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Osteoclasts: more than ‘bone eaters’

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Abstract

As the only cells definitively shown to degrade bone, osteoclasts are key mediators of skeletal diseases including osteoporosis. Bone forming osteoblasts, and hematopoietic and immune system cells, each influence osteoclast formation and function, but the reciprocal impact of osteoclasts on these cells is less well appreciated. Here, we highlight functions osteoclasts perform beyond bone resorption. First, we consider how osteoclast signals may contribute to bone formation by osteoblasts and the pathology of bone lesions, such as fibrous dysplasia and giant cell tumors. Second, we review the interaction of osteoclasts with the hematopoietic system, including the stem cell niche and adaptive immune cells. Connections between osteoclasts and other cells in the bone microenvironment are discussed within a clinically relevant framework.

Keywords

osteoclast; osteoblast; bone remodeling; osteopetrosis; osteoporosis; PTH

Bone remodeling and osteoclasts 101

Bone is a composite tissue of protein and mineral, which undergoes continual remodeling to grow, heal damage, and regulate calcium and phosphate metabolism. This remodeling process is executed by the concerted and sequential effort of bone resorbing osteoclasts and bone forming osteoblasts, acting in what has been termed the basic multicellular unit (BMU) (Figure 1A). Osteocytes, long-lived osteoblast-derived cells that reside within the bone matrix, monitor bone quality and stress, and coordinate remodeling through membrane bound and secreted factors. Skeletal integrity is maintained throughout the lifespan by matching bone formation and resorption, a process referred to as osteoclast:osteoblast ‘coupling.’ Coupling is thoroughly summarized in recent excellent reviews [1, 2] and in Figure 1.

Osteoclasts are multinucleated giant cells that differentiate from myeloid precursors under the influence of the cytokines macrophage colony stimulating factor (MCSF) and receptor

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activator of NF- κ B ligand (RANKL) supplied by osteoblasts and/or osteocytes (Figure 1A) [3]. A decoy receptor for RANKL called osteoprotegerin (OPG), which is also made by the osteoblast lineage, tempers osteoclast differentiation [4]. Osteoclasts degrade bone by the polarized secretion of proteolytic enzymes (e.g. cathepsin K) and acid, which hydrolyze and solubilize the organic and inorganic components of bone, respectively. Proton and enzyme secretion is directed into a resorption lacunae, which is partitioned from rest of the bone microenvironment by a sealing zone of densely packed podosomes that surrounds the apical membrane of the osteoclast [3, 5]. Subsequent to the osteoclastic resorptive phase, coupling mechanisms promote the recruitment and differentiation of mesenchymal derived osteoblast progenitors at the resorption lacunae. After these cells mature into osteoblasts, they line the eroded bone surface and secrete the organic component of bone, termed osteoid, which is mineralized over time by the incorporation of hydroxyapatite [1]. As osteoblasts secrete osteoid, some cells are entrapped within the matrix where they eventually become osteocytes (Figure 1A).

When coupling is deranged, the delicate balance between resorption and formation is lost, resulting in bone disease. The most common skeletal disease is osteoporosis, where resorption exceeds formation, leading to decreased bone density and fractures. Osteoporosis affects almost 9 million individuals in the US and fragility fractures increase morbidity and mortality [6, 7]. In inflammatory arthritis and bone metastases, pathologic osteoclast activation results in peri-articular erosions and painful osteolytic lesions, respectively [8]. Rarer diseases, such as osteopetrosis and pyknodysostosis, result from genetic mutations that affect osteoclast formation or resorptive function, leading to the accumulation of dense, but brittle, bone [9–11]. These diseases highlight the essential role of osteoclasts for normal skeletal development and performance. Since the initial description of the osteoclast in the 1870s [12], research on this cell has focused on understanding its origins, differentiation program and resorptive mechanisms. Increasingly, however, evidence points to a more complex identity for osteoclasts beyond their role as ‘bone eaters.’

Beyond resorption: regulation of the bone remodeling cycle in health and disease by osteoclasts

Coupling: How osteoclasts ‘talk back’ to cells of the osteoblast lineage

Coupling of bone formation to resorption is likely achieved through multiple mechanisms, including signals that stimulate the proliferation of pre-osteoblasts, their recruitment to resorption lacunae, and their differentiation into bone forming cells. Cellular mediators of coupling include osteoclasts, osteoblasts, osteocytes, macrophages and T-cells, which produce a variety factors including Wnt pathway regulators, such as sclerostin, and cytokines such as oncostatin M [13–16]. Among osteoclasts, gene expression and response to stimuli may vary depending on location (for example, trabecular vs. cortical or intramembranous vs. enchondral), suggesting that osteoclasts come in different subtypes [17, 18]. In this section, osteoclast centered coupling mechanisms are reviewed.

Release of matrix derived growth factors (Figure 1A)—Initial efforts to identify osteoclast dependent coupling mechanisms focused on growth factors embedded within

bone matrix. Transforming growth factor β 1 (TGF β 1) released during osteoclast culture on bone induces the migration of mesenchymal stem cells (MSC) *in vitro* and MSC migration to bone surfaces is reduced in *Tgfb1*^{-/-} mice [19]. Similarly, insulin-like growth factor 1 (IGF1) liberated from bone by osteoclastic resorption is proposed to promote osteogenic differentiation [20]. Release of matrix-derived growth factors is also invoked as a mechanism supporting growth of bone metastases (see Box 1). Decreased osteoblast numbers and low bone mass in TGF β 1-deficient mice, and in mice with an osteoblast specific deficiency of insulin-like growth factor receptor 1 (IGFR1), suggest that matrix derived TGF β 1 and IGF1 are important coupling mediators. Moreover, elevated osteoclast numbers in the subchondral bone of mice with experimentally induced osteoarthritis was associated with an increase in local TGF β activity [21]. This increased TGF β was linked to the subsequent formation of islets of pre-osteoblasts and osteoarthritis progression. However, in none of these cases has inhibition of bone resorption *in vivo* been shown to prevent TGF β or IGF1 mediated effects on mesenchymal cells [19–21].

Box 1

Usurping local resources: osteoclasts feed bone invaders

Liberation of growth factors embedded in bone matrix by osteoclasts may promote metastatic tumor growth in bone. Reciprocal stimulation of osteoclasts by cancer cell derived parathyroid hormone related protein (PTHrP) and other factors, could potentiate growth factor release in what has been termed the ‘vicious cycle’ [113–115]. Xenograft experiments utilizing breast cancer cells expressing a TGF β responsive reporter demonstrated osteolytic metastases had high TGF β activity. Inhibition of osteoclastic bone resorption with pamidronate reduced TGF β activity and osteolytic lesions, suggesting that matrix resorption is a relevant source of TGF β for skeletal metastasis *in vivo* [116]. While prophylactic pamidronate treatment decreased frequency of bone metastasis, the drug did not decrease disease progression if administered after tumor cell inoculation. Thus, whether inhibiting the release of matrix growth factors by osteoclasts has a substantive effect on tumor growth is unclear. Several bisphosphonates, as well as the anti-RANKL antibody denosumab, reduce skeletal events in metastatic cancer, but data on whether they prevent bone metastasis are inconsistent [117, 118].

Clastokines: secreted osteoclast derived coupling factors (Figure 1B)—A comparison of osteoblast activity in the two forms of autosomal recessive osteopetrosis (ARO) suggests that osteoclasts produce coupling factors directly, rather than via their resorptive function (reviewed in [22]). ARO results from mutations that inhibit either osteoclast formation (osteoclast-poor) or function (osteoclast-rich) [10, 11, 23, 24]. In osteoclast-rich ARO, bone formation by osteoblasts is maintained, or even increased. For example, patients with osteoclast-rich ARO caused by homozygous mutations in *chloride channel, voltage sensitive 7 (CLCN7)* or *α 3 subunit of vacuolar proton pump (TCIGR1)*, which encode a chloride channel and a subunit of the vacuolar H⁺-ATPase respectively, display normal to increased bone formation rates (BFRs) [25]. Likewise, an increase in dysfunctional osteoclasts in *v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene*

homolog (*Src*^{-/-}) mice, *Ctsk*^{-/-} mice, and mice with conditional deletion of *cathepsin k* (*Ctsk*) in osteoclast precursors, is accompanied by increased osteoblast numbers and BFRs [26–29]. In contrast, *op/op* mice develop osteoclast-poor osteopetrosis due to mutations in colony stimulating factor 1 (*Csf1*) or its cognate receptor, *Csf1r*, and have decreased bone mineralization and abnormal osteoblast organization [30, 31]. Bone formation is also decreased in osteoclast deficient *FBJ murine osteosarcoma viral oncogene homolog* (*c-fos*^{-/-}) mice [32]. Similarly, osteoclast-poor ARO patients have normal to low osteoblast surface [25]. Taken together, this genetic evidence suggests that osteoclasts are a source of coupling activity independent of their resorptive function.

Over the past 15 years, a number of potential osteoclast derived coupling factors (clastokines) have been identified using *in vitro* assays (Table 1). Three recently identified clastokines for which *in vivo* data suggest a role in coupling are collagen triple repeat containing 1 (CTHCR1), sphingosine-1-phosphate (S1P) and complement factor 3a (C3a). *Cthcr1* was identified as a bone morphogenetic protein 2 (BMP2) induced gene in osteoprogenitors. Global deficiency of *Cthcr1* decreased osteoblast number and bone formation *in vivo*, with cell autonomous effects on calvarial osteoblast proliferation and differentiation *in vitro* [33]. More recently, *Cthcr1* was found to be strongly induced in osteoclasts after initiating bone resorption [34]. Similar to the findings of Kimura *et al.*, CTHCR1 promoted osteoblast differentiation and bone formation. However, osteoblast specific deletion of *Cthcr1* did not result in a bone phenotype. In contrast, deletion of *Cthcr1* in osteoclasts resulted in reduced bone mass and BFR, suggesting osteoclasts are the physiologically relevant source of this bone anabolic molecule. The complement cascade component C3 was identified in osteoclast conditioned medium and its cleavage product, C3a, stimulated osteoblast differentiation *in vitro* [35]. Moreover, in mice, a chemical C3a receptor (C3aR) antagonist prevented the increase in BFR that accompanies elevated bone resorption after ovariectomy, resulting in further decreases in bone volume. Sphingosine kinase 1 (SPHK1) and its product, S1P, are induced during osteoclast differentiation. *In vitro*, S1P promotes osteoblast differentiation [36, 37]. The *in vivo* importance of S1P as a clastokine is supported by a recent study that examined mice with myeloid specific deficiency of cathepsin K. As expected, this strain had poorly resorptive osteoclasts. These osteoclasts expressed higher levels of SPHK1, secreted more S1P, and demonstrated elevated osteoblast numbers and BFRs [29]. Separate studies found that myeloid specific ablation of one member of the S1P receptor (*S1pr1*) family resulted in reduced bone mass, while deletion of a second (*S1pr2*) resulted in mildly increased bone mass, suggesting that S1P directly affects osteoclast lineage cells as well [38, 39]. The stage of osteoblast lineage differentiation on which each of these coupling factors acts, and their relative importance in physiologic and pathologic circumstances, remains to be elucidated.

Cell contact mediated mechanisms (Figure 1C)—Bidirectional signaling utilizing cell surface receptor-ligand pairs is a third mechanism contributing to coupling. Several ephrin receptors (Eph) and their ephrin ligands may mediate communication between osteoclasts and the osteoblast lineage (reviewed in [40]). Interaction of osteoclast ephrinA2 with osteoblast EphA2 promotes osteoclast and inhibits osteoblast differentiation from precursors [41]. In contrast, interaction of ephrinB2 with EphB4 promotes osteoblast

differentiation while inhibiting osteoclasts [42]. Reverse signaling through RANKL on osteoblasts may also contribute to coupling. W9, a peptide that directly binds RANKL to inhibit its interaction with RANK, surprisingly increased cortical BFRs *in vivo*, promoted ectopic bone formation in BMP2 impregnated collagen sponges and enhanced osteoblast differentiation *in vitro* [43]. Whether endogenous RANK on osteoclasts could have a similar effect on RANKL remains to be determined. The degree to which cell contact mediated mechanisms contribute to coupling should be further investigated by resolving how osteoclasts interact with osteoblast lineage cells within the BMU in space and time.

Clinical implications of osteoclast derived coupling activity

The goals of osteoporosis treatment are to increase bone mass and prevent fractures. An increase in bone mass can be achieved with anti-resorptives or anabolic agents that promote bone formation. Although either approach will accrue bone, an optimal osteoporosis treatment would engage both mechanisms. However, currently approved osteoporosis treatments target these mechanisms individually and result in detrimental effects on the other half of the bone remodeling cycle because of osteoclast:osteoblast coupling. Thus, increases in bone formation are accompanied by elevated bone resorption, while reduced resorption leads to a concomitant decrease in formation. Since pharmacologic treatment of osteoporosis typically starts with anti-resorptive agents, which include the nitrogen containing bisphosphonates (alendronate, risedronate, ibandronate, zoledronic acid) and denosumab [44], understanding how osteoclasts promote bone formation is highly clinically relevant. Bisphosphonates are deleterious to both osteoclast function and survival as they inhibit prenylation of small GTPases and result in accumulation of toxic nucleotide analogs [45]. Denosumab is a humanized monoclonal antibody to RANKL that blocks osteoclast formation. Both classes of anti-resorptives reduce bone formation by osteoblasts, which may ultimately lead to poor quality bone when these agents are used for many years.

The only agent approved by the FDA to increase bone formation, intermittent parathyroid hormone (PTH), also increases bone resorption. One might think that supplementing PTH treatment with an anti-resorptive could prevent this increase in bone resorption to synergistically increase bone mass. However, osteoclasts may be required to achieve the full anabolic effect of PTH. For example, in animals the anabolic effect of PTH is blunted by bisphosphonates or genetic osteoclast deficiency [46]. Likewise, in humans, treatment of osteoporosis with alendronate and PTH resulted in less of an anabolic effect compared to PTH alone [47, 48]. Other osteoporosis trials however, challenge these findings. For example, dual treatment with zoledronic acid and PTH did not diminish bone accrual [49] and PTH remained anabolic when given with denosumab [50]. The effect of different anti-resorptives on PTH driven bone formation could potentially be explained by a number of mechanisms. First, anti-resorptives may differentially influence the ability of osteoclasts to produce coupling mediators. Second, anti-resorptives may display varying direct effects on osteoblasts or osteocytes. For example, bisphosphonates have been shown to inhibit osteoblast apoptosis [51]. However, the relative potency of different bisphosphonates to suppress cell death in osteoblasts in humans has not been resolved. Moreover, denosumab may have unappreciated effects on osteoblasts by binding membrane bound RANKL. Lastly, different bone compartments (e.g. periosteal, endocortical, cancellous) may variably

require osteoclast derived coupling factors for osteoblast activity [52, 53]. Thus, the interpretation of these studies may be influenced by how and where measurements of bone formation were made.

Newer agents under development for osteoporosis may exploit, or circumvent, the need for osteoclast coupling factors. In contrast to bisphosphonates and denosumab, odanacatib, a small molecule inhibitor of cathepsin K in late stage development, decreases resorption without affecting osteoclast survival and appears to positively affect bone formation. Cathepsin K inhibition has less of an inhibitory effect on bone formation markers than other anti-resorptives, stimulates periosteal and endocortical bone formation in monkeys, and augments the effect of PTH in ovariectomized mice [52, 54–56]. Anabolic agents also in development block the function of sclerostin, an osteocyte derived inhibitor of the Wnt signaling pathway critical for osteoblast differentiation [57]. Patients with mutations in *SOST*, the gene encoding sclerostin, have increased bone density [58], and romosozumab, a monoclonal antibody against sclerostin, substantially increased bone mineral density in women with osteopenia. Romosozumab increased bone formation markers as expected, but intriguingly resulted in sustained decreases in bone resorption markers [57]. The mechanism behind this phenomenon may involve the ability of Wnt signaling to simultaneously decrease RANKL and increase osteoprotegerin expression on osteoblast-lineage cells [59].

Does coupling contribute to pathologic bone lesions?

A number of primary and secondary bone lesions share a common pathology consisting of large osteoclasts within an accumulation of stromal cells that may be of the osteoblast lineage. These conditions include giant cell tumor of bone, fibrous dysplasia and conditions linked by excessive PTH signaling such as brown tumors, osteitis fibrosis cystica, and osteoclast-rich osteopetrosis. Given that osteoclasts likely mediate the proliferation and differentiation of mesenchymal osteoblast precursors during bone remodeling, in this section we consider whether osteoclasts in these lesions directly promote expansion of the stromal component.

Giant cell tumors are locally aggressive osteolytic primary bone tumors for which no etiologic mutation has been identified. Pathologically they are composed of osteoclast precursors, osteoclast-like giant cells and neoplastic stromal cells that differentiate into osteoblasts *in vitro*. Bisphosphonates are used to decrease osteolysis, but may also reduce tumor recurrence and progression [60, 61], suggesting osteoclast-like giant cells promote tumor growth. This hypothesis is substantiated by recent studies showing that the RANKL inhibitor, denosumab, not only eliminated the giant cells, but also decreased the proportion of stromal cells with a concomitant increase in new woven bone formation [62, 63].

Fibrous dysplasia (FD) is a benign fibro-osseous lesion caused by somatic mutations in *guanine nucleotide binding protein, alpha stimulating (GNAS)* in skeletal progenitors. FD is an ancient disease that can be found in Neanderthal bone [64] and presents with pain, skeletal deformity and pathologic fractures. On radiographs, ground glass lesions and endosteal scalloping are observed [65]. Mutations in *GNAS* result in constitutive activation of $G\alpha_s$, one of the G proteins activated by the PTH receptor [66]. Constitutive $G\alpha_s$ activation leads to the focal accumulation of pre-osteogenic stromal cells that form

immature woven bone and cause marrow fibrosis [67, 68]. The stromal cells are strongly positive for RANKL, driving the formation of clusters of osteoclasts within the fibrous elements of the tumors [68, 69]. Bisphosphonate therapy may be useful for treating pain and decreases bone turnover markers in some studies, but it is less clear whether there is an effect on lesion size or healing [70]. Denosumab has now been used in three FD patients refractory to bisphosphonates with improvement in pain, decreases in bone formation markers, and in one case a slowing of the rate of lesion growth [71, 72].

Osteoclast-rich ARO patients develop severe cytopenias due to loss of marrow space, exacerbated in some cases by infiltration of the residual marrow space by stromal or fibroblastoid cells (i.e. fibrosis) [11, 23, 25, 73]. Accumulation of these cells may result from chronic hyperparathyroidism, analogous to that seen in brown tumors [74]. Interestingly, bone marrow (BM) fibrosis has not been reported in osteoclast-poor ARO patients, who presumably also have elevated PTH. It is possible, though speculative, that osteoclasts are required for this fibrotic response to chronic hyperparathyroidism. Support for this concept comes from a mouse model where OPG reversed marrow fibrosis in mice expressing a constitutively active PTH receptor in osteoblasts [75]. In summary, a deeper understanding of how osteoclasts ‘talk back’ to the osteoblast lineage may provide insight into, and new treatment options for, osteoclast-rich ARO, osteitis fibrosis cystica, brown tumors, giant cell tumors and FD.

Osteoclasts and other cells in the bone microenvironment

Osteoclasts and the hematopoietic stem cell (HSC) niche

Providing a niche for hematopoiesis is a major skeletal function and osteoblasts and osteoclasts play a direct role in forming the BM cavity through their bone forming and resorbing activities. However, these cells may also directly regulate the hematopoietic stem cell (HSC) niche. The function of the osteoblast lineage in the development and maintenance of the HSC niche has been the focus of much investigation [76]. Here, we focus on how osteoclasts regulate HSC biology.

Osteoclasts in the formation and maintenance of the HSC niche—Anemia and leucopenia are presenting features of ARO. While altered hematopoiesis may simply reflect a lack of BM space, osteoclasts may play an active role in establishing the HSC niche. ARO patients with *TCIRG1* mutations manifest the most severe hematologic abnormalities [11]. Thus, Mansour *et al.* examined the HSC niche in *oc/oc* mice, which carry a homozygous *Tcirg1* loss of function mutation [77]. *Oc/oc* mice displayed a 50-fold reduction in Lin^{neg}Sca1⁺cKit⁺ (LSK) HSCs, and homing of adoptively transferred wild type (WT) HSCs to *oc/oc* BM was defective. These abnormalities were accompanied by an expansion of BM CD45⁺ mesenchymal cells with limited osteoblast differentiation capacity [77]. Restoration of osteoclast activity rescued these defects. The authors concluded that osteoclasts aid in the formation of the HSC niche through effects on osteoblast differentiation, although a precise mechanism was not identified. It is possible that these CD45⁺ mesenchymal cells represent the same stromal cells observed on tissue pathology of ARO patients discussed in the preceding session. A recent publication measured LSK cells numbers in the BM and spleen of *Ctsk*^{-/-} mice, which have a more modest osteoclast-rich osteopetrosis compared to the

oc/oc strain [78]. A decrease in the total number of LSK cells in the BM of *Ctsk*^{-/-} mice was observed, likely secondary to a decrease in the total cellularity of BM. In contrast, the total number and fraction of LSK cells was increased in spleen. These data are consistent with the hypothesis that osteoclasts contribute to the establishment of the BM HSC niche.

Two studies addressed the role of osteoclasts in maintenance of the HSC niche in adult mice and came to opposing conclusions. First, alendronate or calcitonin reduced LSK numbers in BM while increasing HSCs entering the cell cycle, suggesting the HSCs were beginning to differentiate [79]. In addition, alendronate blocked the increase in LSK cell numbers in response to PTH, an effect mediated by osteoblasts. The authors of this study concluded that osteoclasts maintain ‘niche-type’ osteoblasts. The second study generated chimeric mice lacking either TCIRG1 or RANK in BM derived cells [80]. In competitive repopulation experiments, BM cells from *Tcirg*^{-/-} or *Rank*^{-/-} chimeras engrafted in a secondary host equivalently to WT BM. This suggests that neither chimera had a functional defect in HSCs. However, small numbers of residual WT osteoclast precursors in these chimeras may compensate osteoclast function, as suggested by the modest changes in bone mass and resorption markers seen in this study.

Multiple explanations for the HSC phenotype of *oc/oc*, *Ctsk*^{-/-} or bisphosphonate or calcitonin treated, mice are possible. First, a lack of osteoclast-derived growth factors from bone may play a role. Alternatively, dysfunctional osteoclasts may affect the HSC niche by inhibiting osteoblast differentiation, promoting mesenchymal cell proliferation and/or blocking migration of LSK cells into the BM. The observation that osteoclast-poor ARO patients display only mild hematologic abnormalities argues that dysfunctional osteoclasts may actively prevent the formation of the HSC niche [10, 11, 24]. In support of this model, mouse strains with osteoclast-poor osteopetrosis show higher levels of LSK cells than *oc/oc* mice [81]. To resolve these mechanisms, the HSC niche should be examined in mice with osteoclast-lineage specific mutations that affect formation, function or the secretion of putative coupling factors.

Osteoclasts and the mobilization of HSCs—Under steady state conditions only a small number of HSCs are found in the circulation and lymphatics [82]. In stress situations (e.g. infection, bleeding) HSCs are released from their BM niche, proliferate [83] and differentiate to mature blood cells [82]. Subsequently, a fraction of HSCs are mobilized to the peripheral blood. This phenomenon is exploited during BM transplantation procedures by treating donors with granulocyte colony stimulating factor (G-CSF), which augments mobilization and facilitates the isolation of HSCs from blood. Mobilization involves cytokines, chemokines and proteases, which breakdown interactions between niche support cells and HSCs [82]. The chemokine stromal derived factor-1 (SDF-1), also called chemokine (C-X-C motif) ligand 12 (CXCL12), through its receptor chemokine (C-X-C motif) receptor 4 (CXCR4), is a particularly important mediator of HSC homing and mobilization. Multiple niche cells express SDF-1, which attracts HSCs and maintains their quiescence. Accordingly, a CXCL12 antagonist, AMD3100, promotes HSC mobilization [84]. SCF, another niche cell product, also retains HSCs in the BM through its receptor, c-kit [82].

Since excess G-CSF elevates osteoclast activity and causes osteoporosis in mice and humans [85–88], a number of studies have investigated whether osteoclasts contribute to HSC mobilization. In 1998, Takamatsu *et al.* found that inhibition of bone resorption with bisphosphonates increased peripheral HSC numbers after G-CSF treatment [89]. Likewise, G-CSF induced HSC mobilization was increased in mice with osteopetrosis due to mutations in the genes coding for MCSF, c-Fos and RANKL [81]. Conversely, mice lacking OPG have increased osteoclast activity and reduced LSK mobilization. Together, these studies argue against an important role for osteoclasts in mobilization.

Other studies have reached the opposite conclusion. Kollet *et al.* showed that mobilizing stimuli, such as bleeding, or treatment with pro-inflammatory molecules and SDF-1, increased osteoclast number [90]. Excess RANKL, which augments osteoclastogenesis, increased LSK cells as well. This effect was blunted in young, female protein tyrosine phosphatase receptor type, E deficient (*Ptpre*^{-/-}) mice, which display a transient defect in osteoclast activity. Moreover, RANKL treatment reduced immunostaining for stem cell factor (SCF) and SDF-1 on endosteal cells, while inhibitors of matrix metalloproteinase (MMP) and cathepsin K, two abundant enzymes in osteoclasts, prevented RANKL induced LSK mobilization [90]. This study suggests osteoclasts promote mobilization by degrading the BM components serving to retain HSCs. The second study examined responses to uridine diphosphate-glucose (UDP-glc), a pyrimidine derivative released at sites of inflammation and cancer [91]. Kook and colleagues found that injection of UDP-glc into mice promoted mobilization of an HSC population distinct from that induced by G-CSF. UDP-glc increased the expression of RANKL in BM lysates and histology suggested it elevated osteoclast numbers as well. UDP-glc could not mobilize HSCs in *op/op* mice, which lack osteoclasts because of a mutation in *Csfr1*. Thus, UDP-glc may be a novel HSC mobilizing factor that acts via osteoclast activation.

The data in the preceding two paragraphs are seemingly in disagreement, but a number of issues should be considered when interpreting these results. First, since bisphosphonates may not block the ability of osteoclasts to secrete cathepsin K or other enzymes [92, 93], failure of these drugs to inhibit mobilization does not exclude a role for osteoclasts. Second, while the analysis of mice lacking CSF1 (*op/op*), c-Fos or RANKL may appear definitive [81], the bones of these mutants are abnormal and an absence of osteoclasts may have profound secondary effects on osteoblasts or other niche cells. In addition, *op/op* mice lack macrophages [94], which may also contribute to mobilization [82]. Third, the marked reduction in HSC mobilization in young, female *Ptpre*^{-/-} is out of proportion to their modest bone phenotype [90]. Since this is a germline mutation, a direct function for protein tyrosine phosphatase epsilon (PTPε) in HSCs or other niche cells is possible. Fourth, cells other than osteoclasts may produce cathepsin K and MMPs in bone. Fifth, a function for the RANKL:RANK axis outside of the skeletal system has been recently appreciated [95–98]. Thus, ascribing effects of exogenous RANKL on mobilization solely to its ability to promote osteoclastic bone resorption may be incorrect. Additional experiments are still needed to resolve whether osteoclasts promote HSC mobilization.

Negative regulation of T-cells by osteoclasts

Bone is a common target for treatment-resistant infections and many common metastatic cancers. Thus, the bone microenvironment is conducive to pathologic processes that might otherwise be eradicated by an effective immune response. Here, we consider the emerging concept that osteoclast lineage cells are immunosuppressive through direct effects on adaptive immunity.

A double agent: osteoclast progenitors as myeloid derived suppressor cells (Figure 2)—Although osteoclasts are clearly within the myeloid lineage, only recently has the cellular identity of the osteoclast precursor (OCP) been addressed. Jacquin *et al.* demonstrated that the primary murine BM OCP resides in a CD11b^{low/-}CD115⁺ population that is otherwise lineage negative [99]. Recently, an OCP population in mice characterized by high expression of Ly6C, which is similarly CD11b^{low/-}CD115⁺, was identified [100]. To demonstrate that these cells were *bona fide* OCPs, they were adoptively transferred into either osteoclast deficient hosts [101], or hosts given lipopolysaccharide (LPS) into the calvaria to promote local osteoclastogenesis. In each case, osteoclasts derived from the transferred OCPs were identified. Testing whether adoptive transfer of these OCPs might exaggerate bone loss in a T-cell dependent model of rheumatoid arthritis (RA) led to the surprising finding that these cells attenuate joint inflammation [100]. This led to the hypothesis that OCPs may be myeloid derived suppressor cells (MDSCs).

Initially identified in tumor microenvironments, MDSCs have been found in the context of autoimmunity as well, and are characterized by expression of CD11b and either Ly6C (monocytic MDSC) or Ly6G (granulocytic MDSC) [102]. Similar to monocytic MDSCs, CD11b^{low/-}Ly6C^{hi} OCPs did not express Ly6G and inhibited T-cell proliferation *in vitro* through nitric oxide produced in response to T-cell derived interferon γ (IFN γ) [100]. Interestingly, differentiation of OCPs into osteoclasts did not diminish their ability to inhibit T-cell proliferation. Likewise, Grassi *et al.* showed that human osteoclasts inhibit T-cell responses *in vitro*, despite expressing class II major histocompatibility complex (MHC) and co-stimulatory molecules [103]. These data revealed an unexpected duality: OCPs, and perhaps even the osteoclast, can function as MDSCs *in vitro* and *in vivo*.

The duality of OCPs as MDSCs has also been noted in 3 cancer models [104–106]. An expansion of CD11b⁺Gr-1⁺ MDSCs with osteoclast differentiation capacity was found in mice injected with either a murine myeloma or a breast cancer cell line [104, 106]. When CD11b⁺Gr-1⁺ MDSCs from tumor challenged mice were co-inoculated with cancer cells into secondary hosts, they formed osteoclasts and increased tumor burden. In a third report, Sawant *et al.* isolated CD11b⁺Gr-1⁺ MDSCs from the BM of mice with bony breast cancer metastases and showed they differentiated into osteoclasts when cultured with RANKL [105]. It is tempting to speculate that CD11b⁺Gr-1⁺ MDSCs promote a hospitable bone microenvironment for tumor cells by inhibiting anti-tumor immune responses and releasing matrix-derived growth factors through bone resorption.

Induction of regulatory T-cell responses by osteoclasts (Figure 2)—Although osteoclasts derive from the same lineage as macrophages and dendritic cells, only recently

has the ability of osteoclasts to present antigen to T-cells been investigated. Li *et al.* showed that human osteoclasts express Class I and Class II MHC molecules, as well as co-stimulatory molecules, and induced both allo- and antigen- specific CD4⁺ and CD8⁺ T-cell responses [107]. In contrast, murine osteoclasts reportedly express only Class I MHC, suggesting they preferentially activate CD8⁺ T-cells [108]. CD8⁺ T-cells primed by osteoclasts lacked cytolytic activity and expressed high levels of forkhead box P3 (FoxP3), a marker of regulatory T-cells (T_{reg}). Accordingly, osteoclast-primed CD8⁺ T-cells (OC-iTc_{reg}) suppressed antigen-specific naïve T-cell proliferation [108]. Two follow-up studies on OC-iTc_{reg} demonstrated that these cells inhibit osteoclasts *in vitro* through IFN- γ , interleukin 6 (IL6) and IL10, and *in vivo* after both RANKL challenge and ovariectomy [109, 110]. IFN γ produced by effector T-cells similarly inhibits osteoclast differentiation *in vitro* and in an arthritis model [8]. These data suggest that osteoclasts temper immune responses by driving regulatory CD8⁺ T-cell differentiation, which can feedback and inhibit osteoclast activity. Future work will determine the relevance of these interactions to pathologic states in bone and define the mechanisms osteoclast lineage cells utilize to control T-cell polarization. In addition, these data should be incorporated with the emerging appreciation that regulatory T-cells suppress osteoclast differentiation through membrane bound Cytotoxic T-Lymphocyte Antigen 4 [111, 112].

Concluding Remarks

For decades, osteoclast related research has deciphered the cellular and molecular mechanisms governing the differentiation and bone resorbing activity of this cell. This investigation led to the discovery and clinical application of anti-resorptive agents, which have prevented countless fractures and improved the quality of life of patients with bone cancer. The studies outlined in this review highlight additional dimensions of osteoclast biology. Osteoclasts may directly regulate osteoblast differentiation, the HSC niche, T-cell activation and skewing, and the proliferation of tumor and stromal cells in bone. However, much remains to be learned before the role of osteoclasts in all aspects of skeletal biology can be fully appreciated. Key future directions include defining: i) whether osteoclasts communicate with bone resident osteocytes to regulate remodeling; ii) the spatiotemporal relationship of osteoclast derived bone anabolic signals (i.e. bone matrix factors, clastokines and cell surface molecules) with osteoblasts and their precursors in the BMU paradigm; and iii) whether subsets of osteoclasts differentially interact with other cells depending on the microenvironmental context, as suggested by recent reports [17, 18]. In addition, it will be important to resolve conflicting reports, such as those that support or oppose a direct regulatory role for osteoclasts in the HSC niche, and to add to emerging data on how osteoclast lineage cells influence immune responses occurring at the bone interface.

To investigate these questions in adult animals, investigators must move beyond the reliance on mouse models with germline mutations. Undoubtedly, the dramatic skeletal phenotypes that occur when genes important for osteoclast formation and function are mutated in rodents and humans have fueled our understanding of this cell. However, these skeletal phenotypes are often so severe that answering more subtle questions about how osteoclasts communicate with other cells in bone is challenging. In mouse models, we suggest that systems for the temporal deletion of conditional alleles in osteoclasts and their precursors be

established. Moreover, clinical research in humans with emerging therapeutics which specifically target key regulators of bone remodeling, such as RANKL, cathepsin K and sclerostin, could include nested translational studies that specifically address their effects on the immune system, HSCs and tumor growth, where appropriate. In these ways, a clear picture of osteoclast biology beyond their role as ‘bone eaters’ will emerge.

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Highlights

- Osteoclasts modify the skeletal environment beyond their ability to resorb bone.
- Bone forming osteoblasts and their precursors are regulated by osteoclasts.
- Cancer, hematopoietic and immune cells are targets of osteoclast-derived factors.
- Insight into the osteoclast may improve outcomes for patients with bone diseases.

Figure 1. Osteoclasts-osteoblast interactions in the basic multicellular unit (BMU)

A. Osteoclasts (OC) differentiate from OC precursors (OCP) under the influence of MCSF and RANKL produced by osteoblast (OB) lineage cells including osteocytes. As OCs create a resorption pit, growth factors, including TGF β and IGF1, are released from the bone matrix. These growth factors may recruit mesenchymal osteoblast progenitors and promote their differentiation into mature cells that secrete osteoid to fill the area of resorbed bone. Some OBs differentiate further into matrix embedded osteocytes. **B.** OCs produce a number of clastokines (see Table 1) that may recruit OB progenitors and promote their proliferation and differentiation. **C.** Cell-cell contact mechanisms may also mediate OC-OB communication. Bidirectional signaling from OC ephrins and OB Eph receptors and reverse signaling through RANKL on OBs have both been invoked. Abbreviations: MCSF, macrophage colony stimulating factor; RANKL, receptor activator of NF- κ B ligand; TGF β , transforming growth factor β ; IGF1, insulin-like growth factor 1.

Figure 2. Immunoregulation by osteoclasts

Osteoclast precursors (OCPs) and osteoclasts (OCs) inhibit CD4 and CD8 T-cell proliferation via nitric oxide (NO) production in response to T-cell derived interferon γ (IFN γ). IFN γ in turn inhibits differentiation of OCPs into mature OCs. OCs also present antigen through major histocompatibility complex Class I (MHCI) to skew CD8+ T-cells toward an induced T_{reg} phenotype termed OC-iTc_{reg}. OC-iTc_{reg} in turn inhibit OCP differentiation to mature OC through IFN γ , interleukin 10 (IL10) and IL6.

Table 1

A summary of putative clastokines that couple bone resorption to osteoblastic bone anabolism.^a

Clastokines supported by <i>in vivo</i> data			
Factor		Evidence for effect on bone formation	Citation
CTHCR1	collagen triple repeat containing 1	CTHCR1 is produced by mature OC and stimulated OB differentiation in cell and organ cultures OC specific deletion of <i>Cthrc1</i> resulted in decreased BFR and BV/TV	[33, 34]
C3a	complement component 3a	C3 identified as an OB differentiation promoting factor in OC conditioned media C3a promotes OB differentiation <i>in vitro</i> A C3aR antagonist augmented bone loss after ovariectomy by reducing BFR	[35]
S1P	sphingosine-1-phosphate	S1P promotes OB differentiation Increased S1P from cathepsin K deficient OCs augmented OB differentiation <i>in vitro</i> OC specific <i>Ctsk</i> ^{-/-} have increased OB numbers and BFR	[29, 36, 37]
Sema4D	semaphorin 4D	OCs express Sema4D <i>Sema4d</i> ^{-/-} OC conditioned media promotes, and Sema4D inhibits, OB differentiation <i>Sema4d</i> ^{-/-} mice have increased BFR and BV/TV, which is rescued by BM transplant Sema4D antibody reduces bone loss after ovariectomy	[119]
TRAcP	tartrate resistant acid phosphatase	Secreted TRAcP promotes OB differentiation TRAcP overexpressing transgenic mice have increased BFR <i>Tracp</i> ^{-/-} mice phenotype suggests role in endochondral ossification	[120–122]
CT-1	cardiotrophin-1	OC express CT-1 CT-1 promotes OB differentiation Neonatal <i>Cl-1</i> ^{-/-} have decreased OB numbers and BV/TV	[123]
Clastokines supported by <i>in vitro</i> data only			
CXCL16	chemokine (C-X-C motif) ligand 16	CXCL16 identified as an OB pro-migratory factor in TGFβ1 treated OC conditioned media	[124]
LIF	leukemia inhibitory factor	OC derived LIF suppresses TGFβ1 induced OB migration	[124]
afamin	afamin	Expressed by OC Promotes OB cell line migration <i>in vitro</i>	[125]
BMP6	bone morphogenic protein 6	Induced during OC differentiation Promotes OB differentiation <i>in vitro</i>	[37, 126]
Wnt10b	wingless-type MMTV integration site family, member 10b	Induced during OC differentiation Promotes OB differentiation <i>in vitro</i>	[37]
SOST	sclerostin	Sclerostin is higher in conditioned media of OCs prepared from aged versus young mice and correlates with inhibition of mineralization Sclerostin neutralizing antibodies relieve mineralization inhibition by OC conditioned media from aged mice	[127]
HGF	hepatocyte growth factor	Expressed by OC Promotes OB proliferation	[128, 129]
PDGF-BB	platelet derived growth factor BB	Produced by non-resorbing OC Induces MSC/OB precursor migration Inhibits OB differentiation.	[130–132]

^a Abbreviations: BFR, bone formation rate; BM, bone marrow; BV/TV, fraction of bone volume in total volume; MSC, mesenchymal stem cell; OC, osteoclast; OB, osteoblast.