A quest for clarity in bone erosion: The role of sequestosome 1 in Paget’s disease of bone

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Alteentments in the SQSTM1 gene are a putative cause of Paget’s disease of bone, yet results are conflicting about how these mutations impact osteoclasts, the cell type believed to be the main pathological contributor. In this issue of JBC, Zach et al. provide important new evidence that the protein encoded by SQSTM1, p62, negatively regulates osteoclastogenesis and demonstrate that aged p62-deficient mice develop bone phenotypes similar to those of Paget’s disease. These findings help to clarify the role of this important protein and present new opportunities to interrogate bone biology.

Bones are in a constant state of flux, broken down by osteoclasts and reformed by osteoblasts, and pathologic bone conditions such as Paget’s disease of bone (PDB)\(^2\) result from an imbalance between these two processes. PDB patients have larger and more numerous osteoclasts, which leads to characteristic bone erosions along with an increase in rapidly formed, highly disorganized bone as the osteoblastic rebuilding tries to keep pace with the dominant osteoclastic resorption (1). PDB is the second most common bone disease after osteoporosis and causes bone deformation, pain, and susceptibility to bone fracture (2). Mutations in the gene sequestosome 1 (SQSTM1) are found in \(~40\%\) of PDB patients, and its protein product, p62, is up-regulated during osteoclastogenesis (3), suggesting it plays a key function in PDB initiation or development. However, p62’s role is not well defined, and previous studies produced conflicting results as to how this protein participates in osteoclast biology.

Osteoclasts and osteoblasts, along with other bone marrow cells, are constantly in communication to manage proper levels of bone growth. For example, osteoblasts excrete the protein RANKL, which stimulates production of multinucleated osteoclasts from precursor cells in a process called osteoclastogenesis (Fig. 1), which can be detected by readouts such as activation of the transcription factors Nfatc1 and NF-κB. An early study of osteoclastogenesis using precursor cells from p62-deficient mice showed decreased osteoclast formation and decreased Nfatc1/NF-κB activation (3). However, subsequent studies showed the opposite result: Osteoclasts harboring mutations in p62 displayed increased size and nucleation (4–6), along with activation of Nfatc1 and NF-κB (7, 8). Further discrepancies were seen in in vivo models of p62 deficiency. Mice generated with the equivalent of a human P392L mutation in SQSTM1 (the most frequent mutation in PDB) display a variety of phenotypes including decreased bone volume (5), local osteolytic lesions (6), or no phenotypic changes (4). So what does p62 really do?

Zach et al. (9) approached this question with similar strategies to past work, but with two important differences. First, they worked with a previously generated mouse model of p62/sequestosome 1 deficiency, which deleted exons 1–4 of the gene and had not been previously investigated for bone phenotypic changes. Second, they used a much reduced cell count in their in vitro differentiation protocols that avoids artificial inhibition of osteoclastogenesis. Consistent with previous findings, they found p62 was up-regulated during osteoclastogenesis in WT cultures. Furthermore, p62 deficiency led to an increase in osteoclast nucleation and differentiation and increased sensitivity to RANKL early in culture (i.e. days 3–4). However, no difference in osteoclast number was seen at later time points. These data led the authors to conclude that p62 is a negative regulator of osteoclastogenesis, differing from the findings of Duran et al. (3) but in agreement with other studies (4–8). Interestingly, Zach et al. (9) found that p62 deficiency did not disrupt Nfatc1 or NF-κB signaling, in contrast to two previous reports (7–8). So, how does this regulation play out in vivo?

Zach et al. (9) next investigated the skeletal phenotype of p62-deficient mice, in which previous reports indicated varying degrees of bone volume change. Zach et al. (9) found that p62-deficient femurs were similar to WT femurs at 3 and 6 months, but there was an increase in bone mass at 9, 12, and 15 months. Histologically, p62-deficient mice had more mature osteoclasts in the lower femurs. Interestingly, closer examination of bones from 21-month-old mice revealed osteolytic lesions in the p62-deficient mice that are consistent with a PDB-like phenotype. Two serum markers of bone turnover were also significantly increased in p62-deficient mice.

The work of Zach et al. (9) brings some clarity to the inconsistent findings about p62 in PDB, and importantly makes available an animal model that mimics human disease. Bone phenotypes depend highly on the mouse background strain, which may explain the differences between the mouse model used by Zach et al. (9) and those of previous.
For example, Chang et al. (10) found that osteoblast-specific depletion of p62 resulted in low bone mass due to impaired macrophage-dependent osteoclast differentiation. Understanding the role of p62 in cells of the marrow compartment may help uncover non-cell autonomous effects on osteoclasts and osteoblasts. We look forward to these answers and more, as the quest for insights into PDB continues.

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