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Lipid Kinases and Bone Homeostasis: Lessons Learned From Phosphoinositide 3-Kinase Isoform–Specific Knockouts

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Bone homeostasis is maintained throughout life by coordinated osteocyte regulation of bone resorption by osteoclasts and bone formation by osteoblasts. Many pathologic bone states such as osteoporosis, inflammatory arthritis, and bone metastasis have increased numbers, survival, or function of osteoclasts resulting in enhanced bone resorption outpacing bone formation. Differentiation, survival, recruitment, and resorptive activity of osteoclasts and their precursors are highly regulated by intracellular signals stimulated by soluble mediators, extracellular matrix, and adjacent cells. In this issue of *Arthritis & Rheumatology*, Gyo ri and colleagues describe a specific role for the enzyme phosphoinositide 3-kinase β (PI3K β) in the regulation of osteoclast-mediated bone resorption (1), suggesting that a specific inhibitor of this enzyme isoform may be a novel antiresorptive agent.

Osteoclasts are multinucleated cells containing numerous mitochondria and lysosomes, and they are adherent to bone surfaces. Osteoclast migration, polarization, and resorption are dependent on effective actin cytoskeleton reorganization into specialized podosomes, podosome belts, and actin rings. When attached to bone, mature osteoclasts form stable sealing zones consisting of an actin ring and ruffled borders made of infoldings of plasma membrane. During the process of bone resorption, the osteoclast cytoskeleton further rearranges such that secretory vesicles polarize toward and insert into the ruffled border plasma membrane to deliver their cargo of matrix-degrading molecules, including collagenase and other enzymes, into resorption lacunae (2).

Osteoclast differentiation is induced by stimulation of myeloid precursors with macrophage colony-stimulating factor (M-CSF) and RANKL in conjunction with costimulatory signals mediated by the immunoreceptor tyrosine–based activation motif (ITAM) adaptor proteins DNAX-activation protein 12 (DAP-12) and Fc receptor γ -chain (FcR γ) (3,4). RANK and costimulatory ITAM signaling cooperate to increase intracellular calcium, induce the transcription factor NF-ATc1, and promote osteoclastogenesis. DAP-12 is a key adaptor protein that is involved in the development and activation of osteoclasts, which involves

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RANKL and M-CSF signaling and integrin activation (5). Once activated by Src kinase, DAP-12 recruits Syk and PI3Ks leading to the generation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃), followed by recruitment of pleckstrin homology domain-containing proteins including Akt, phospholipase C γ (PLC γ), TEC kinases, and Vav (6). As such, DAP-12-induced recruitment of PI3K in part regulates osteoclast cytoskeletal rearrangement, spreading, migration, resorption, and survival.

PI3Ks are key enzymes in intracellular signaling from cell surface receptors and are involved in the regulation of immunity, metabolism, cardiovascular disease, and cancer. PI3Ks mediate cell growth, survival, differentiation, and migration. PI3Ks are divided into 3 classes (I, II, and III), and each class has different tissue specificities and physiologic functions. The PI3K classes are based on structure, function, and lipid specificity. Class I PI3Ks are heterodimeric molecules composed of a regulatory subunit (p85 α , p55 α , p50 α , p85 β , p55 γ , p101, or p87) and a catalytic subunit (p110 α , p110 β , p110 δ , or p110 γ) that phosphorylates the primary substrate phosphatidylinositol 4,5-bisphosphate, producing PIP₃. The regulatory p85 subunit keeps the kinase inactive during the basal state and then brings the catalytic p110 kinase to its substrate upon p85 recruitment to phosphotyrosine residues in receptors or adaptor proteins such as DAP-12.

Class I PI3K α , PI3K β , and PI3K δ are activated downstream from receptor tyrosine kinases (RTKs) or non-RTKs such as growth factor receptors and DAP-12. PI3K γ is downstream of G protein-coupled receptors. The outputs of PI3Ks include catalytic activity, scaffolding function, and downstream effectors such as protein kinase B (also called Akt) and small GTPases (7). The creation of PI3K isoform-specific inhibitors for use in cancer therapies recently allowed differing effects of selective PI3K inhibitors to be characterized in osteoclasts (8,9). Isoform-specific inhibitor studies revealed a role for PI3K α , PI3K β , and PI3K γ in RANKL-induced survival but not basal osteoclast survival. Additionally, resorption was specifically inhibited by PI3K β and PI3K γ inhibitors, while osteoclast attachment to bone surfaces was dependent on PI3K δ in vitro. These studies contrasted with those using cells derived from p85-deficient mice lacking normal class I PI3Ks, which exhibited defects in osteoclast resorption in vivo and in vitro but normal osteoclast differentiation and survival. PI3K γ -deficient mice showed increased bone mass in vivo potentially related to impaired osteoclast precursor migration (10). Thus, the exact role of individual PI3K isoforms in osteoclasts has been difficult to dissect through the use of inhibitors alone.

Gyori and colleagues utilized a p110 β -deficient mouse and identify a specific role for PI3K β in osteoclast development, bone resorption, and bone homeostasis (1). They show that PI3K β is required for normal bone remodeling under basal conditions in vivo and that mice deficient in PI3K β have a high bone mass phenotype with increased trabecular number. Histomorphometric analysis of PI3K β -deficient mouse bones revealed plump, poorly spread osteoclasts with significantly shallower resorption pits compared to wild-type mouse bones. In vitro-derived osteoclasts from PI3K β -deficient mice were reduced in number and size, with very poor resorptive capacity. However, despite their crenated appearance, PI3K β -deficient mouse osteoclasts were able to induce osteoclast-specific gene expression similar to that in wild-type mouse osteoclasts. PI3K β -deficient mouse osteoclasts had normal

survival and apoptosis, which suggests that the isoform-specific inhibitor may have had partial off-target effects. Thus, loss of PI3K β leads to small, poorly functional osteoclasts with normal survival and gene expression very similar to DAP-12-deficient mouse osteoclasts (11–13).

To define the mechanism of how PI3K β regulates osteoclast resorption, Gyori and colleagues examined actin ring formation and found that PI3K β -deficient mouse osteoclasts failed to form normal actin rings, instead forming patches of F-actin in the cell periphery.

Indeed, using enhanced green fluorescent protein–tagged Lifeact, which binds to F-actin, time-lapse videomicroscopy revealed the inability of PI3K β -deficient mouse osteoclasts to form actin rings, while wild-type mouse osteoclasts formed long-lived actin rings under the same conditions. Additionally, PI3K β -deficient mouse osteoclasts accumulated secretory vesicles instead of discharging them through the actin ring–stabilized ruffled border. Vesicle accumulation led to decreases in cathepsin K secretion and ineffective matrix degradation (1). Thus, PI3K β is required for normal osteoclast cytoskeleton organization and intracellular vesicle release necessary for bone resorption.

As demonstrated in the current study by Gyori et al, osteoclast cytoskeleton organization is essential for normal bone homeostasis and bone resorption. The significance of this finding is evidenced by the interest in the growing list of molecules that direct the actin cytoskeleton and are required for bone homeostasis. Indeed, many of these molecules are directly upstream (c-Fms, integrins, c-Src, DAP-12, FcR γ) or downstream (PLC γ , regulator of G protein signaling 10, Vav, Rac, Rho, cell division control protein 42) of PI3K. Specific PI3K p110 β inhibitors are currently in development for thrombosis disorders and as cancer therapies. With our new understanding of the unique role of PI3K β in osteoclast biology and bone homeostasis, such isoform-specific inhibitors may also be useful as therapeutic agents for osteoclast-mediated diseases such as osteoporosis, inflammatory arthritis, and metastatic bone disease.

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