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SPOTLIGHT

Open Thy Lattice Osteoclast, Resorb me!

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Osteoclasts degrade bone using Cathepsin K and two metalloproteinases: MMP9 and MMP14. In addition to cleaving collagen, Zhu et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202206121) discover that MMP9 and MMP14 also proteolyze galectin-3 on the cell surface. This process drives a galectin-3/LRP1 signaling axis that supports the hard tissue-resorbing function of osteoclasts.

Skeletal integrity is maintained by the coordinated activity of bone-forming osteoblasts and bone-resorbing osteoclasts. Understanding the cellular and molecular events at the origin of systemic or local bone loss occurring in highly prevalent conditions such as osteoporosis and rheumatoid arthritis is paramount to designing efficient preventive and therapeutic strategies. The osteoclast, a hematopoietic-derived multinucleated cell with complex morphological and functional features and a unique bone-resorbing capacity, has been at the center of attention for decades (1). Efforts have focused on understanding how these highly specialized cells differentiate, adhere to, and migrate along bone surfaces and how their unique subcellular bone-resorbing compartment is generated, maintained, and functions. Decreasing the bone-resorbing activity of osteoclasts by targeting essential molecules is among the most favored therapeutic strategies for osteoporosis (2).

During differentiation, osteoclasts undergo cytoskeletal reorganization resulting in cell polarization with a distinct apical membrane facing the bone surface. Adhesion is mediated by specific F-actin-containing structures, the podosomes, and resorption requires podosome packing to delineate a sealing zone where secreted protons and proteases dissolve and degrade the mineralized matrix (3). Macrophages, dendritic cells, neutrophils, endothelial cells, and megakaryocytes also use podosomes or podosome-like structures instead of focal adhesions to adhere and migrate, but podosome assembly into a resorptive organelle is unique to osteoclasts.

In this issue, Zhu et al. (4) shed light on osteoclast biology that may have therapeutic implications for bone loss and beyond. As any good research, the study started by a critical review of previously known facts followed by a simple question. Osteoclasts degrade type I collagen, the main component of the bone matrix, using a network of enzymes that includes Cathepsin K and two matrix metalloproteinases: the secreted MMP9 and the membrane-anchored MMP14, which are functionally redundant. Osteoclasts lacking both MMPs can form normally but cannot resorb bone (5). As MMPs have many documented substrates, including signaling molecules, the authors asked whether these two MMPs have functionally relevant, matrix degradation–independent activities.

To answer this question, Zhu et al. (4) first conducted an unbiased gene expression profile comparison of wild-type and mmp9/14-deficient (DKO) osteoclasts differentiated on plastic tissue culture dishes. The reasoning behind isolating and differentiating bone marrow progenitors away from any collagen-containing substrate was to prevent any possible confounding effects arising from MMP9/14 matrix-degradation products generated in the wild-type arm of the comparison. Surprisingly, a significant number of transcripts were differentially expressed between the two genotypes. The authors then systematically assessed the most changed biological processes. They excluded a contribution of altered mitochondrial activity and energy metabolism while showing that changes in transcripts related to cytoskeletal organization and small GTPases-mediated signals were reflecting a defect in sealing zone formation in DKO osteoclasts. RhoA is a major determinant of cytoskeletal organization, podosome arrangement and sealing zone formation (6). Zhu et al. (4) observed a marked decrease in GTP-loaded active RhoA in DKO osteoclasts and could rescue sealing zone formation and resorption defects in these cells by transducing a constitutively active form of RhoA. They also found that intact MMP9/14 proteolytic activity was required for RhoA activation. Collectively, these experiments indicate that MMP9/14 cleave one or several proteins upstream of RhoA, triggering the cytoskeletal changes essential to sealing zone formation. This novel MMP9/14 proteolytic activity was more crucial to the bone-resorptive function of osteoclasts than the contribution of the two MMPs to overall collagen degradation. By crossing the current MMP-related literature with the observed over-representation of glycosylation-related genes in their transcriptomic analyses, and through a rigorous process of educated guess/experimentation/elimination/validation, the authors

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demonstrated that the essential activity of MMP9/14 is the cleavage of an inhibitory lattice-like network formed by the carbohydrate-binding protein galectin-3 (7) on the cell surface. For example, they elegantly showed that treatment with an antibody neutralizing galectin-3 lattice formation but not carbohydrate-binding potential reversed impaired RhoA activation, cytoskeletal organization, and bone resorption of DKO osteoclasts.

At this point, Zhu et al. (4) could have closed the chapter after having validated their unexpected discovery using human osteoclast cultures and ex vivo bone explants, which they did. They could have proposed and discussed several plausible explanations for how the cleavage of the galectin-3 lattice by MMPs could impact on RhoA activity in osteoclasts. For example, previous studies have shown that, in other cell types, galectin-3 binds $\alpha_v\beta_3$ (8), the major integrin expressed in osteoclasts, and that galectin-3 modulates integrin-mediated RhoA signaling (9). Instead, the authors went further embarking on a "fishing expedition" to identify galectin-3-binding proteins at the osteoclast surface. Biotin/streptavidin pull-down followed by mass spectrometry revealed a number of galectin-3 glycosylated binders, including a few integrins and receptors, but the top hit was Lrp1 (the ApoE receptor), another surprise. The authors then demonstrated that the novel galectin-3/Lrp1 interaction they uncovered is functionally relevant to RhoA activation, sealing zone formation and bone resorption. While gene manipulation experiments in mice have shown that Lrp1 modulates osteoclast differentiation and numbers, these findings imply that Lrp1 also modulates osteoclast bone-resorbing activity once the osteoclast is fully differentiated and provide additional clues to the association of LRP1 polymorphisms with low bone mass.

Overall, this paper shows how two metalloproteases, a sugar-binding, lattice-forming protein, and a member of the LDL receptor family modulate in concert the biological function of a highly specialized cell type. It also provides a nice example of how a hypothesis-driven thorough study can lead to a discovery that opens new avenues for future work. One obvious task is to identify the molecule(s) connecting Lrp1 to RhoA in osteoclasts. In other systems, Lrp1 utilizes one or more co-receptors to elicit cell signaling responses (10), and the authors list a few candidates from their pull-down experiments that could also be explored. It is also worth investigating whether this novel biological rheostat is utilized by all osteoclast subtypes, extends to other hard tissue-resorbing cells, and/or is perverted in pathological conditions. The importance of galectin-3 cleavage by MMP9 for cartilage matrix remodeling during endochondral ossification (11) and the numerous reports connecting galectin-3 to tumour invasion and metastasis (7) support this possibility.

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