from the endoplasmic reticulum. Oscillating Ca^{2+} allows calcineurin-sensitive NFATc1 autoamplification. Finally, NFATc1 together with other transcription factors (AP-1, PU.1, MTF, etc.) controls the expression of the typical osteoclast genes, e.g., cathepsin K, TRAP, calcitonin receptor, OSCAR, and also genes that regulate cell fusion, i.e., dendritic cell-specific transmembrane protein (DC-STAMP) and the ATPase, H^+ transporting, lysosomal 38 kDa, V0 subunit d2 (Atp6v0d2) [35–38].

The third key player in osteoclast signaling is the M-CSF receptor (M-CSFR; c-fms). M-CSFR signaling promotes RANK expression, thereby making the progenitor cells more sensitive to RANKL. M-CSFR together with $\alpha_v\beta_3$ integrin also affect the cytoskeleton by changing c-src, Sky, as well as Rho small GTPases, Vav3, Rac1/2, and Arp2/3 signaling [7, 39]. Together, M-CSFR, RANK, and ITAM signaling control the proliferation, differentiation, fusion, activity, and survival of osteoclasts. All these synchronized steps occur in the unique environment of the BMU (Fig. 3).

The control of bone remodeling

Osteoclasts are not stand-alone cells in bone remodeling; they team up with the bone-forming osteoblasts and the “amazing” osteocytes [40], which are former osteoblasts that are buried in their own matrix, provide the signals to control osteoclastogenesis during bone remodeling, and also control aspects of modeling [41, 42]. Targeted depletion of RANKL in osteocytes but not in osteoblasts results in an osteopetrotic phenotype, causing a paradigm shift in the functional understanding of bone remodeling. Bone resorption is also linked with osteocyte apoptosis [43], which provided new hypothesis about the pathophysiology of osteoporosis induced by loss of steroid hormones, glucocorticoids, or unloading [44]. The osteocytes can also release minerals from their lacunae walls [45], which is particularly impressive during lactation, but act presumably independent of the BMU.

The concept of formation-resorption coupling also advanced, for example, by studies with the sphingosin-1-phosphate (S1P) released by osteoclasts [46] and the EphB4-ephrin-B2 signaling complex [47], complement component 3a [48], and semaphorins [49]. Besides these “osteoclastokines,” molecules released from the bone matrix, for example, TGF- β [50], can target mesenchymal cells. Accumulating evidence supports the central role of the cellular envelope to cover the space where all three phases of bone remodeling—resorption, reversal, and formation—take place [4]. This concept was described in 2001 [51] and has since then been advanced to explain the reversal phase of bone remodeling [4, 52]. Research on the BMU has a renaissance, providing new hypothesis on the functional coupling on bone resorption and bone formation in health and disease.

This model allows the formulation of a hypothesis why blocking cathepsin K reduces osteoclast function while bone formation can even be increased [53, 54], and why bisphosphonates, even though the osteoclasts are visible, does not [55]. That almost no osteoclasts are visible with denosumab is not surprising, as their formation is blocked [56]. Bisphosphonates have a negative impact on the canopy coverage; in contrast, the pharmacological inhibition of cathepsin K (odanacatib) increases the extent of the osteoclast-canopy interface [57]. The key to the future bone-anabolic therapies evidently lies in preservation of the canopy coverage of the bone-remodeling compartments, which is basically the BMU.

The past and present of “molecular and cellular basis of bone resorption”

Osteoclast research has an exciting history and a challenging future that extended the borders of bone research. The authors would like to refer the readers to superb review series published at the millennium change in *Nature* [58, 59] and *Science* [60, 61], and [62] the recent

comprehensive works [63], one of which is entitled “The Great Beauty of the osteoclast” [8]. Primers for osteoimmunology [64] along with clinical aspects beyond osteoporosis, e.g., rheumatoid arthritis [65, 66], also needs to be mentioned. Readers should also refer “Bonekey Rep” for comprehensive updates on recent aspects of bone research. The molecular and cellular basis of bone resorption should always be understood in the context of the BMU.

Conflict of interest

The author declares that there are no actual or potential conflicts of interest in relation to this article.

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