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COMMENTARY

Inhibition of cathepsin K: blocking osteoclast bone resorption and more

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Approved therapeutic agents that were developed for the treatment of osteoporosis, improve bone mass and reduce fracture susceptibility by decreasing or increasing bone remodeling, consequently altering the balance of bone formation and bone resorption to favor an increase in bone mass.[1–3] Through the tight coupling of the processes of bone remodeling, the antiresorptive drugs such as bisphosphonates and the anti-RANKL antibody reduce bone resorption by blocking osteoclast function or differentiation and indirectly reduce bone formation.[2]

Following the approval of multiple bisphosphonates for the treatment of osteoporosis in the late 1990s, there has been continued interest in new targets for the development of osteoporosis therapies. The enzyme cathepsin K (CatK) has received significant interest because of its abundant and relatively restricted expression in osteoclasts.[4] Genetic defects in CatK activity in humans (pycnodysostosis) and in mice with mutation or deletion of the *CatK* gene leads to osteoporosis.[4] Global deletion of *CatK* in mice leads to marked reduction of bone resorption and increases the number of osteoclasts and bone formation rate by ~2–3 times compared with that of wild-type mice.[5] The high bone formation phenotype observed in *CatK* knockout mice suggests this enzyme may be involved in mediating changes in osteoclast-derived coupling factors that regulate bone formation. However, a number of non-osteoclastic bone cells, such as osteoblasts and osteocytes, have been reported to express CatK mRNA albeit at very low levels compared with that in osteoclasts.[6,7] Hence, a direct and intrinsic role of CatK in these cells in the global knockout model cannot be completely ruled out.

Mechanistically, Lotinun *et al.*[8] have defined the pivotal role of CatK in bone remodeling through osteoclast functions *in vivo*. Targeted deletion of *CatK* in cells at the early stage of osteoblast differentiation using Osx-cre revealed no obvious bone phenotype. This finding provided compelling evidence that CatK does not directly regulate bone formation, at least not during skeletal remodeling and modeling including periosteal cortical expansion. Interestingly, these data also suggest that CatK may not have a significant role in the late-differentiated osteocyte in the maintenance of bone homeostasis or in response to mechanical loading. Recently, CatK expression has been shown to be increased in osteocytes in mice during lactation, leading to a process known as osteocytic osteolysis.[7] Hence, the role of CatK in osteocytic perilacunar matrix remodeling could be evaluated in cells of the osteoblast lineage using the conditional *CatK* knockout mice.

This work also provided important genetic evidence for a coupling mechanism that regulates the communication from osteoclasts to other cells that mediate bone formation during remodeling. Mice with targeted deletion of *CatK* in the hematopoietic cell lineage driven by Msx-1-cre or CD11b-cre have the high bone mass and high bone formation rate that was observed in global *CatK* knockout mice. Although Msx-1-cre targets several neuronal and hematopoietic cell types, the CD11b-cre conditional knockout is more specific to the monocyte/macrophage lineage. *CatK* is deleted only in male CD11b-cre knockout mice, which show a similar overall but milder bone phenotype as compared with that of the sex-matched global or Msx-1-conditional knockout mice.[8] The milder phenotype in CD11b-*CatK* knockout mice may be because of lower efficiency of CatK ablation in osteoclasts or an additional contribution from other hematopoietic cell types. Of note, the bone phenotype of global *CatK* deletion appears to be gene-dose-dependent when comparing bone mineral density of heterozygote with homozygotes in skeletally mature animals.[5] It is thus tempting to propose that levels of CatK activity in osteoclasts directly regulate the rate of bone removal activity as well as the generation of 'coupling' signals that mediate bone formation.

How does CatK inhibition in osteoclasts maintain or increase bone formation? Genetic or pharmacological inhibition of CatK selectively reduces the osteoclast's ability to break down demineralized bone matrix proteins, but these cells remain viable and continue to create shallow resorption pits. Other cellular activities are preserved as well, such as secretion of acid and proteins including CatK itself.[9] In addition, the number of osteoclasts increases under CatK inhibition, which is likely due to increased activity of RANKL/OPG as a feedback

mechanism, as observed in *CatK*$^{-/-}$ mice.[8,10] Therefore, it is reasonable to propose that these non-resorbing osteoclasts during CatK inhibition maintain the signals for the reversal and formation phases within the bone-remodeling unit. These signaling molecules can be classified into two categories: (a) soluble or membrane-bound osteoclast-derived cytokines or clastokines that are growth factors made by osteoclasts; and (b) matrix-derived growth factors (MDGFs) or matricines that are released from the bone matrix during the bone resorption process.

Lotinun *et al.*[8] demonstrated that expression of sphingosine-1-phosphate, a known clastokine,[11] is increased in conditioned media of osteoclasts derived from *CatK* knockout mice because of upregulation of sphingosine kinase 1 (Sphk1). It is unknown how gene ablation of CatK results in this increased expression of Sphk1 in osteoclasts. It is also unknown whether Sphk1 is upregulated in osteoclasts under pharmacological inhibition of CatK, which demonstrates a high bone formation phenotype in rabbits and monkeys. However, reduction of CatK activity in osteoclasts by either pharmacological inhibition[9] or genetic deletion as in *CatK*$^{-/-}$ osteoclasts[8] leads to dramatic retention of intracellular vesicles, which could shift the balance of membrane trafficking for peptide/protein synthesis, transport and degradation in these cells during bone resorption.

With CatK inhibition, osteoclasts *in vitro* continue to make shallow resorption pits with a slight reduction of total resorption surface,[9] suggesting the cells are capable of acid secretion and initial degradation of bone matrix proteins. There is additional evidence supporting the concept of accumulation of MDGFs in the local microenvironment of the bone-remodeling unit with CatK inhibition. Insulin-like growth factor-1, a late osteoblastic differentiation factor, has been demonstrated to be increased in conditioned media of osteoclasts in the presence of a CatK inhibition.[12] Acidification by resorbing cells can release active transforming growth factor-β to promote recruitment of osteoblast progenitors.[13] CatK, in addition to its collagenase activity, also shows gelatinase and peptidase activities.[14] Indeed, many MDGFs may be physiological substrates of CatK in the bone resorption lacunae. Hence, Lotinun *et al.*[8] proposed a mechanistic model in which inhibition of CatK leads to reduction of the rate of bone removal, while maintaining the local concentration of 'coupling' factors, including clastokines and MDGFs, to promote bone formation. As compensation for the reduction of bone resorption, increased number of non-resorbing osteoclasts further enhances the release of these 'coupling' factors.

The fine-tuned balance of bone remodeling is likely coordinated by local concentrations of a complex mixture of clastokines and MDGFs within the bone-remodeling unit. Inhibition of CatK and the release of bone formation factors are discussed in Lotinun *et al.*[8] in the context of their known positive functions in the promotion of osteoblast recruitment and/or differentiation. It is also likely that CatK controls the release of negative regulators of bone formation. Sclerostin is known as a physiological inhibitor of osteoblast differentiation, which is produced predominantly by osteocytes.[15] Inhibition of CatK in osteoclasts could indirectly regulate sclerostin expression through the osteocyte network. More recently, osteoclasts in aging mice have been shown to produce sclerostin[16] and osteocytes in lactating mice have been shown to express CatK.[7] The colocalization of both the protease and sclerostin under these high bone-remodeling status suggests that CatK could potentially modulate bone formation via the Wnt signaling pathway.

The complexity of this mechanism is evident as one observes that the bone phenotype observed in the osteoclast-conditional knockouts of *CatK* in mice cannot be completely translated into larger species treated with CatK inhibitors.[4] In ovariectomized nonhuman primates, inhibition of CatK activity with selective CatK inhibitors results in the reduction of trabecular bone remodeling, maintenance or enhancement of endocortical bone formation, and stimulation of periosteal bone formation.[17–19] Combinations of clastokines and MDGFs released from trabecular or cortical bones by osteoclasts in response to CatK inhibitors may explain the bone site-specific regulation of remodeling.

The effects of CatK inhibitors on periosteal modeling are difficult to explain using a proposed remodeling-based model. CatK expression in osteocytes or in cells within the monocyte–macrophage lineage could directly be responsible for the modeling-based periosteal cortical bone formation observed in preclinical species. Future research using the same genetic approach in mice will define whether the role of CatK on periosteal bone formation is regulated by the osteocyte network or directly by the periosteal monocytes–macrophages (or osteomac) lineage. More importantly, results from clinical studies with selective CatK inhibitors[4,20] will further our understanding of the novel mechanism by which CatK inhibition affects bone remodeling, and whether the treatment-related high bone formation observed in preclinical species is translated into humans.

Conflict of Interest

Le Duong, PhD is currently an employee of Merck & Co.

References

1. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord* 2010;**11**: 219–227.
2. Baron R, Ferrari S, Russell RG. Denosumab and bisphosphonates: different mechanisms of action and effects. *Bone* 2010;**48**:677–692.
3. Baron R. Osteoporosis in 2011: osteoporosis therapy--dawn of the post-bisphosphonate era. *Nat Rev Endocrinol* 2011;**8**:76–78.
4. Duong LT. Therapeutic inhibition of cathepsin K - reduction of bone resorption while maintaining bone formation. *BoneKEy Reports* 2012;**1**:67; doi:10.1038/bonekey.2012.67.
5. Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan SB *et al.* Bone density, strength, and formation in adult cathepsin K (-/-) mice. *Bone* 2009;**44**:199–207.
6. Mandelin J, Hukkanen M, Li TF, Korhonen M, Liljestrom M, Sillat T *et al.* Human osteoblasts produce cathepsin K. *Bone* 2006;**38**:769–777.
7. Wysolmerski JJ. Osteocytes remove and replace perilacunar mineral during reproductive cycles. *Bone* 2013;**54**:230–236.
8. Lotinun S, Kiviranta R, Matsubara T, Alzate JA, Neff L, Lüth A *et al.* Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation. *J Clin Invest* 2013;**123**: 666–681.
9. Leung P, Pickarski M, Zhuo Y, Masarachia PJ, Duong LT. The effects of the cathepsin K inhibitor odanacatib on osteoclastic bone resorption and vesicular trafficking. *Bone* 2011;**49**:623–635.
10. Kiviranta R, Morko J, Alatalo SL, NicAmhlaoibh R, Risteli J, Laitala-Leinonen T *et al.* Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases via an increased RANKL/OPG ratio. *Bone* 2005;**36**:159–172.
11. Ryu J, Kim HJ, Chang EJ, Huang H, Banno Y, Kim HH. Sphingosine 1- phosphate as a regulator of osteoclast differentiation and osteoclast-osteoblast coupling. *EMBO J* 2006;**25**:5840–5851.
12. Fuller K, Lawrence KM, Ross JL, Grabowska UB, Shiroo M, Samuelsson B *et al.* Cathepsin K inhibitors prevent matrix-derived growth factor degradation by human osteoclasts. *Bone* 2008;**42**:200–211.

13. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z *et al.* TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nat Med* 2009;**15**:757–765.
14. Lecaille F, Bromme D, Lalmanach G. Biochemical properties and regulation of cathepsin K activity. *Biochimie* 2008;**90**:208–226.
15. Costa AG, Bilezikian JP. Sclerostin: therapeutic horizons based upon its actions. *Curr Osteoporos Rep* 2012;**10**:64–72.
16. Ota K, Quint P, Ruan M, Pederson L, Westendorf JJ, Khosla S *et al.* Sclerostin is expressed in osteoclasts from aged mice and reduces osteoclast-mediated stimulation of mineralization. *J Cell Biochem* 2013;**114**:1901–1907.
17. Jerome C, Missbach M, Gamse R. Balicatib, a cathepsin K inhibitor, stimulates periosteal bone formation in monkeys. *Osteoporos Int* 2012;**22**:3001–3011.
18. Masarachia PJ, Pennypacker BL, Pickarski M, Scott KR, Wesolowski GA, Smith SY *et al.* Odanacatib reduces bone turnover and increases bone mass in the lumbar spine of skeletally mature ovariectomized rhesus monkeys. *J Bone Miner Res* 2012;**27**: 509–523.
19. Cusick T, Chen CM, Pennypacker BL, Pickarski M, Kimmel DB, Scott BB *et al.* Odanacatib treatment increases hip bone mass and cortical thickness by preserving endocortical bone formation and stimulating periosteal bone formation in the ovariectomized adult rhesus monkey. *J Bone Miner Res* 2012;**27**:524–537.
20. Costa AG, Cusano NE, Silva BC, Cremers S, Bilezikian JP. Cathepsin K: its skeletal actions and role as a therapeutic target in osteoporosis. *Nat Rev Rheumatol* 2011;**7**: 447–456.